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Rice breeding strategies of North Eastern India for resilience to biotic and abiotic stresses: A review

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ABSTRACT

In north eastern region, rice is grown in diverse agro-climatic conditions viz., upland, jhum, terraces, lowland and deep water. The region is considered to be a hot spot for rice diversity and have reported to have valuable landraces, wild rice and primitive cultivars. The major rice production constraints in the region are large acreage under traditional cultivars, biotic and abiotic stresses, and lack of wide scale technological intervention. Among abiotic stresses, soil acidity, iron toxicity, aluminium toxicity, low light intensity, low temperature, flooding and moisture deficit stress are the major problems that lead to lower productivity of rice. Under biotic stress, rice blast is the major disease that ultimately affects rice in all the rice growing ecosystem of the region and cause huge economic loss. Hence, it would be logical to prioritize the rice research on the basis of prevailing constraints under rainfed areas of north eastern India.Breeding of suitable rice varieties by exploitation of landraces and local cultivars adapted to the regions would be the most sustainable strategy to boost rice production. A systematic collection, conservation, characterization and documentation of rice germplasms for resistance to stresses, grain quality and other agronomic important traits will accelerate the successful utilization of the germplasms in classical and innovative rice breeding work.

Key words: *Rice, germplasm, stress, breeding, diversity*

The north east (NE) region of India constitutes eight states (Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura). The region is consider as a hot spot for agro-biodiversity and is reported to have 9650 cultivars of rice, 15 races and 3-sub races of maize, 300 of taros, 230 of yams, 17 species and 52 cultivars of citrus, 16 taxa of banana, 700 taxa of orchids and 19 taxa of sugarcane (Satpathy and Sarma 2001). Rice is the principal staple food crop of the NE region and occupying maximum area under cultivation (Kuotsuo *et al.* 2014). The total rice production of NE region is estimated to be ~5.5million tones with average productivity of 1.57 t/ha, which is much below the national average of 2.08 t/ha (Pattanayak *et al.* 2006). In NE region, rice is grown mainly under four ecosystem, *viz.*, direct seeded rainfed upland, transplanted in terraced land, low lying wet land areas and flood prone valley areas. The production constraints of rice are widely related with certain abiotic and biotic stresses endemic to the region. The productivity of rice could be enhanced by systematic planning and designing of effective breeding system related with various aspect of rice. Except in some part of Assam and Tripura, rice is usually grown in only one season (mono-cropping). Rainy seasons in the NE region prevails for 7-8 months and hence there is opportunity to take two crop of rice by selection of appropriate varieties in *pre-kharif* and main *kharif* season. Traditional landraces are of long duration in nature and hence only one crop is grown during main *kharif* season (Kumar *et al.* 2016c). Systematic planning could execute cultivation of two crop of rice *i.e.,* cultivation of short duration variety during *pre-kharif* and medium duration variety during main *kharif* season. Development of short duration rice varieties having cold tolerance at reproductive stage could be most appropriate for post-flood seasons (Viraktamath *et al.* 2010). The current review paper highlight the richness of rice genetic diversity, specialty rice and breeding varieties for various biotic and abiotic stresses encountered by rice plants in the region.

Rice diversity and its utilization

NE region of India is a reservoir of large amount of rice germplams including wild rice. NE region is also being considered as the secondary centre of origin and enriched with landraces and primitive cultivars of special importance (Durai *et al.* 2015). The rice germplams grown in higher altitude of Arunachal Pradesh are intermediate between *indica* and *japonica*. Kampti cultivar of Arunachal Pradesh is rich in aroma with soft glutinous type endosperm (Durai *et al*. 2015). In Manipur, rice is grown in variable environment ranging from higher altitude, jhum cultivation to deep water rice. The state is enriched with very special black rice (Chakhao) along with other short grain indigenous rice. In Meghalaya genetic diversity are widely distributed in *Khasi*, *Garo* and *Jaintia* hills. In upland and jhum cultivation, farmers prefer mostly the local genotypes of special local taste. Most of these indigenous rice landraces are medium to longer duration (130-170 days). Sikkim is a high altitude hill state of NE India where agriculture is practiced only in~11.7% of geographical area. The majority of the local landraces of the state have been reported for cold tolerant. Tripura is covered by diverse topography and rice ecosystem includes *jhum*, wetland and deep water rice. In Assam, rice is cultivated in diverse ecology ranging from, flood prone areas of the Brahmaputra, Barak valley of Assam and also in upland *jhum*. Aromatic rice varieties of Assam are known as *Joha*. There are two variants of *Joha* rice available in Assam *viz., Kala Joha* and *Amru Joha*. Along with cultivated *Oryza* species, five wild species along with their different forms occurs in NE region. *Oryza nivara* and *O. sativa f. spontanea* are frequently observed in rice field as these are morphologically very similar with cultivated rice. Other species of *Oryza* reported in the region are *O. officinalis*, *O. granulata*, *O. rufipogon* and *O. meyeriana* (Hore 2005). Apart from species of *Oryza* several other related taxa of *Oryza viz., Hygrorhiza aristata*, *Leersia hexandra*, *Porteresia coarctata* and *Zizania latifolia* are commonly found in the region. *Zizania latifolia* is mostly observed in Loktak lake of Manipur. Several intermediate forms or weed like species are also found *i.e.,*Tulsibaon, bogibaon and Kenkubaon in deep water and water logged conditions in the region (Hore 2005).

Breeding for disease resistance in rice

The unique ecosystem of NE region provides a suitable environment for many fungal, bacterial and viral diseases in rice due to the favourable weather conditions congenial for the disease development and spread. Among various diseases recorded in the region, rice blast (*Magnaporthe oryzae*), sheath blight (*Rhizoctonia solani*), sheath rot (*Sarocladium oryzae*), false smut (*Ustilaginoide avirens*) and bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) holds economic significance. Rice blast is economically most important and prevalent disease of rice in whole NE region, resulting in the huge economic losses. Due to high rainfall and relative humidity, high incidence of leaf and neck blast was reported from the NE region (Ngachan *et al*. 2014). Screening of a large array of rice germplasm under uniform blast nursery and artificial inoculation has led to identification of nine germplasm lines *viz.*, Meghalaya Lakang, KumtaMah, KemenyaPepeu, Wainem, Thekrulaha, Vishkv, PhouralUtlou, MesaoTsuk and Gum Dhan resistant to both leaf and neck blast under Manipur conditions (Anonymous 2015). An analysis of virulence spectrum of rice blast pathogen indicated that race profile of rice blast pathogen in NE region is highly diverse and distinct compared to other rice growing regions of India. Akhanaphou, a unique rice landrace of NE India, having its origin in Manipur has been reported to exhibit high field resistance to leaf and neck blast. Recently two novel quantitative trait loci (QTLs), *qLNBL-5* and *qLNBL-7* were identified conferring resistance to leaf and neck blast diseases (Aglawe *et al.* 2017). Employing the data generated on virulence spectrum of rice blast pathogen, now the breeding focus has been on gene deployment/pyramiding based durable management strategy. Sheath blight is another economically important disease affecting rice in NE region of India. The disease is most prevalent in pockets where the relative humidity is very high (>95%), the temperature is moderate (28-32°C) and N application is high. The disease is more prevalent in the lowlands of NE region where water stagnation is common, however under upland conditions; sheath blight is not a severe problem. At present the management of sheath blight in the region relies on the use of chemicals. Studies conducted at various parts of world have suggested that partial or quantitative resistance exists in different varieties to *Rhizoctonia solani* (Liu *et al.* 2009). A major QTL conferring resistance to sheath blight was identified to be located at chromosome 9 (Liu *et al.* 2009). QTLs responsible to sheath blight pathogen of rice in NE region needs to be defined/validated and employed for breeding resistance. Sheath rot disease has its sporadic occurrence in the region, particularly in lowlands and high yielding; nitrogen responsive cultivars are most susceptible to the disease. Availability of resistance to sheath rot disease is limited due to its complex nature (Bigirimana *et al.* 2015). A high pathogenic variability was reported to exist among the *S. oryzae* isolates (Ayyadurai *et al.* 2005); hence breeding efforts needs to be focused keeping in view this pathogenic variability. False smut is an emerging disease of rice in NE region of India, recorded to have its high incidence during last five years. Different popular varieties and local landraces of NE region were having various level of susceptibility to false smut disease. Hybrids are more susceptible to the disease. Resistance mechanism to false smut in rice is not fully understood. Two major genes having equivalent additive and polygenes model for explaining inheritance of false smut resistance was proposed (Li *et al.* 2012). Once the genetic locus responsible for false smut resistance is identified, they can be transferred to popular varieties of NE region. Bacterial leaf blight (BLB) is a disease occurring in the pockets of NE region of India receiving little higher temperature with high relative humidity. Marker assisted pyramiding of these resistance genes into popular rice cultivars of NE region and identification of novel résistance genes against BLB from the germplasm of region will help devising a durable location specific management strategy for BLB.

Breeding for insect and pest

Brown plant hopper [*Nilaparvata lugens* (Stål.)] is one of the most destructive pests of rice in NE region which cause moderate to severe yield loss. Identification of resistant varieties is very important as the biotypes of the pest is changing its behaviour from time to time and the earlier released resistant rice varieties becomes susceptible to the pest. The Brown plant hopper (BPH) resistance genes have been identified in wild species *Oryza australiensis*, *Oryza officinalis*, *Oryza glaberima*, *Oryza eichengiri*, *Oryza rufipogon*, *Oryza minuta* and Indian cultivars (Zhang 2007). The use of resistant rice varieties is the most economical and efficient method for controlling the BPH as compared to chemical control. Therefore, it is imperative to identify BPH resistance genes from diverse sources and incorporate them into rice cultivars. Krishna *et al.* (1977) reported 69 resistant or moderately resistant lines from screening of 890 cultivars of NE region of India. Khush *et al.* (1985) found that resistance to brown plant hopper in rice cultivar, ARC10550 was governed by a single recessive gene which was designated as *bph5*. Li *et al.* (2010) evaluated 1200 accessions of common wild rice (*Oryza rufipogon*) and identified six accessions for broad spectrum resistance against several biotypes of BPH. Whorl maggot mainly infects the rice plant where stagnation of water occurs for longer duration and drainage of water is not adequate. The tolerant genotypes have the ability to compensate the loss occurred by whorl maggot by faster growth. The rice cultivar IR 40 and the wild rice *viz.*, *Oryza branchyantha* and *O. ridleyi* were identified as resistant to whorl maggot. In India, attempts were made to screen lines having resistance to whorl maggot and few lines such as RP 2418-5, RP 2418-10 and RP 2419-3 (Sain and Hakim 1988) and IR 9209-48-3-2 and UPR 82- 1-7, have been identified to be least susceptible to whorl maggot in Punjab. Gundhi bug has become a major problem in NE states, especially, Manipur since early 2000s (Krishnaiah and Varma 2012). It is one of the major pests responsible for crop damage at vegetative and reproductive stage particularly at milking stage. Both the adults and nymphs feed on grains at the milking stage and reduce yield by as much as 30%.

Coarse-grain and bearded cultivars are resistant to the rice bug. Rice stem borers damage crop plants in nursery stage, vegetative stage and reproductive stage leads to drastic reduction in yield. Several species of rice stem borers are reported from NEH region and yellow stem borer is considering as predominant. Progress on the development of rice varieties with high level of resistance to stem borers in India and other part of the world is very much slow due to lack of suitable germplasm, screening techniques and a poor understanding of the genetics of resistance. In the recent years some efforts have been made at national and international levels to decipher genes from moderately resistant genotypes. Several landraces from the NE collection have been identified for moderate level of resistance that includes, ARC 6107, ARC 6044, RYT 2908 (vegetative stage), ARC 6215, ARC 6579, ARC 5757 (heading stage), and ARC 5500 (Kalode *et al.*1989). Gall midge (*Orseolia oryzae*) is one of the major pests which are causing significant yield loss of rice (Murlidharan and Pasalu 2005). In India, gall midge damage causes an average annual yield loss of ~ 477 thousand tons of grain or 0.8% of the total production amounting to US$ 80 million (Bentur *et al.* 2003). Currently, a total of 13 biotypes of the Asian rice gall midge are reported. The Indian biotypes have been identified and characterized on the basis of differential reactions to genetically defined host plants (Sardesai *et al.* 2001). A new gall midge biotype existing in Manipur was identified as biotype 6 (Singh 1996), which differs from the other six biotypes in India (Lakshmi *et al.* 2006). Breeding of resistant rice varieties has proved to be a viable and ecologically sound approach for management of this pest. Through a special germplasm collection and screening program, 6730 landraces from NE states were screened, out of which 43 accessions were identified as tolerant to gall midge (Sastry *et al.* 1971). Sain and Kalode (1994) reported 337 lines as primary donors, out of which a large proportion come from the landraces of NE states of Assam (34%) and Manipur (7%), followed by those from Madhya Pradesh (20%), Tamil Nadu (8%), Odisha (5%) and Kerala (4%). Besides, cultivated rice, several of the accessions of wild species of *Oryza*, like *O. brachyantha*, *O. eichingeri*, *O. granulata*, and *O. ridleyi* were also reported to be gall midge resistant (Israel *et al.* 1959).

Breeding for weed tolerance

Weed is a great problem especially in upland rice ecosystem of NE region (Kumar *et al.* 2016a; Chatterjee *et al.* 2016; Prasad *et al.* 2013). Weeds are among the most important biological constraints for rice production in the region (Kumar *et al.* 2017). For effective weed control, proper herbicide application, timing and method are required which are often not met thereby resulting in poor weed control (Mandal *et al.* 2011a). Therefore, attention must be paid for alternative technologies which offer sustainable and economical weed control method in rice (Kumar *et al.* 2015; Roy *et al.* 2011). Genetic variation among diverse range of genotypes in their ability to compete with weeds has been documented for many crops, including rice (Kumar *et al.* 2016b). Crop competitiveness against weeds is composed of tolerance to weed infestation (Kumar *et al.* 2016c), which is the ability to maintain high yields under weedy conditions (Kumar *et al.* 2016d). A better understanding of genetic mechanisms by which a rice genotype becomes more competitive to weeds would not only serve to assist plant breeders in developing competitive cultivars more quickly and effectively but would also justify the use of plant breeding to increase crop-competitive ability. Competitive ability of rice genotypes is a much complex trait and could not be justified by selection of one or few characteristics. Plant height, specific leaf area index, tillering ability, dry matter portioning of leaves, crop duration, root biomass and shoot biomass at an early stage will be important traits while selecting the weed tolerant rice genotypes under upland rice ecosystem (Kumar *et al.* 2016d). In NE region, farmers prefer to grow traditional tall type varieties despite of low yield due to fast initial growth and exhibit weed tolerant ability under upland and jhum rice cultivation. Early seedling vigor is desirable trait under direct seeded rice (DSR) for enhancing the initial crop establishment and the ability to compete against weeds. Recently, Singh *et al.* (2017) phenotype a set of 253 BC_3F_4 lines derived from cross between Swarna and Moroberekan and identified several QTLs (*qEV3.1*, *qEUE3.1*, *qSHL3.1*, *qSL3.1*, *qSFW3.1*, *qTFW3.1*, *qRDW3q*, *EV5.1*, *qEUE5.1*, *qSHL5.1*, *qSL5.1*, *qSFW5.1*, *qSDW5.1*, *qTDW5.1*) for early seedling vigor and related traits under direct seeded system. Further, there is need to explore a range of diverse genotypes and design an effective breeding methodology for weed tolerant in order to achieve reasonable yield in upland rainfed dry land ecosystem.

Breeding for abiotic stresses

Rice cultivation in the NE region of India is exposed to different abiotic stresses that include problematic soil (acid soil), low temperature, metal toxicity (Fe & Al), low light intensity, intermittent flooding and drought (Ngachan *et al.* 2014). Recently researchers are using geostatistics and krigging tools to study the spatial variability of soil physico chemical properties to achieve a better understanding of complex relations between soil properties and environmental factors (Tripathi *et al*. 2015). Rice plant encounter several abiotic stresses right from nursery, vegetative to reproductive stage causing significant loss in yield depending upon intensity and severity of stresses. Identification and introgression of target genes/QTL(s) conferring high level of resistance/tolerance to agronomically desirable varieties through marker assisted selection (MAS) would be the most efficient strategy to achieve potential yield under stress environment. Soil quality is one of the important issue responsible for widespread yield stagnation and productivity declines in the rice cropping system (Shahid *et al*. 2013).

Breeding for low-Phosphorous (P) tolerance

In NE India, rice productivity is less than 40% of the national average due to acidic soils and poor availability of phosphorus (Anonymous 2012). Deficiency of phosphorous in acid soils are usually tackled by application of adequate amount of suitable phosphatic fertilizer and other management practices (Kumar and Meena 2016; Bhattacharyya *et al.* 2015). Several researchers has reported considerable genotypic variation for P uptake in diverse traditional varieties of rice and these traditional varieties are likely to possess genes/QTLs for high P acquisition. Phosphorus uptake1 (Pup1), a major quantitative trait locus (QTL) derived from Kasalath is reported to exhibit 78.8% phenotypic variance for P-uptake and the only available QTLs for maker assisted selection in rice (Wisuwa *et al.* 2002). Tyagi *et al*. (2012) have identified four genotypes containing *Pup1*, Sahbhagidhan, Dagaddeshi, Pynthor and Paijong, adapted to north eastern and eastern part of India, as potential donors for rice breeding for P deficiency tolerance. A major candidate gene *OsPSTOL1* (Phosphorus starvation tolerance 1) in the Pup1 QTL has been cloned and gene based makers are available for maker assisted gene pyramiding in different genetic background of rice (Pariasca-Tanaka *et al*. 2014). Recent years, a specific group of rice (aus-type varieties) that originates from NE region of India with poor and problematic soils has been recognized as a valuable source for novel genes/QTLs for tolerance to P and other nutrient deficiency.

Breeding for iron toxicity

Fe toxicity is one of the most emerging abiotic stress constraints in valley (low land) area of NE region. However, exact area and annual loss incurred due to iron toxicity is not yet fully studied in the region. Fe toxicity is commonly characterized by high amounts of reducible Fe, low pH, low redox potential, low cation exchange capacity (CEC) and low exchangeable potassium content (Ottow *et al*. 1982). Rice cultivars differs in their ability of iron toxicity tolerance and selection of rice cultivars with superior iron tolerance has paramount importance in the view of breeding of high yielding varieties with inbuilt iron toxicity tolerance (Shahid *et al*. 2014). Genetic differences in adaptation and tolerance for iron toxicity have been exploited for development of iron toxic tolerant cultivars (Nozoe *et al*. 2008).Therefore, pyramiding of genes/QTLs from different sources could result in higher level of tolerance in new varieties. Physiological mechanism of tolerance, such as root exclusion, iron compartmentalization within organs or true tissue tolerance, have been proposed but remain unexplored in diverse gene pool of rice (Matthus *et al*. 2015). Sahrawat and Sika (2002) reported African cultivated rice (*O. glaberrima*) could be a good source of tolerance to Fe toxicity as compared to Asian cultivated rice (*Oryza sativa*). Therefore, there is need to exploit genetic variability available among the different accession of *O. glaberimma* for tolerance to iron toxicity. Identification and incorporation of Fe toxicity tolerance QTL(s) in elite genetic background would be beneficial in order to get higher productivity of rice in iron toxic soils. Currently, application of DNA markers in breeding for tolerance to Fe toxicity by marker-assisted selection is very much limited because QTLs reported are for small effects and even for the few major ones, large confidence intervals and/or lack of validation in other genetic backgrounds and environment (Sikirou *et al*.

2015). Dufey *et al*. (2015) constructed an integrated map with the previous QTLs identified using the annotated physical map of the rice reference variety Nippon bare and highlighted four candidate regions (CR), with a high QTL density. Chrisanawati *et al*. (2016) identified three STS markers *i.e*., OsIRT1, OsIRT2 and OsFRO2 associated with iron toxicity tolerance in double haploid lines of rice. Nugraha *et al*. (2016) identified SNPs markers, TBGI380435 related to heavy metal transport detoxification and marker, TBGU313277 associated with proline transporter, probably associate with tolerance to iron toxicity in rice. Identification of several small effects QTLs for Fe toxicity tolerance showed that mechanism of tolerance may involve additive/cumulative effects of several minor genes. Manipulation of several major and minor QTLs/ genes may have a significant impact on phenotype. Acidic soils with iron toxicity often lead to zinc toxicity. Therefore, identification of QTLs associated with zinc toxicity tolerance is equally important while breeding for iron toxicity tolerance. Recently, Liu *et al*. (2016) identified a unique QTL, *qFRSDW11* associated with iron and zinc toxicity tolerance. NE regions has a rich reservoir of germplasm in form of landraces, primitive cultivars, wild rice and these untapped potential germplasm could be targeted for identification of some novel QTLs for iron toxicity.

Breeding for Aluminum toxicity

Aluminum toxicity in acid soils can be managed by application of lime, that is expensive, ineffective in sub-soil and in some cases may have a deleterious effect on the soil structure (Rao *et al*. 1993). Development of aluminum toxicity tolerant cultivars is most effective and sustainable approach to manage the problem. Genetics of aluminum tolerance revealed a complex multi-genic trait and therefore, for characterization of aluminum tolerance traits, composite screening methods could be much reliable than single screening method. Exploitation of natural variation and mapping of QTL through association mapping are most impressive tools for development of Al-stress tolerant cultivars. Kumar *et al*. (2016*) identified few rice cultivars *viz*., Posimot, Epyo, Aaha and VR-14 having aluminum toxicity tolerance under acid soil conditions of Meghalaya. Tolerant genotypes could be used in hybridization programme for development of high yielding genotypes with inbuilt tolerance of aluminum tolerance. The identification of DNA markers that are linked for aluminum tolerance loci in particular gene pools provides an important starting point for transferring and pyramiding genes that may contribute to the sustainable improvement of crop productivity in aluminum-rich soils (Nguyen *et al.* 2001). The isolation and characterization of genes responsible for aluminum tolerance is likely to be necessary to gain a comprehensive understanding of this complex trait. STAR1/2, the major Al tolerance gene identified in rice has conferred a basal Al tolerance in rice which is probably because, unlike temporary environmental stresses such as water and temperature stresses, Al toxicity is continuous in soil (Pattanayak and Pfukrei 2013). Recent progress in genomic research may lead to identification of more number of tolerant genes/QTLs and accelerate development of rice varieties having inbuilt tolerance to Al toxicity.

Breeding for cold tolerance

Low temperature is a major limiting factor for rice productivity in high altitudes of NE region of India. Cold stress affects rice plants by causing seedling mortality, spikelet sterility and eventually significant yield losses (Shimono *et al*. 2002). Identification of new genetic sources of cold tolerance is very much crucial for rice breeders to develop cold-tolerant rice cultivars in temperate and high altitude regions. There is a significant amount of genetic variability available for cold tolerance among rice gene pools and this variability often reflect the place of origin, natural selection as well as breeding history. Dissecting cold stress-mediated physiological, biochemical and genetic causes will facilitate in breeding of rice varieties for cold tolerance. The booting stage is found to be the most critical and cold sensitive stage for rice plants. On cold damage/injury at the booting stage, many evaluation methods were proposed in past such as, long cold water irrigation (Han and Koh 2000), constant temperature deep cold water irrigation (Futsuhara and Toriyama 1964), short-term low temperature management (Tsumoda *et al*. 1968), natural low temperature treatment (Dai *et al*. 2002) and identification of artificial climate chamber (Farrell *et al*. 2006). Cold-tolerant genetic resources have been identified and being used as a donor material for incorporating cold tolerance into new high yielding varieties (Abe *et al*.1989). Cruz *et* *al*. (2006) reported that reduction rate in spikelet fertility in response to low temperature can be used for evaluating cold tolerance per se. The ICAR Research Complex for NEH Region Umiam has identified Upper Shilong (1900 MSL) a site for cold tolerance research work, where temperature is quite low during reproductive stage (flowering and seed formation stage) of rice growth. About 1000 cultures of rice and segregating populations were screened at Upper Shillong and cultivar, Khonorullo was found to be the best followed by DuIlo-6, Ryllo Red 2, Ryllo Red 5, IR 3941-23, Jungruh *etc*. (Ngachan *et al*. 2014).The identified genotypes have low yield potential but possess unique adaptive cold tolerance traits and these traits could be targeted for development of high yielding cold tolerant rice variety. Many cold-tolerance related QTL have been identified in the past 20 years for reproductive stage, germination stage and vegetative growth. The QTL *Ctb1*, *qCTB2a*, *qPSST-3*, *qLTB3* are related to cold tolerance at the reproductive stage; *qCTP11* is related to cold tolerance at the germination stage; and *qCtss11* and *qCTS4a* are related to cold tolerance at the seedling stage (Zhang *et al*. 2014).

Breeding for low light intensity

Light intensity is one of the most vital environmental factors that affect growth and development of rice. The solar radiation requirements of a rice crop differ at various growth phases (Yoshida and Parao 1976). Cloudy weather during the vegetative growth phase slightly affects rice growth and development, while shading during reproductive phase has a pronounced effect on spikelet fertility and grain quality. There has been several report that increasing light intensity will lead to increase in total number of tillers, number of effective tillers (Emmanuel and Mary 2014) and plant height and leaf area (Liu *et al*. 2009). Rice crop suffers from low light intensity in NE India and ~50% of the total requirement *i.e.*, 1600 bright sunshine hours during growth period. Rice cultivars adapted to various geographical conditions in the NE India differ in their light tolerant capacities. Cultivars that are traditionally being grown are supposed to have greater tolerance for low-light stress than recently introduced cultivars. Therefore, identification of such rice cultivar will be essential in breeding for low light tolerant high yielding varieties of rice. Hence, in the future, new research should focus on strengthening the tolerance of rice plants to low light intensity.

Breeding for submergence/deep water

NE region of India falls under high rainfall zone and during monsoon, flood is one of the major problems occurring in low lying areas of Assam, Tripura, Manipur and Meghalaya. Rice is a reasonably flooding-tolerant crop but water logging for longer duration significantly affect the crop growth and survival. Flooding of rice field may be flash or intermittent or stagnant prolonged flooding. Flash-floods or submergence are highly unpredictable and may occur at any growth stage of the rice crop and the yield loss may range between less than 10 and 100% depending on associated factors such as water depth, duration of submergence, temperature, turbidity of water, nitrogen fertilization, light intensity and age of crop (Mohanty *et al*. 2001). Several local landraces generally grow in low-laying areas where water stagnation beyond 50 cm for more than a month in the season. These local landraces are slightly tolerant to deep water conditions due to inherent internodal elongation ability. The important local cultivars of north eastern region suitable for deep water rice are Padmapani, Panikekoa, Panindra, Padmanath, Sabita, Rayada B3, Maguribao, Negheribao (Bhowmick *et al*. 2000). Screening of the elite germplams for flood tolerance and its utilization in hybridization programme is one of the major objectives of conventional plant breeding for development of flood tolerance rice variety. In the past breeders have concentrated on using tall traditional cultivars such as FR13A and Kurkaruppan to incorporate strong submergence tolerance in modern varieties. At International Rice Research Institute (IRRI), Phillipines, submergence tolerance gene "*Sub1-A*" has been identified in FR-13 and successfully transferred in breeding lines, IR 49830-7-1-2-3 and IR-40931-33 using conventional breeding approaches (Sarkarung *et al*. 1995). Xu *et al*. (2006) reported clusters of three genes at Sub1 locus encoding putative ethylene response factors, which includes three ethylene responsive factors (ERF) like genes, *Sub1A*, *Sub1B* and *Sub1C*. *Sub1A-1* was later reported to be tolerance specific allele and a primary determinant of submergence tolerance. There is also possibility of novel gene/QTL in tolerant cultivars of NE accessions and therefore exploring elite germplasms for submergence or deep water rice has to be taken in priority.

Breeding for drought tolerance

Drought is one of the major abiotic constraints, affecting rice production and yield stability in a wider range of rainfed ecosystem. The water requirement by conventional transplanted rice varies from 1300-1900 mm of water (Kumar *et al*. 2017). In the present scenario, increasing water scarcity has threatened productivity and sustainability of irrigated rice systems; hence alternative water saving rice production technique has gained focus (Kumar *et al*. 2016). Although high rainfall occurs in NE region of India especially during monsoon season but short period of drought/intermittent drought is very much common in jhum or upper terraces due to low water retention capacity. The success of a drought breeding programme greatly depends on extent of available genetic variability in the population subjected to selection, the selection criterion, and availability of suitable selection protocol/screening. Selection for genotypes for drought resistance can be performed by measuring yield under stress and or measuring a secondary trait correlated to yield under stress. NE region of India is a rich reservoir of germplasm and these germplasm need to be well characterized for various morphological and physiological traits which are directly or indirectly contributing to drought tolerance.Several efforts have been made to identify major QTLs with a large and consistent effect on grain yield under drought (Bernier *et al*. 2007). At the International Rice Research Institute (IRRI), traditional and improved donors were used in developing mapping populations for the identification of major qDTYs (Swamy and Kumar 2011). *qDTY12.1* was the first reported large-effect QTL for grain yield under drought environment in a population of 436 random F_3-derived lines from a cross between upland rice cultivars Vandana and Way Rarem (Bernier *et al*. 2007). Anupam *et al*. (2017) identified seven droughts tolerant QTLs in 74 Tripura rice germplasms and observed *qDTY2.1*was present in the maximum number of germplasm while *qDTY1.1* was present in the least. Development of near isogenic lines (NILs) for vegetative and reproductive stage QTLs and pyramiding of these QTLs not only allow sustainable rice cultivation in areas affected by drought but also open a new way to grow rice in new areas.

Conclusion and future scope

The potential landraces and wild species of the region are source of valuable gene(s)/QTL (s) for several economic important traits. Therefore, it is utmost necessary for systemic collection, characterization and documentation of germplasm for its successful utilization in breeding programme. Farmers of the region are still conserving the germplam in their traditional way and hence there is need to keep balance between introduction of HYVs/Hybrids and cultivation of indigenous varieties in the view of protection of the traditional varieties of special value. The valuable landraces are reported to have gene(s) for several biotic and abiotic stresses and therefore these genes must be introgressed in the elite varieties through marker assisted selection. The nutritional dense cultivars could serve as a source materials for biofortification programme and these valuable cultivars should be quantified for several nutritional and biochemical characters. In future, there is need to prioritize the rice research on the basis of prevailing the local constraints under rainfed areas of NE India in order to achieve sustainable and profitable rice production.

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RGG1 transgenic rice plants and their physiological characteristics in relation to salinity stress

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ABSTRACT

The heterotrimeric G-protein complex, comprising of G α , G β , and G λ subunits. It is an evolutionarily conserved-signaling molecular machine which transmits signals from transmembrane receptors to downstream target proteins. Now-a-days their functions in plant stress-signalling have been reported. Here we report the physiological function of rice G-protein λ subunit (RGG1) rice (Oryza sativa L. *cv. IR64) plants under salinity stress in* T_3 *generation. The overexpression of CaMV35S promoter driven RGG1 in transgenic rice confers high salinity (200 mM NaCl) stress tolerance. Agronomic parameters were studied and found to be higher in the transgenic plants with respect to wild type (WT) plants.*

***Key words:** Antioxidative enzymes, G-protein gamma interacting partners, oxidative stress; hormones, RGG1, salinity stress tolerance, transgenic rice*

Gene Bank Accession Number of RGG1: GU111573.1

Locus: GU111573

Salinity is a widespread soil problem limiting crop productivity worldwide, especially in the tropical and irrigated fields where salinization has caused deterioration of agricultural lands (Mahajan and Tuteja 2005; Munns and Tester 2008). Several studies have demonstrated that the introduction of foreign genes into crop plants provides resistance against biotic as well as abiotic stresses (Xiong *et al*. 2006; Mazzucotelli *et al*. 2008; Chen *et al*. 2013). It was studied earlier that some RNA/DNA helicases also play an important role in the abiotic stress resistance (Liu *et al*. 2002; Tuteja and Tuteja 2006; Vashisht and Tuteja 2006; Kant *et al*. 2007; Li *et al*. 2008). Recently, the over-expression of a mitochondrial helicase OsSUV3 has been reported to impart salinity stress tolerance in rice plants without yield loss (Tuteja *et al*. 2013).

Rice (*Oryza sativa*) is an important cereal crop that provides a staple diet for almost half of the world's population and is the major food crop cultivated in Asia. The quality and yield of rice is greatly affected by environmental stresses such as salinity, drought, heat and cold. Abiotic stresses decrease both the growth and productivity of crops by reducing photosynthesis, decreasing seedling fresh weight, germination percentage and biomass and increasing the generation of reactive oxygen species (ROS) (Hadiarto and Tran, 2011). The heterotrimeric G-proteins are composed of G α (39-52 kDa), G β (34-36 kDa) and G λ (7-10 kDa) subunits (Gilman 1987; Tuteja and Sopory 2008). G-proteins transduce the signals from the outside environment to inside possibly through regulators (Colaneri *et al*. 2014). Subunits of G-protein have been reported in several plants such as arabidopsis, lotus, lupin, pea, rice, soybean, spinach, tobacco, tomato and wild oat (Jones and Assmann 2004; Assmann 2002; Mishra *et al*. 2007; Yadav *et al*. 2012). Plant G-proteins have been reported to regulate the ion channels, cell proliferation and developmental events and are involved in plant responses to stress, light, hormones, innate immunity, and in controlling shoot meristem size (Jones 2002; Jones and Assmann 2004; Perfus-Barceoch *et al*. 2004; Chen *et al*. 2006; Bommert *et al*. 2013; Cheng *et al*. 2015; Maruta *et al*. 2015). Pea G-proteins have been shown to be regulated under stress (Mishra *et al*. 2007; Bhardwaj *et al*. 2011). In recent studies, it was found that G-protein alpha null mutation confers prolificacy potential in maize (Urano *et al*. 2015), and type B heterotrimeric G-protein gamma-subunit regulates auxin and ABA signaling in tomato (Subramaniam *et al*. 2016). Furthermore, the interactome of arabidopsis G-protein reveals that G-proteins are multifunctional and play significant role in the development and combat against environmental stresses (Klopffleisch *et al*. 2011).

In the current study, we have developed transgenic rice plants IR64 (*Oryza sativa* L., cv. IR64) by over-expressing RGG1 gene. We observed that the over-expression of RGG1 leads to the enhancement of salinity stress tolerance by coping with stress-induced oxidative damage.

MATERIALS AND METHODS

Polymerase chain reaction and Western-blot analysis

Integration of the RGG1 gene was checked by PCR using 35sCamv forward primer (5'-AGAAGACGTTCCAACCACGTCTT-3') and RGG1 gene specific reverse primer (5'-TCACAAAAACCAGCATTTGCATCTG-3'). The crude plant extract from WT and over expressing lines was prepared using the method described (Hurkman and Tanaka 1986). Equal amount of crude proteins were denatured and separated using SDS PAGE, electroblotted onto polyvinylidene fluoride (PVDF) membrane and then probed with mouse polyclonal antibodies (1:1,000 dilution) raised against full length RGG1 and the crude extract from WT plant was taken as negative control. Western blot analysis using anti-RGG1 (1:5000) primary and alkaline phosphatise conjugated anti-mice (Sigma) secondary antibodies (1:1000 dilution) was performed to check the production of the protein by the transgenic lines. The blot was developed as per manufacturer's protocol (Sigma). The alkaline phosphatase conjugated secondary anti-mouse IgG antibody (Sigma-Aldrich http://www.sigmaaldrich.com) was used at 1:10000 dilution.

Isolation of RNA and quantitative real-time PCR

25-days-old rice (*Oryza sativa* cv. IR64) seedlings samples were harvested. Leaf samples of the wild type (WT) plants as well as T_3 transgenic lines (L1-L5) were used for RNA isolation and qRT-PCR was performed as described earlier (Tuteja *et al*. 2013). For qRT-PCR, the RGG1 gene specific primers (forward 5'-GCGCTTTCTCGAGGAACTTGAAG-3' and reverse 5'-CTTGCCAGTCTTGGGACAGATGGTTTG-3') were used. The expression was normalized to α-tubulin (forward 5'-GGTGGAGGTGATGATGCTTT-3' and reverse 5'-ACCACGGGCAAAGTTGTTAG-3') and calculated using the $2^{-\Delta\Delta Ct}$ method from three independent experiments (Livak and Schmittgen 2001).

Measurement of photosynthetic activities, agronomic attributes and endogenous ion content of T_3 transgenic plants

45-days-old seedlings of transgenic and WT plants were allowed to grow in 200 mM NaCl in a tank till maturity. The different photosynthetic parameters like stomatal conductance (gs), net photosynthetic rate (PN), and intercellular CO_2 concentration (Ci) were recorded in fully expanded leaves using an infrared gas analyser (IRGA; LI-COR, http://www.licor.com) on a sunny day between 10:00-11:30 am. The different agronomic characteristics were measured at 0 and 200 mM NaCl treatment in T_3 transgenic and WT plants using the method described (Tuteja *et al*. 2014). The endogenous ions (phosphorous, potassium and sodium) content were measured as described earlier (Tuteja *et al*. 2013).

Biochemical assays of RGG1 transgenic plants in T_3 generation

Biochemical analysis like lipid peroxidation, catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and proline were carried out by using 25-days-old WT and transgenic rice seedlings exposed for 24h to salt stress. The electrolytic leakage was measured as previously described (Garg *et al.* 2012).

Statistical analysis

The experiment was arranged in a randomized block design. For various growth parameters of the WT and RGG1 T_3 transgenic plants, values are presented asmeans of three replicates (each plant was considered a replicate). Here the 'mean of three replicates' represents the 'mean of three independent plants. Data were analysed statistically and standard errors were calculated. Least significant differences (LSDs) between the mean values (n = 3) of control (WT) and RGG1 overexpressing transgenic rice lines (L1-L5) were calculated by one-way analysis of variance (ANOVA) using SPSS 10.0 (SPSS, Inc., now IBM, http://www-01.ibm.com/software/analytics/spss). A comparison between the means was performed using Duncan's multiple range tests. The WT and transgenic lines at $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered statistically significant.

RESULTS AND DISCUSSION

Polymerase chain reaction, Western-blot analysis and transcript profile analysis of T_3 RGG1 transgenic rice plants

The T_3 transgenic rice plants were developed using the T-DNA construct of the RGG1 gene (Figure 1a). Phenotypically the transgenic rice plants were significantly taller (L1-L5) than the WT (Figure 1b). The integration of the transgene (RGG1) was confirmed by PCR using 35S forward and the gene specific reverse primers. The expected size band of 430 bp was observed (Figure 1c). The western blot results show that the protein is expressed to almost similar levels in all the transgenic lines L1-L5 (Figure 1d).The RGG1 transcript level was up-regulated significantly by 10- to 12-folds in comparison with the WT plants (Figure 1e).

Agronomic performance of RGG1 T_3 transgenic plants under stress

Under 200mM NaCl stress condition the RGG1 transgenic plants showed better performance in several growth parameters, such as plant height, root length, root dry weight and leaf area, as compared to the WT plants (Table 1). Several yield characters, such as days required for flowering, number of tillers per plant, panicles per plant, filled grain per panicle, chaffy grain per panicle, 100 grain weight at 200 mM NaCl were recorded and found to be almost similar to the WT plants grown in water (0 mM NaCl). However, the WT plants did not survive till flowering stage under 200 mM NaCl stress (Table 2).

Table 1. Phenotypic attributes of WT, VC and T_3 generation of *RGG1* overexpressing transgenic lines (Line 1, Line 2, Line 3, Line 4 and Line 5) of rice (*Oryza sativa* L. cv. IR64) under 0 and 200 mM NaCl

Yield attributes	Control WT plants		200 mMNaCl grown T1 RGG1 transgenic plants									
			Line 1		Line2		Line 3		Line 4		Line 5	
	0	200	0	200	0	200	0	200	0	200	0	200
Plant height(cm)	62±3.2[a]	31.6±1.4[b]	72±3.2[a]	71±3.7[a]	81±3.1[a]	66±3.2[a]	76±3.1[a]	73±3.1[a]	74±3.2[a]	68±3.2[a]	75±3.1[a]	69±3.3[a]
Root length (cm)	28±0.8[ab]	12.8±0.02[b]	28±1.0[a]	25±1.0[ab]	27±1.5[a]	19±1.5[ab]	29±1.3[a]	26±1.0[a]	28±1.0[a]	22±1.1[ab]	29±1.4[a]	19±1.4[ab]
Root dry weight (g)	2.6±0.13[b]	1.2±0.01[c]	2.8±0.14[a]	2.1±0.14[a]	3.2±0.12[a]	2.6±0.17[a]	2.9±0.2[a]	2.7±0.15[a]	2.9±0.16[a]	2.5±0.13[a]	3.6±0.13[a]	2.6±0.16[a]
Leaf area (cm^2/plant)	85±2.3[ab]	41.72±2.0[c]	96±1.0[a]	78±1.7[ab]	96±1.0[a]	76±1.4[ab]	93±1.6[a]	81±1.6[ab]	95±1.0[a]	84±1.8[ab]	98±1.0[a]	88±1.6[ab]
Net photosynthetic rate (PN, µ mol CO_2 $m\text{-}2s^{-1}$)	9.17±0.8[b]	5.04±0.21[c]	10.35±0.6[a]	7.27±0.8[a]	10.55±0.1[a]	9.38±0.3[a]	10.07±0.4[a]	8.17±0.6[a]	10.38±0.6[a]	8.29±0.8[a]	10.54±0.2[a]	7.37±0.2[a]
Stomatal conductance (gs, m mol $m^{-2}s^{-1}$)	227±11.4[a]	109.3±5.3[b]	242±10.9[a]	209±11.6[a]	255±10.9[a]	212±10.7[a]	248±10.2[a]	219±10.3[a]	244±10.8[a]	205±11.5[a]	256±10.7[a]	211±10.8[a]
Intracellular CO_2 (Ci, µ mol mol^{-1})	227±11.3[a]	101.1±4.4[b]	225±10.2[a]	208±10.2[a]	227±10.5[a]	202±10.2[a]	224±11.5[a]	209±10.2[a]	228±10.2[a]	204±10.3[a]	232±10.5[a]	206±10.1[a]
Phosphorus (%)	0.221± 0.014[b]	0.125± 0.002[c]	0.226± 0.010[a]	0.235± 0.010[a]	0.224± 0.011[a]	0.247± 0.011[a]	0.223± 0.011[a]	0.263± 0.011[a]	0.225± 0.010[a]	0.236± 0.011[a]	0.226± 0.012[a]	0.247± 0.011[a]
Potassium (%)	0.148± 0.002[b]	0.094± 0.002[c]	0.146± 0.002[a]	0.129± 0.002[a]	0.146± 0.002[a]	0.139± 0.002[a]	0.147± 0.001[a]	0.148± 0.005[a]	0.142± 0.002[a]	0.139± 0.002[a]	0.147± 0.002[a]	0.136± 0.003[a]
Sodium (%)	0.005± 0.001[a]	0.066± 0.001[a]	0.004± 0.001[a]	0.048± 0.001[a]	0.005± 0.001[a]	0.041± 0.001[a]	0.005± 0.001[a]	0.047 ±0.001[a]	0.006± 0.001[a]	0.041± 0.002[a]	0.004± 0.001[a]	0.048± 0.001[a]

Each value represents mean of three replicates ± SE. Means were compared using ANOVA. Data followed by the same letters in a row are not significantly different at $P > 0.05$

Table 2 Comparison of various yield parameters of WT and T_3 generation of *RGG1* over expressing transgenic lines (Line 1, Line 2, Line 3, Line 4 and Line 5) of rice (*Oryza sativa* L. cv. IR64) under 0 or 200 mM NaCl, respectively.

Yield attributes	Control WT plants		200 mM NaCl grown T_1 *RGG1* transgenic plants									
			Line 1		Line2		Line 3		Line 4		Line 5	
	0	200	0	200	0	200	0	200	0	200	0	200
Time required for flowering (days)	91±2.3[a]	ND*	93±3.6[a]	87±2.2[a]	92±3.0[a]	90±2.7[a]	93±2.6[a]	90±2.7[a]	94±3.6[a]	90±2.1[a]	93±3.1[a]	89±2.4[a]
No. of tillers/plant	22±1.0[c]	ND	23±0.12[ab]	22±1.2 [ab]	22±0.15 [ab]	21±1.2[ab]	22±0.12[a]	19±1.0[a]	21±0.12[ab]	20±1.3 [ab]	23±0.14 [ab]	21±1.2[ab]
No. of panicle/plant	18±0.5[c]	ND	22±0.11[ab]	21±1.1[ab]	26±0.13[ab]	22±1.1[ab]	25±0.15[a]	21±1.2[a]	27±0.11[ab]	20±1.0[ab]	24±0.13[ab]	21±1.0[ab]
No. of filled grain/panicle	84±3.1[b]	ND	96±3.2[a]	83±3.2[a]	93±3.23[a]	85±4.1[a]	95±3.27[a]	86±3.2[a]	96±3.3[a]	81±3.1[a]	95±3.23[a]	88±4.0[a]
No. of chaffy grains/panicle	13±0.21[a]	ND	07±0.12[b]	05±0.11[b]	05±0.06[b]	04±0.22[b]	07±0.21[b]	05±0.11[b]	08±0.12[b]	06±0.11[b]	05±0.05[b]	04±0.21[b]
Straw dry weight (g)	56±1.3[b]	ND	55±3.05[a]	47±2.3[a]	68±2.1[a]	60±1.6[a]	63±1.8[b]	55±1.3[a]	59±3.04[a]	48±2.1[a]	62±2.2[a]	61±1.4[a]
100 grain weight	2.78±0.1[a]	ND	2.68±0.12[b]	2.61±0.11[a]	2.68±0.12[b]	2.66±0.10[a]	2.64±0.14[b]	2.61±0.10[a]	2.68±0.12[b]	2.61±0.11[a]	2.68±0.12[b]	2.63±0.11[a]
Seed weight per plant	37.64±1.2[a]	ND	52.99±1.4[b]	45.49±0.3[b]	62.07±0.5[a]	47.48±1.1[b]	63.36±1.2[b]	47.13±1.1[b]	61.99±1.2[b]	42.28±1.1[b]	61.19±1.2[b]	48.6±1.3[b]

ND-No data, * control plants did not survived till harvesting under 200 mM NaCl. Each value represents mean of three replicates ± SE. Means were compared using ANOVA. Data followed by the same letters in a row are not significantly different at $P > 0.05$ as determined by least significant difference (LSD) test. a, b, c indicate significant differences at $P > 0.05$ level as determined by Duncan's multiple range test (DMRT).

Photosynthetic characteristics and endogenous ion contents of RGG1 T_3 transgenic plants under stress

The photosynthetic characteristics of transgenic as well as WT plants were measured after one week of induction of salt (200 mM NaCl) stress. The photosynthetic rate declined by 35% in WT as compared with RGG1 transgenic lines. The stomatal conductance, net photosynthetic rate and intracellular CO_2 were also higher in transgenic lines as compared to the WT plants (Table 1). In the presence of NaCl (200 mM), the WT plants accumulated excess sodium whereas the transgenic lines had reduced amounts of sodium in their leaves. Salt-treated T_3 transgenic lines showed higher accumulation of phosphorus and potassium (Table 1).

Analysis of antioxidant enzymes activities and response of ion leakage, proline content malondialdehyde (MDA) in T3 RGG1 transgenic plants

The overexpression of RGG1 resulted in increased enzymatic activities of CAT, APX and GR due to salt treatment (200 mM NaCl) in transgenic plants (Fig. 2a-c). The changes induced by the presence of salt in the accumulation of MDA, ion leakage and proline antioxidant machineries in T_3 transgenic lines (L1-L5) were compared with rice seedlings of WT plants. The levels of MDA, ion leakage were significantly reduced while proline content were increased in RGG1 transgenic lines as compared to the WT under salt (200 mMNaCl) stress (Fig. 2d-f). The increased detoxification of ROS led to reduced membrane lipid peroxidation *i.e.*, MDA production and membrane damage as indicated by electrolyte leakage.

G-proteins are ubiquitous in nature, and are known to be involved in diverse cellular and metabolic processes, including their new emerging role in plant abiotic stress tolerance (Misra 2007). Salinity is a multigenic trait which controls the whole plant machinery and rice productivity is severely affected due to this stress. Although G-gamma subunits were initially regarded as a passive partner in the G beta-gamma dimer whose only function was to anchor the dimer to the plasma membrane, they have now emerged as an important member of the heterotrimer, providing multiple physiological functions in plants (Jones and

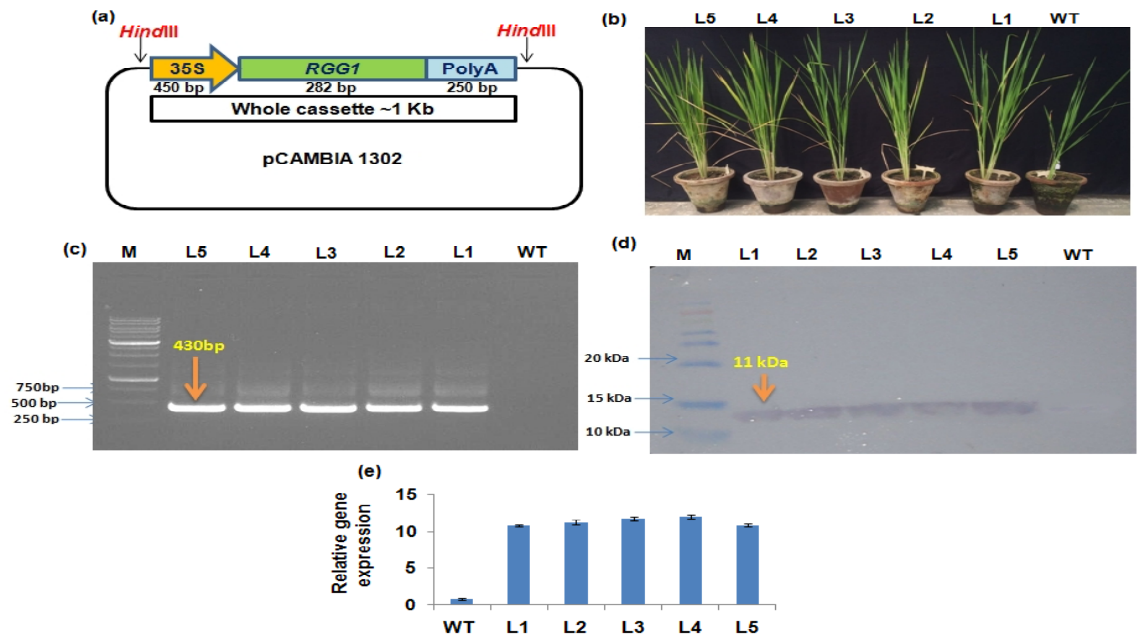

Fig. 1 Analysis of RGG1 over expressing transgenic T_3 IR64 rice plants. (a) The OsRGG1 gene cloned in pCAMBIA1302 vector at HindIII site. (b) Transgenic plants (L1- L5) along with WT. (c) PCR analysis of the RGG1 over expressing transgenic (T_3) lines along with wild type (WT), positive control (PC) and negative control (NC) shows the amplification of the 430 bp fragment. (d) Western blot analysis showing the production of RGG1 protein (~11kDa). (e) Real time PCR analysis of the RGG1 over expressing transgenic (T_3) lines (L1- L5) along with WT

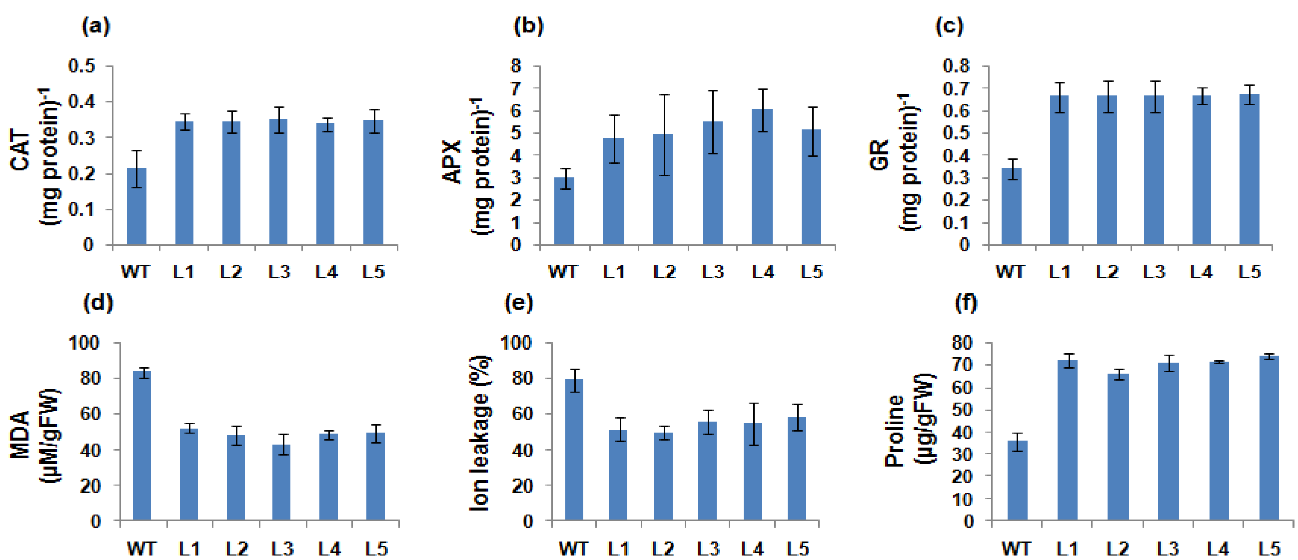

Fig. 2. Biochemical analysis of RGG1 over expressing T_3 transgenic lines (L1- L5) along with WT. (a) Catalase (CAT) activity, one unit of enzyme activity defined as 1 µ mol H_2O_2 oxidized min^{-1}. (b) Ascorbate peroxidase (APX) activity, one unit of enzyme activity defined as 1 µ mol of ascorbate oxidized min^{-1}. (c) Glutathione reductase (GR) activity, one unit of enzyme activity is defined as 1 µ mol of GS-TNB formed min^{-1} due to reduction of DTNB (d) Lipid peroxidation expressed in terms of malondialdehyde (MDA) content. (e) Percent electrolytic leakage. (f) Level of proline accumulation.

Assmann 2004; Perfus-Barbeoch *et al*. 2004; Trusov *et al*. 2007; Zhang *et al*. 2008; Trusov *et al*. 2009; Dupre *et al*. 2009; Klopffleisch *et al*. 2011; Trusov and Botella 2012; Urano *et al*. 2013; Cheng *et* *al*. 2015; Maruta *et al*. 2015). The present study was conducted in order to study the function of RGG1 in providing salinity stress tolerance in rice (*Oryza sativa* L. cv. IR64).

The RGG1 overexpressing transgenic lines retained more chlorophyll than WT under salinity stress, which is in agreement with the earlier reports (Sanan-Mishra *et al*. 2005; Moradi and Ismail 2007; Dang *et al*. 2013; Singh *et al*. 2012; Sahoo *et al*. 2012). The photosynthetic activities like net photosynthesis rate (Pn), stomatal conductance (gs), and intercellular CO_2 concentration (Ci) were decreased by salinity stress but a lesser reduction was observed in RGG1transgenic lines as compared to WT plants. The better control over photosynthesis apparatus under salinity stress may be due to retention of chlorophyll content in these transgenic lines. It has been reported earlier also that tolerance in PDH45 and SUV3 overexpressing rice plants in stress results due to maintenance of the photosynthetic apparatus (Gill and Tuteja 2010; Tuteja *et al*. 2013). Under salinity stress plant produces more ROS, which can cause serious damage to plasma membrane, chloroplasts and mitochondria by peroxidation and de-esterification of membrane lipids and damage to nucleic acids and proteins (Gill and Tuteja 2010).

The antioxidant enzymes such as APX, GPX and GR showed significantly higher activity under salinity stress in T_3 transgenic lines as compared to WT plants, which help in scavenging the ROS production during the stress. To protect the plants from the injurious effects of H_2O_2, plants produce more APX through the AsA-GSH cycle, where APX uses ascorbate as hydrogen donor and GR catalyses the NADPH dependent reduction of GSSG (oxidised form) to GSH (reduced form) and maintains high ratio of GSH/ GSSG (Gill and Tuteja 2010).

Higher concentration of potassium and lower concentration of sodium were found in leaves of RGG1 overexpressing transgenic lines than WT plants under salinity stress. It indicates that the overexpression of RGG1 may restrict the entry of sodium ions in the leaves of transgenic lines thereby contributing towards protection of photosynthetic machinery from salinity stress.

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Comparison of morpho-physiological traits and root architecture of tolerant and susceptible rice genotypes under both phosphorus and water stressed and normal condition

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ABSTRACT

A comparison study of root architecture and morpho-physiological traits were taken up using nine tolerant and three check germplasm lines. The experimental work was done under rain protected condition using an innovative approach. A significant differences was observed in the root and shoot length of Pup1 positive genotypes than Pup1 negative germplasm lines under both phosphorous supplemented and deficient soil conditions. A higher uptake of phosphorus was noticed in Pup1 positive plants of leaf phosphorus analysis under water stressed condition as compared to normal. The higher p-uptake genotypes showed higher tiller number and high plant dry weight. The Pup 1 negative genotypes showed low root width and low root density than the positive plants. The Pup1 positive plants showed higher root dry weight, root length and root density under both phosphorus and water stress condition.

Key words: *Phosphorus deficient soil, phosphorus supplement soil, phosphorus stress tolerant, root density, root volume, root weight*

Rice is grown in a wide range of environments, but more than 40% of global rice production is from rain-fed ecosystem with limited control on water which is often associated with drought, flood or other calamities. Moreover 60% of rain-fed soils are deficient in one or more nutrients (Haefele and Hijmans 2007). In these soils, phosphorus (P) is one of the most important macro nutrients that is limited in availability. There is no substitute for P in food production and it is considered as the most limiting mineral nutrient for plants across all arable land (Kochian 2012). Most of the P is tightly bound to the soil. It is present either as unavailable form or slowly available form, which is not immediately accessible by plants. However, plants have evolved many adaptations to low concentrations of available phosphate in the soil. The induction of high affinity phosphate transporters and the secretion of acid phosphatase and organic acids contribute to the mobilization of phosphate from organic and inorganic substrates and active uptake of phosphorus (P) from the rhizosphere (Gardner *et al*. 1983; Tadano and Sakai 1991; Mucchal *et al*. 1996; Mucchal and Raghothama 1999; Liu *et al*. 2001; Kai *et al*. 2002; Wasaki *et al*. 2003a). It is also clear that P is efficiently utilized once inside the plant tissue (Duff *et al*. 1989, 1991). P deficiency causes reduction of leaf expansion and the number of leaves (Marschner 1995). Phosphorus (P) is of unequivocal importance for the production of food crops. It is often referred as the "energizer" since it helps to store and transfer energy during photosynthesis. It is a vital component of ATP, the 'energy currency' of the cell. It forms the basic component of many organic molecules, nucleic acids and proteins (Lea and Miflin 2011). Only 1% of P is present as available form in the soil solution which is found in irrigated ecosystems, where rice is grown in water logged conditions. There is a scarcity of P in most of the soils and hence soils are to be replenished with P fertilizers regularly. The major QTL for phosphorus uptake was mapped on the long arm of chromosome 12 and is referred as *Pup1*. *Pup1* region in the chromosome contain the transcription factor gene *OsPTF1* which confers tolerance to P deficiency (Yi *et al*. 2005). Moreover, the research experiments showed that the *Pup1* enables the plants to increase P uptake by 3- to 4-fold primarily because it conferred strong and high root growth rates despite of P deficiency in soils (Ismail *et al*. 2007). Root morphological and physiological studies indicated that the *Pup1* gene expresses in root tissue where it either leads to higher root growth per unit P (higher internal efficiency) or improves P uptake per unit root size (external efficiency) (Wissuwa 2003). Therefore, varieties with *Pup1* locus might contain the morphologically and physiologically favorable root structure for the efficient usage of P uptake. It was observed in experiments that rice with *Pup1* extract up to 3 times as much naturally occurring soil phosphorus, tripling the grain yield and dry weight (Fredenburg 2006). The *Pup1* region sequenced by Heuer *et al*. (2009) confirmed that 278-kbp sequence of Kasalath rice variety was significantly different from the syntenic regions in Nipponbare rice variety due to large insertions or deletions (INDELs) that is directly linked with P deficiency tolerance. It is reported that the impact of *Pup1* on enhancing yield in P-deficient soil under drought stress is significantly high (Bernier *et al*. 2009; Venuprasad *et al*. 2009). *Pup1* is present in 80-90% of the upland and lowland/irrigated varieties. Underlying *Pup1* is a single kinase gene, OsPupK46-2 which is located in the indel which is closely associated with P deficiency and is highly conserved in the drought tolerant accessions in the rice germplasm (Chin *et al*. 2010, 2011). This gene underlying the *Pup1* locus increases early root growth and P acquisition efficiency under low-P conditions in several different genetic backgrounds and is subsequently named Phosphorus-starvation tolerance 1 (PSTOL1), which encodes a serine/ threonine kinase of the LRK10L-2 subfamily (Gamuyao *et al*. 2012). The *PSTOL1* gene also plays a role in lignification of rice roots in response to drought and P-stress (Tyagi *et al*. 2012). They also hypothesized that two QTLs *Pup1* and *Yld12.1* might be pleiotropic and introgression of this region might help select simultaneous P deficiency tolerance as well as for yield under drought. According to Chin *et al*. (2011), *pup1* has been successfully introgressed into two irrigated rice varieties, namely IR64 and IR74 and three Indonesian upland varieties, namely Dodokan *et al*. (2012) have identified four genotypes containing *Pup1*, namely Sahbhagi dhan, Dagaddeshi, Pynthor and Paijong, adapted to North Eastern and Eastern part of India, as potential donors for rice breeding for P deficiency tolerance. This study was carried out to compare the root architecture of tolerant and susceptible rice genotypes under phosphorus deficient and water stressed situation with normal condition.

MATERIALS AND METHODS

Plant materials

Twelve rice genotypes comprising of 9 tolerant and three check genotypes as suggested by Pandit *et al.* 2016 were selected for the present study (Table 1). Seeds of these germplasm lines were collected from ICAR-National Rice Research Institute (NRRI), Cuttack.

Experimental site

An experiment was carried out in a raised brick structured tank at ICAR-National Rice Research Institute (NRRI), Cuttack (latitude 25.30N, longitude 85.15E) during dry season of 2015. The tank was made collapsceable type by providing low proportion of sand to cement (20:1) in the walls. The inside wall length was 18ft, inside breadth-6.5ft, 3ft height above ground and 1.5ft below ground with each tank was partitioned into two sub-tanks by a middle wall with size 18'x3"x3' (above ground). The tanks were filled with phosphorus deficient soil (around 660cft) collected from other lands having loamy sand with pH 4.21, organic carbon 0.573 %, having available nitrogen, phosphorous, exchangeable potassium of 150, 14.08 and 25.54 kg ha^{-1}, respectively. To estimate soil phosphate, Olsen *et al*. (1954) method was adopted. The soil height was maintained up to 3ft in each tank. The tank soil was leveled uniformly and irrigated. Six moisture meter probes were inserted in each tank to assess the moisture content of the tank. One/two seeds of individual genotypes were sown 2cm below the soil. The experiment was replicated twice with split plot design in four main plot (stress, no stress, with application of phosphorus and without application of phosphorus) and twelve genotypes in subplots. After germination, single seedling was maintained. Both water stress and no stress (control) tanks were fertilized at the rate of 80, 40 and 40 kg ha^{-1} N, P_2O_5 and K_2O, respectively. Phosphorus was not applied in one tank to study *Pup1* action. Nitrogen was applied on three occasions, *viz*., 1/3rd each at basal, maximum tillering and panicle initiation stages, while the P_2O_5 and K_2O were applied as basal application.

P uptake ability and assessment of phenotypic traits in phosphorus deficient and supplemented soil

For the soil experiment, the Pup1 positive and checks (12 rice genotypes) (Table 1) *viz*., Bowdel, Lalsankri, Karni, Dinoroda, N-22, Bamawypan, Tepiboro, Dular , Surjamukhi, and three check varities Kasalath, IR-64, and Kalinga-III, 1 plant from each variety were grown in a tank described above with natural light conditions for 45 days. Soil was kept aerobic, but well watered without draining at all times. Thereafter the stress was imposed in one tank and other kept as such for another 15days. Eight quantitative morpho-physiological characters were measured in each plant. Tiller number, leaf area, root length (cm), shoot length (cm), root density (mass per volume), root dry weight (g) and shoot dry weight (g) were measured. Root volume was measured by measuring the spilled content of water. P uptake in rice leaves was also quantified both in P deficient and P supplemented soil during the said period.

Statistical analysis

Physiological and biochemical data were analyzed following the split plot design as outlined by Gomez and Gomez 1984 and Panse and Sukhatme 1985.

RESULTS AND DISCUSSION

Morpho-physiological studies indicated that the *Pup1* gene express in root tissue where it either leads to higher root growth per unit P or improves P uptake per unit root size (Wissuwa *et al*. 2002). It is also reported that the impact of *Pup 1* and other QTLs on enhancing yield in P-deficient soil under drought stress is significantly high (Bernier *et al*. 2009; Venuprasad *et al*. 2009). Hence, genotypes with *Pup1* locus might have contained the morphologically and physiologically favourable root structure for the efficient usage of P uptake. Evaluation of phenotypes for relative tiller number (tiller number under P-deficiency relative to non-stress tiller number) has been used as an indirect estimate for P uptake (Ni *et al*. 1998). Employing a relative parameter allows for comparisons in stress response between a variety of diverse genotypes without confounding effects due to substantial differences in tillering ability. Without variation under optimum P supply, the relative tiller number entirely depends on the number of tillers produced under P deficiency.

Association between *Pup1* containing genotype, water stress and shoot/ root traits in soil experiment

Table 1. Twelve rice genotypes used for root architecture study under water and phosphorus stressed and normal conditions

Sl.No.	Genotype	Pup1K-20 240bp (Kasalath allele)/ 243bp (Nipponbare allele) 59^{0}C	Pup1-K42 918bp was obtained at 57^{0}C	Pup1-K46 523bp was obtained at 59^{0}C	Closely associated microsatellite marker RM28073 656bp was obtained at 57^{0}C	Closely associated microsatellite marker RM28102 168bp was obtained at 57^{0}C
1	Bowdel	+ve	+ve	+ve	+ve	+ve
2	Lalsankri	+ve	+ve	+ve	+ve	
3	Karni			+ve	+ve	+ve
4	Dinoroda		+ve		+ve	+ve
5	N-22		Non-specific	+ve	+ve	+ve
6	Bamawypan*		+ve	+ve	+ve	+ve
7	Tepiboro		+ve	+ve	+ve	+ve
8	Dular*		Non-specific	Non-specific	-ve	Not detected
9	Surjamukhi*		+ve	+ve	-ve	-ve
10	Kasalath	+ve	+ve	+ve	+ve	+ve
11	IR-64	-ve check	-ve check	-ve check	-ve check(600bp)	-ve check(155bp)
12	Kalinga-III	Not detected	Not detected			

* drought tolerant genotypes and have deeper rooting ability.

Table 2. Morpho-physiological effect of stress and no stress on rice plant in P-deficient and P-sufficient soil during vegetative stage of the crop (water stress imposed 45DAS)

Shoot dry weight (g)

	Bowdel	Lalsankri	Karni	Dinoroda	N-22	Bamaw-ypan	Tepiboro	Dular	Surja-mukhi	Kasalath	IR-64	Kalinga-III
S0	42.05	61.85	59.7	59.34	19.04	22.95	29.35	31.84	23.35	99.04	89.67	91.2
S1	23	29.92	16.8	27.66	23.27	35.52	20.82	21.41	12.59	39.76	23.02	20
P0	23.76	26.29	35.9	28.04	20.43	24.8	31.33	12.74	12.41	38.01	45.04	46.5
P1	41.29	65.48	40.6	58.97	21.88	33.68	18.84	40.52	23.53	100.8	67.65	64.6
	Sem	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**	
Stress	9.823	ns	22.2	S x P	13.89	ns	22.2	V× S/P	0.775	2.208		
P	9.823	ns	22.2	Varieties	0.548	1.561	0.506	S/P× V	97.05	435.8		

Root dry weight (g)

	Bowdel	Lalsankri	Karni	Dinoroda	N-22	Bamaw-ypan	Tepiboro	Dular	Surja-mukhi	Kasalath	IR-64	Kalinga-III
S0	11.79	14.71	17.8	16.6	11.41	16.86	9.25	13.19	13.4	25.02	15.13	31.7
S1	11.94	4.09	2.57	12.23	9.62	16.11	9.86	17.43	4.94	11.9	8.11	5.19
P0	8.96	5.52	9.74	7.13	10.62	16.33	9.8	6.53	8.04	12.01	7.77	16.2
P1	14.76	13.29	10.6	21.7	10.42	16.63	9.32	24.09	10.31	24.91	15.47	20.7
	Sem	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**	
Stress	2.69	ns	6.08	S x P	3.804	ns	6.08	V× S/P	1.221	3.479		
P	2.69	ns	6.08	Varieties	0.863	2.46	0.797	S/P× V	8.601	36.45		

Total biomass (g)

	Bowdel	Lalsankri	Karni	Dinoroda	N-22	Bamaw-ypan	Tepiboro	Dular	Surja-mukhi	Kasalath	IR-64	Kalinga-III
S0	53.84	76.56	77.5	75.94	30.45	39.81	38.6	45.04	36.75	104.4	104.8	123
S1	34.94	34.01	19.4	52.63	32.9	51.63	30.68	38.84	17.53	51.66	31.13	25.2
P0	32.72	31.8	45.7	35.17	31.05	41.13	41.13	19.27	20.44	50.02	52.81	62.8
P1	56.05	78.77	51.2	93.4	32.29	50.31	28.15	64.61	33.84	106.1	83.12	85.3
	Sem	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**	
Stress	11.67	ns	26.4	S x P	16.5	ns	26.37	V× S/P	0.82	2.336		
P	11.67	ns	26.4	Varieties	0.58	1.652	0.535	S/P× V	136.7	614.3		

% Nitrogen content

	Bowdel	Lalsankri	Karni	Dinoroda	N-22	Bamaw-ypan	Tepiboro	Dular	Surja-mukhi	Kasalath	IR-64	Kalinga-III
S0	1.6	1.73	1.4	1.95	1.95	2.1	1.75	1.38	1.9	1.59	1.68	1.51
S1	1.99	2	2.02	1.82	1.53	2.3	1.82	2.08	1.77	1.73	1.64	1.93
P0	2.03	1.98	1.77	1.86	1.82	2.06	1.75	1.75	1.68	1.53	1.9	1.75
P1	1.56	1.75	1.65	1.9	1.66	2.34	1.82	1.71	1.99	1.79	1.42	1.68
	Sem	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**	
Stress	0.04	0.171	0.09	S x P	0.05	ns	0.09	V× S/P	0.05	0.15		
P	0.04	ns	0.09	Varieties	0.04	0.1	0.03	S/P× V	0	0.01		

% Phosphorus content

	Bowdel	Lalsankri	Karni	Dinoroda	N-22	Bamaw-ypan	Tepiboro	Dular	Surja-mukhi	Kasalath	IR-64	Kalinga-III
S0	0.24	0.25	0.33	0.21	0.21	0.23	0.28	0.2	0.3	0.18	0.19	0.25
S1	0.19	0.22	0.2	0.2	0.19	0.23	0.21	0.17	0.16	0.1	0.12	0.18
P0	0.23	0.21	0.3	0.19	0.21	0.25	0.24	0.18	0.23	0.15	0.14	0.29
P1	0.2	0.26	0.24	0.21	0.19	0.22	0.25	0.19	0.24	0.13	0.17	0.13
	Sem	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**	
Stress	0.008	0.038	0.02	S x P	0.012	ns	0.019	V× S/P	0.004	0.012		
P	0.008	ns	0.02	Varieties	0.003	0.008	0.003	S/P× V	0	0		

% Potash content

	Bowdel	Lalsankri	Karni	Dinoroda	N-22	Bamaw-ypan	Tepiboro	Dular	Surja-mukhi	Kasalath	IR-64	Kalinga-III
S0	1.6	1.36	1.28	1.27	1.39	1.35	1.23	1.27	1.52	1.29	1.18	1.54
S1	1.91	1.66	1.6	1.84	1.69	1.48	1.82	1.73	1.5	1.67	1.36	1.42
P0	1.95	1.49	1.38	1.79	1.64	1.36	1.72	1.66	1.79	1.53	1.38	1.51

Contd.............

Contd.............

P1	1.56	1.53	1.5	1.33	1.44	1.48	1.33	1.34	1.23	1.43	1.17	1.45
	Sem	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**	
Stress	0.088	ns	0.2	S x P	0.124	ns	0.199	V× S/P	0.075	0.213		
P	0.088	ns	0.2	Varieties	0.053	0.15	0.049	S/P× V	0.013	0.049		

Tiller number per plant

	Bowdel	Lalsankri	Karni	Dinoroda	N-22	Bamaw-ypan	Tepiboro	Dular	Surja-mukhi	Kasalath	IR-64	Kalinga-III
S0	9	25.25	15.5	16	10	4.75	11.5	7	6.25	20	14	8.7
S1	8.5	25.75	11.75	17.5	22	21.75	19.25	14	12	15.5	12.5	12.7
P0	6.75	19.5	9.5	8.25	16.25	8.75	12.25	8.25	6.5	12.75	11.5	12
P1	10.75	31.5	17.75	25.25	15.75	17.75	18.5	12.75	11.75	22.75	15	14.5
	Sem	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**	
Stress	3.028	ns	6.844	S x P	4.282	ns	6.844	V× S/P	3.536	10.22		
P	3.028	ns	6.844	Varieties	2.536	7.227	2.34	S/P× V	20.957	74.849		

* drought tolerant genotypes and have deeper rooting ability. S0- stress, S1-irrigated & P0-phosphorus not applied, P1-phosphorus applied

Table 3. Contribution of assimilates and inorganic ions towards development of plant parts

% root partitioning

	Bowdel	Lalsankri	Karni	Dinoroda	N-22	Bamawypan	Tepiboro	Dular	Surjamukhi	Kasalath	IR-64	Kalinga-III
S0	36.02	17.83	22	42.54	39.08	37.96	24.33	49.67	46.77	27.42	16.52	27.8
S1	30.97	14.7	15.5	40.7	29.91	33.65	28.76	40.99	30.51	26.57	26.5	33.7
P0	37.28	18.29	15.6	45.74	33.99	35.76	25.87	46.13	37.04	23.83	17.77	30.6
P1	29.71	14.24	21.9	37.49	35	35.85	27.21	44.53	40.25	30.16	25.25	30.9
	Sem	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**	
Stress	5.028	ns	11.4	S x P	7.11	ns	11.36	V× S/P	0.53	1.511		
P	5.028	ns	11.4	Varieties	0.375	1.068	0.346	S/P× V	25.54	114.5		

% shoot partitioning

	Bowdel	Lalsankri	Karni	Dinoroda	N-22	Bamawypan	Tepiboro	Dular	Surjamukhi	Kasalath	IR-64	Kalinga-III
S0	63.98	82.17	78	57.46	60.92	62.04	75.67	50.33	53.23	72.58	83.48	72.2
S1	69.03	85.3	84.6	59.3	70.34	66.35	71.24	59.01	69.49	73.43	61.64	66.4
P0	62.72	81.71	84.5	54.26	66.01	64.24	74.13	53.87	62.96	76.17	82.73	69.5
P1	70.29	85.76	78.1	62.51	65.25	64.15	72.79	55.47	59.75	69.84	62.39	69.1
	Sem	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**	
Stress	5.569	ns	12.6	S x P	7.875	ns	12.59	V× S/P	0.498	1.419		
P	5.569	ns	12.6	Varieties	0.352	1.003	0.325	S/P× V	31.24	140.2		

Root density

	Bowdel	Lalsankri	Karni	Dinoroda	N-22	Bamawypan	Tepiboro	Dular	Surjamukhi	Kasalath	IR-64	Kalinga-III
S0	0.08	0.1	0.12	0.11	0.07	0.11	0.06	0.09	0.08	0.12	0.09	0.21
S1	0.07	0.03	0.02	0.15	0.06	0.1	0.06	0.1	0.03	0.08	0.05	0.03
P0	0.06	0.04	0.07	0.05	0.07	0.1	0.06	0.04	0.04	0.08	0.05	0.11
P1	0.09	0.09	0.07	0.22	0.07	0.1	0.06	0.15	0.07	0.12	0.09	0.13
	Sem	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**	
Stress	0.02	ns	0.04	S x P	0.03	ns	0.04	V× S/P	0	0.01		
P	0.02	ns	0.04	Varieties	0	0.01	0	S/P× V	0	0		

Root length (cm)

	Bowdel	Lalsankri	Karni	Dinoroda	N-22	Bamawypan	Tepiboro	Dular	Surjamukhi	Kasalath	IR-64	Kalinga-III
S0	48.75	48	57	55.88	58.75	57.25	46.25	50.55	81.5	70.3	69.88	55.6
S1	63.68	56.38	47.3	76.3	71.3	70.75	65.38	72.75	54.18	58.38	77.13	55.1
P0	51.18	51.5	49.3	67.75	63.55	64.25	62.38	58.25	75.55	51.05	71.13	52
P1	61.25	52.88	55	64.43	66.5	63.75	49.25	65.05	60.13	77.63	75.88	58.8
	Sem	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**		**Sem**	**CD(5%)**	**CV(%)**	
Stress	2.666	ns	6.03	S x P	3.771	ns	6.027	V within S/P		5.996	17.09	

Soil P level determined by Olsen *et al.* (1954) was found as 14.08kg ha^{-1} at the 4.21 pH level. Significant differences were observed in the root length between *Pup1* positive variety group and *Pup1* negative variety group in both phosphorous supplemented and deficient soil conditions (Table 2). Significant differences were not observed in the shoot length between the *Pup1* positive variety group and *Pup1* negative variety group under both phosphorous supplemented and deficient soil conditions. Out of the twelve rice genotypes used in this study, Karni showed the highest content of P in the plants of P deficient soil followed by Kasalath and Bowdel which were to a tune of 20%, 13.33% and 13.04%, respectively. Similarly, during water stress situation also a significant difference in P uptake was observed when the leaf P availability was measured. A similar result was reported by Sarkar *et al*. 2011. Among these twelve genotypes, five genotypes showed significantly higher P content in leaves both under P deficient and P sufficient soils were Karni, Bowdel, Dinoroda, Kasalath and Kalinga III. It has been found that genotypes with high P-uptake ability have significantly higher plant dry weight (93.4g $plant^{-1}$) than that of average 63.59g $plant^{-1}$. Similar trend was also observed in tiller number per plant as reported by (Ni *et al*. 1998) and in N and K uptake. Significant differences were observed in the root length and root density between the *Pup1* positive genotype group and *Pup1* negative rice genotype group under phosphorous supplemented and deficient soil conditions. The *Pup1* gene positive group has increased the dry weight of root by 16.01g under P supplemented condition and by 9.89g under P deficient soil condition. Under both condition although the shoot length was not significant, shoot dry weight was highly significant at *Pup1* gene positive variety group has increased the root density by about 50% when compared with null *Pup1* group (Table 3). However, the comparative studies among the genotypes revealed that Bowdel, Lalsankri and Karni had better root dry weight having 14.76g, 13.29g and 9.74g, respectively than the rest. When the root width and root volume traits were analysed, it was noted that all individual varieties in *Pup1* negative group, Kalinga III and IR64 had produced low root width and low root density comparatively to other 10 genotypes that contained *Pup1* in P deficient condition. Gupta and Guhey (2011) also reported similar type of finding. Gloria *et al*. (2002) reported that the water deficit in rice caused a larger reduction in leaf area than shoot dry matter, demonstrating the greater sensitivity of leaf enlargement to water stress than dry matter accumulation. As the main organ of plants that take up nutrients, roots play an important role in phosphorous acquisition from soils which was clearly revealed in dry matter partitioning of assimilates towards development of plant organs. In this study root and shoot related traits of *Pup1* positive varieties and *Pup1* negative varieties were analysed by pooling the respective data in order to confirm the general contribution from *Pup1* locus to root and shoot growth. Results revealed that *Pup1* positive genotypes integrate different root traits that contribute to the adaptation to low phosphorous availability and therefore more tolerance to phosphorous deficiency is appeared as compared to *Pup1* negative genotypes during water stress conditions also. Similar result was also reported by Kottearachchi *et al*. 2013.

The comparative study revealed that there is a significant difference between rice with *Pup1* positive genotypes and the rice with *Pup1* negative genotypes, in root width, root dry weight, root volume and shoot dry weight under phosphorus and water stressed condition as compared to normal condition. Phenotypic data corresponding to *Pup1* containing genotype in water stress have indicated the performance of root traits thereby making them useful in utilizing in breeding programs.

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Effect of weed management practices on yield and yield attributes of wet direct seeded rice under lowland ecosystem of Assam

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ABSTRACT

Field experiment was conducted at Agricultural Farm of the Regional Rainfed Lowland Rice Research Institute, Gerua, Assam during early ahu (summer) season of 2015 and 2016 to study the influence of different weed management practices on yield and yield attributes of wet direct seeded rice sown through drum seeder. The dominant weed flora in the experimental field consisted of sedges Cyperus difformis L.*, Scirpus incurvatus* Roxb.*, grasses Leptochloa chinensis* L.*, Echinochloa glabrescens, Echinochloa colona* L. *and broad-leaved weed Ludwigia adscendes* L. *The composition of grasses, sedges and broad-leaved weeds in weedy check plot at 60 DAS was 15.1, 71.5 and 13.4%, respectively. There was 37.3% reduction in the grain yield of rice due to competition with weeds in the weedy plots. All the weed control treatments significantly reduced weed population, dry matter and increased grain yield of rice compared to weedy check. Hand weeding twice recorded grain yield of 5.64 t ha^{-1} resulting in 54.5% increase over weedy check. Mechanical weeding followed by one hand weeding recorded reduction in grain yield of 9.2% over hand weeding twice. Application of flucetosulfuron at 25 g ha^{-1} (5.42 t ha^{-1}), bispyribac sodium at 30 g ha^{-1} (5.40 t ha^{-1}), azimsulfuron at 35 g ha^{-1}(5.38 t ha^{-1}) and bensulfuron methyl+pretilachlor at 60+600 g ha^{-1} recorded grain yield on par with the hand weeding twice. Tank mix application of azimsulfuron+bispyribac sodium recorded 2.2% and 1.9% only, increase in grain yield over its single application.*

Key words: *Drum seeder, rice yield, wet direct seeded rice, weed management practices*

Rice (*Oryza sativa* L.) is the foremost staple food in Asia, where about 92% of the global rice is produced and consumed (IRRI 2012). It is the main staple food in the Asia and the Pacific region, providing almost 39% of calories (Yaduraju and Rao 2013). The term 'rice is life' is most appropriate for India as this crop plays vital role in country's food security and is the backbone of livelihood for millions of rural house hold (Pathak *et al*. 2011). Rice is the major food crop of North East India and occupies an area of 3.5 m ha, which accounts for 7% of the area and 6.5% of the country's rice production (Kumar *et al.* 2016). In context to Assam, rice is consumed by about 90% of the state population and is grown over an area of 2.64 mha occupying around 74.25% of the cropped area (Pegu and Hazarika 2016). Though rice occupies the dominant portion of the cropped area but the productivity is low due to flood prone fragile ecosystem. One of the important issue responsible for widespread yield stagnation and productivity declines in the rice cropping system is soil quality (Shahid *et al*. 2013). There is a urgent need to focusthe attention on lowland rice ecosystem for increased productivity, profitability,employment-generation potential and soil sustainability which could be ensured by adopting modern scientific agricultural practices (Roy *et al*. 2011; Kumar *et al*. 2016).

Rice is commonly grown by transplanting seedlings into puddled soil (wet tillage) in Assam. This production system is labour, water, and energy-intensive and is becoming less profitable as these resources are becoming increasingly scarce (Kumar and Ladha 2011). These factors demand a major shift from transplanting to direct seeding of rice in irrigated ecosystem. About 89% of total flood prone lowlands in India are distributed in five states of eastern India *viz.*, Asaam, West Bengal, Bihar, Odisha and Eastern Uttar Pradesh. Rice farming is risky in those areas during *Kharif* season due to unpredictable hydrology. Cultivation of rice during dry season offers a great potential for boosting and stabilizing the yield from this fragile ecosystem. In Assam, farmers are shifting to summer rice cultivation (*boro* and *early ahu*) by utilizing the harvested rain water stored in small ditches, village ponds and by tapping the ground water using shallow tube wells. Sowing pre-germinated seeds in wet (saturated) puddle soils offer a good alternative method of crop establishment under such situation (Saha *et al*. 2012; Satapathy *et al*. 2016).

Weeds are recognized as major biological constraints that hinder the attainment of optimal rice productivity (Rao and Nagamani 2010; Prasad *et al*. 2013; Hossain *et al*. 2016). Uncontrolled weed growth in transplanted rice causes 45-51% loss in yield (Veeraputhiran and Balasubramanian 2013), whereas weed growth under direct-seeded rice causes yield loss up to 80% (Jabran *et al*. 2012). The failure and success of the drum seeded rice depends on weed management practices. Therefore, the present study was carried out to evaluate the efficacy of different weed management practices on performance of wet direct seeded early ahu rice in lowland ecosystem of Lower Brahmaputra valley zone of Assam.

MATERIALS AND METHODS

Field experiment was carried out during early *ahu* (summer) seasons of 2015 and 2016 at the agricultural farm of Regional Rainfed Lowland Rice Research Station, Gerua, Assam which is located at 26° 14' 59" N latitude, 90° 33' 44" E longitude and at an altitude of 49 m above mean sea level and characterized in the long-term by sub-tropical monsoon type climate with annual average rainfall of 1500 mm. The meteorological weekly average maximum and minimum temperature during the crop growing period from last week of January to first week of June varied from 24.7 ^{0}C to 32.9 ^{0}C and 7.4 ^{0}C to 17.6 ^{0}C respectively in the first year (2015) and 24.1 ^{0}C to 33.9 ^{0}C and 8.6 ^{0}C to 14.6 ^{0}C respectively in the second year (2016). Total pre-monsoon rainfall during the crop growing season in 2015 was 852.5 mm whereas it was 725.3 mm in 2016. The soil was sandy clay loam in texture, having acidic pH (5.2), high organic carbon (0.96%), medium available nitrogen (276 kg ha^{-2}), medium available Phosphorus (17.5 kg ha^{-1}) and medium in available Potassium (226.3 kg ha^{-1}).

The experiment was laid out in randomized block design and replicated thrice. The treatments consisted of T_1: Azimsulfuron at 35 g ha^{-1} at 20 DAS, T_2: Flucetosulfuron at 25 g ha^{-1} at 20 DAS, T_3: Bispyribac sodium at 30 g ha^{-1} at 20 DAS, T_4: Bensulfuron- methyl +Pretilachlor at 60 + 600 g ha^{-1} at 10 DAS, T_5: Azimsulfuron+ Bispyribac sodium at 22+25 gha^{-1} at 20 DAS, T_6: Flucetosulfuron at 25 g ha^{-1} at 5 DAS fb Bispyribac sodium at 25 gha^{-1} at 20 DAS, T_7: Manual weeding twice at 20 and 40 DAS (Recommended practice), T_8: Mechanical weeding by paddy weeder at 20 DAS fb Manual weeding at 40 DAS, T_9: Weed free check (weeding at 15, 30, 45 and 60 DAS) and T_{10}: Weedy check (Table 1).

Seeds of the variety '*Naveen*' at 30 kg ha^{-1} were soaked in plain water for 48 hours and after filtration seeds were incubated in gunny bags for 48 hours for sprouting. Pre germinated seeds were sown by using 12 row drum seeder at 20 cm x 10 cm spacing on puddled saturated soil. The field was maintained at saturation for initial 10 days after sowing (DAS) to facilitate better establishment of seedlings and thereafter that a water depth of 3-5 cm was maintained till grain filling stage of the crop. A fertilizer dose of 80-40-40 kg ha^{-1} of N- P_2O_5- K_2O was applied as urea, diammonium phosphate (DAP) and muriate of potash (MOP) in the field. One third of nitrogen, full dose of P_2O_5 and one third of K_2O were applied as basal dose at the time of final land preparation and incorporated well in to the soil. Remaining two third of nitrogen and potash was applied in two equal splits at maximum tillering and panicle initiation stage. Weed management was done as per schedule. The herbicides were sprayed with knapsack sprayer fitted with flat fan nozzle using 350 litter of water per hectare. Furadon 3G at 30 kg ha^{-1} was applied at immediately after sowing to protect the seeds from bird damage and to provide insurance

Table 1. Treatments employed in the experiment

Treatments	Trade name of the herbicide	Technical name of the weedicide	Dose (ha^{-1})	Time of application (DAS)
Azimsulfuron @ 35 g ha^{-1} at 20 DAS	Segment	Azimsulfuron- 50% DF	35 g.	20
Flucetosulfuronat @ 25 g ha^{-1} at 20 DAS	ICH- 110	Flucetosulfuron- 10% WP	25 g	20
Bispyribac sodium @ 30 g ha^{-1} at 20 DAS	Nominee gold	Bispyribac Sodium- 10% SC	30 g	20
Bensulfuron-methyl +Pretilachlorat @ 60+ 600 g ha^{-1} at 10 DAS	Londax Power	Bensulfuron Methyl 0.6% + Pretilachlor 6% GR	60+600 g	10
Azimsulfuron+Bispyribac sodium @ 22+25 g ha^{-1} at 20 DAS	Segment + Nominee gold	Azimsulfuron- 50% DF + Bispyribac Sodium- 10% SC	22 + 25 g	20
Flucetosulfuron@ 25 g ha^{-1} at 5 DAS fbBispyribac sodium @ 25 gha^{-1} at 20 DAS	ICH-110 fb. Nominee gold	Flucetosulfuron 10% WP fb. Bispyribac Sodium- 10% SC		5 and 20
Manual weeding twice at 20 and 40 DAS	-	-	-	20 and 40
Paddy weeder at 20 DAS fb Manual weeding at 40 DAS	- -	-	-	20 and 40
Weed free (weeding at 15, 30, 45 and 60 DAS)	-		-	15,30,45 and 60
Weedy check	-	-	-	-

against insect pest. Chloropyriphos at 0.05 % was sprayed at PI stage to protect the crop from stem borer and leaf folder attack. The crop was harvested at maturity.

A quadrate measuring 0.5 m x 0.5 m was randomly placed at four sites in each experimental plot to record weed density at 45 and 60 DAS. Weeds were counted and collected for recording dry weight by drying in oven at 70 ^{0}C until constant weight. The data of weed density and dry matter was converted to m^{-2}. The data on count and dry matter of weeds were subjected to square root transformation [(x+ 0.5)] for statistical analysis.

Weed control efficiency was computed using weed dry weight. To determine WCE of individual treatment, the following formula was used and expressed in percentage.

$$WCE = \frac{WDc - WDt}{WDc} x100$$

Where, WCE = Weed control efficiency

WDC = Weed dry weight in weedy check

WD t = Weed dry weight in treated plot

Weed index was determined by using the following formula (Gill and Vijayakumar 1966) and expressed in percentage.

$$\text{Weed index} = \frac{Ywfc - Yt}{Ywfc} x100$$

Where,

Yw fc = Yield of the crop in weed free check

Y t = Yield of the crop in plot under treatment.

Plant height of selected randomly tillers from each of the experimental unit was recorded from soil level to the tip of flag leaf with the help of a meter scale. Days to 50% flowering and maturity were recorded plot wise. At physiological maturity plant samples from each plot were harvested manually and separated into straw and panicles. The dry weight of straw was determined after oven drying at 70^{0}C to constant weight. Panicles were hand-threshed and the filled spikelets were separated from unfilled spikelets with a blower. All unfilled spikelets were counted to determine the number of unfilled spikelets. Grain yield was determined from each plot and adjusted to the standard moisture content of 0.14 g H_2O g^{-1} fresh weight (Kumar *et al*. 2016, 2017).

The data on observed parameters were subjected to analysis of variance (ANOVA) for randomized block design. The results were presented at 5% level of significance (P> 0.05) and critical difference (CD) values were calculated to compare the treatments.

RESULTS AND DISCUSSIONS

The weed flora of the experimental site was similar during both the years and comprised of sedges *Cyperus difformis* L., *Scirpus incurvatus* Roxb, *Cyperus iria* L., *Fimbristylis miliacea* (L.)Vahl; grasses *Leptochloa chinensis* (L.) Nees, *Echinochloa glabrescens* Munro ex Hook. f., *Echinochloa crusgalli* (L.), P. Beauv., *Echinochloa colona* (L.) Link; broad-leaved weeds (BLW) *Ludwigia adscendes* (L.) Hara, *Ludwigia octovalvis* (Jacq.) P. H. Raven, *Monochoria vaginalis* (Burm.f.) C. Presl ex Kunth, *Marsilea quadrifolia* L.; aquatic weeds *Chara zeylanica* var. diaphana (F. Meyen) R.D. Wood and *Hydrilla verticillata* (L.f.) Royle (Table 2). The composition of grasses, sedges and broad-leaved weeds in weedy check plot at 60 DAS was 15.1, 71.5 and 13.4%, respectively. Similarly Rasid *et al*. (2012) and Naseerudin and Subramanyam 2013 reported the dominance of sedges in W-DSR. *Cyperus difformis*, *Echinochloa glabrescens* and *Ludwigia adscendes* were the dominant weeds among sedges, grasses and BLW respectively.

All the weed control treatments significantly reduced density and dry matter of weeds as compared to that in weedy check. The highest dry matter of weeds in weedy plot might be due to increased population and continuous growth as no weed management practice was applied in weedy check (Table 3). Among the weed control practices hand weeding twice recorded lowest weed dry matter (2.41 g m^{-2}) at 45 DAS, however, at 60 DAS tank-mix application of azimsulfuron + bispyribac sodium recorded lowest weed dry matter (2.8 g m^{-2}). Efficacy of tank mix application of bispyribac sodium + azimsulfuron in terms of lowering weed dry biomass, broad spectrum weed control and increase in grain yield of direct seeded rice reported by Ghosh *et al*. (2017). However, in this study tank -mix application of azimsulfuron + bispyribac sodium does not show any significant effect in terms of weed control efficiency and increase in grain yield over single application of azimsulfuron or bispyribac sodium alone. This might be due to dominance of sedge weeds (71.5%) in the experimental plots. Efficacy of single slot application of azimsulfuron or bispyribac sodium in controlling broad leaved weeds and sedges in rice field is reported by Singh *et al*. (2010); Naseerruddin and Subramanyam, 2013 and Duary *et al*. (2015). Similarly effectiveness of bispyribac sodium in terms of weed control efficiency and increase in rice grain yield in W-DSR also reported by Yadav *et al*. (2011). Application of bensulfuron methyl + pretilachlor recorded significantly reduction in weed population and dry matter which resulted increase in grain yield of rice. Similar findings are also reported by Saha and Rao 2010 and Singh *et al*. (2014). Early post emergence application of flucetosulfuron at 25 g ha^{-1} recorded significantly reduction in weed population and dry matter which resulted increase in grain yield of W-DSR. Bhimal and Pandey (2014) reported the usefulness of flucetosulfuron in terms of reduction in weed dry matter and increase in grain yield

Table 2. Weed flora associated with wet direct seeded rice sown by drum seeder

Scientific name	Common name	Group	Family
Cyperus difformis L.	Small flower Umbrella Sedge	Sedge, Annual herb	Cyperaceae
Scirpus incurvatus Roxb.	Bulrush	Sedge, Annual herb	Cyperaceae
Cyperus iria L.	Rice Flat Sedge	Sedge, Annual herb, Occasionally perennial	Cyperaceae
Fimbristylis miliacea (L.) Vahl	Forked Fringe rush	Sedge, Annual/Perennial herb	Cyperaceae
Leptochloa chinensis (L.) Nees	Chinese Sprangle top	Grass, Annual herb	Poaceae
Echinochloa glabrescens Munro ex Hook. f.	Cockspur Grass	Grass, Annual herb	Poaceae
Echinochloa crusgalli (L.) P. Beauv.	Barn yard Grass	Grass, Annual herb	Poaceae
Echinochloa colona (L.) Link	Jungle Rice	Grass, Annual herb	Poaceae
Ludwigia adscendes (L.) Hara	Water Primose	Broad leaf, Perennial herb	Onagraceae
Ludwigia octovalvis (Jacq.) P. H. Raven	False Primose	Broad leaf, Perennial herb	Onagraceae
Monochoria vaginalis (Burm.f.) C. Presl ex Kunth	Oval-leafed	Broad leaf, Annual/ Perennial	Pontederiaceae
Marsilea quadrifolia L.	Four leaf clover	Broad leaf, Perennial	Marsileaceae
Chara zeylanica var. diaphana (F. Meyen) R.D. Wood	Stonewort	Submerged Aquatic	Characeae

Table 3. Effect of weed management practices on weed population, dry matter and WCE in Wet direct seeded rice sown by drum seeder (mean of two years)

Treatments	Weed Population (m^{-2})		Weed dry weight (gm^{-2})		WCE (%)	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Azimsulfuron @ 35 g ha^{-1} at 20 DAS	4.5 (19.7)	4.2 (17.0)	2.51 (8.7)	4.6 (20.9)	91.4	90.6
Flucetosulfuronat @ 25 g ha^{-1} at 20 DAS	4.5 (19.4)	5.2 (27.0)	3.32 (11.5)	5.4 (28.7)	89.1	87.1
Bispyribac sodium @ 30 g ha^{-1} at 20 DAS	4.9 (23.5)	4.1 (16.0)	3.29 (11.1)	4.3 (17.9)	88.9	91.0
Bensulfuron-methyl +Pretilachlorat @ 60+ 600 g ha^{-1} at 10 DAS	4.6 (20.5)	3.9 (14.4)	3.06 (9.3)	4.0 (15.7)	90.9	93.7
Azimsulfuron+Bispyribac sodium @ 22+25 g ha^{-1} at 20 DAS	4.2 (17.4)	3.8 (13.9)	2.93 (8.4)	2.8 (7.4)	91.0	94.6
Flucetosulfuron @ 25 g ha^{-1} at 5 DAS fb Bispyribac sodium @ 25 gha^{-1} at 20 DAS	3.4 (11.3)	3.8 (14.0)	2.69 (7.1)	3.9 (14.9)	93.1	90.8
Manual weeding twice at 20 and 40 DAS	6.4 (40.7)	6.4 (40.9)	2.41 (5.7)	4.0 (15.3)	94.5	93.6
Paddy weeder at 20 DAS fb Manual weeding at 40 DAS	8.4 (69.5)	8.7 (74.9)	4.31 (19.3)	5.4 (28.2)	78.6	86.6
Weed free (weeding at 15, 30, 45 and 60 DAS	0.7 (0.0)	0.7 (0.0)	0.70 (0.0)	0.70 (0.0)	100.0	100.0
Weedy check	20.7 (426.0)	21.7 (469.3)	10.29 (118.1)	14.36 (219.0)	-	-
CD (p=0.05)	2.3	2.58	1.44	1.35	-	-

*DAS=Days after sowing

Table 4. Effect of weed management practices on growth of wet direct seeded rice sown by drum seeder (mean of two years)

Treatments	Plant height (cm)	Tillers (m^{-2})	Panicle length (cm)	Days to 50% flowering	Spikelet fertility (%)
Azimsulfuron @ 35 g ha^{-1} at 20 DAS	107.0	608.5	23.2	90.3	77.5
Flucetosulfuronat @ 25 g ha^{-1} at 20 DAS	109.4	633.2	23.1	91.4	79.7
Bispyribac sodium @ 30 g ha^{-1} at 20 DAS	106.5	637.4	23.3	91.4	77.1
Bensulfuron-methyl +Pretilachlorat @ 60+ 600 g ha^{-1} at 10 DAS	109.0	639.3	23.8	91.7	78.3
Azimsulfuron+Bispyribac sodium @ 22+25 g ha^{-1} at 20 DAS	108.2	622.4	23.7	90.8	78.9
Flucetosulfuron @ 25 g ha^{-1} at 5 DAS fb Bispyribac sodium @ 25 gha^{-1} at 20 DAS	106.8	615.0	23.4	92.7	77.9
Manual weeding twice at 20 and 40 DAS	108.3	626.3	23.3	91.7	79.6
Paddy weeder at 20 DAS fb Manual weeding at 40 DAS	108.0	618.0	23.0	91.5	76.4
Weed free (weeding at 15, 30, 45 and 60 DAS	108.0	646.4	24.2	91.7	80.6
Weedy check	97.5	533.3	21.6	92.0	74.9
CD (p=0.05)	4.83	61.19	1.0	NS	NS

*DAS=Days after sowing

of transplanted rice.

All weed control treatments recorded significantly higher plant height, number of tillers m^{-2} than weedy check. Various weed management practices does not recorded any significant difference in days to 50% flowering and spikelet fertility % against weedy plot. We observed low filled spikelet percentage which might be due to occurrence of continuous rain during flowering period, but it does not affect the grain yield because of higher number of effective tillers per unit area.

Hand weeding twice recorded grain yield of 5.64 t ha^{-1} resulting in 54.5% increase over weedy check. Mechanical weeding followed by one hand weeding recorded reduction in grain of 9.2% over hand weeding twice. The yield reduction might be due to poor control of weeds at early crop growth stage by paddy weeder. Single shot herbicides like

Table 5. Effect of weed management practices on yield and yield components of wet direct seeded rice sown by drum seeder (mean of two years)

Treatments	Panicles (m^{-2})	Grains panicle $^{-1}$	Test weight(g)	Grain yield(t ha^{-1})	WI (%)
Azimsulfuron @ 35 g ha^{-1} at 20 DAS	374.0	89.2	21.7	5.38	7.6
Flucetosulfuronat @ 25 g ha^{-1} at 20 DAS	383.5	88.0	21.4	5.42	7.0
Bispyribac sodium @ 30 g ha^{-1} at 20 DAS	361.0	88.0	21.6	5.40	7.3
Bensulfuron-methyl +Pretilachlorat @ 60+ 600 g ha^{-1} at 10 DAS	378.5	89.4	21.8	5.39	6.9
Azimsulfuron+Bispyribac sodium @ 22+25 g ha^{-1} at 20 DAS	387.9	91.9	21.6	5.50	5.5
Flucetosulfuron @ 25 g ha^{-1} at 5 DAS fbBispyribac sodium @ 25 gha^{-1} at 20 DAS	366.5	88.1	21.1	5.35	8.1
Manual weeding twice at 20 and 40 DAS	389.0	95.3	21.1	5.64	3.2
Paddy weeder at 20 DAS fb Manual weeding at 40 DAS	353.5	85.4	21.6	5.12	12.2
Weed free (weeding at 15, 30, 45 and 60 DAS	398.7	99.3	21.5	5.82	-
Weedy check	283.9	71.8	21.0	3.65	37.3
CD (p=0.05)	37.15	9.76	NS	0.35	-

DAS=Days after sowing

flucetosulfuron at 25 g ha^{-1} (5.42 t ha^{-1}), bispyribac sodium at 30 g ha^{-1} (5.40 t ha^{-1}) and azimsulfuron at 35 g ha^{-1}(5.38 t ha^{-1}) recorded 48.5%, 47.9% and 47.4% increase in grain yield over weedy check. However, the herbicide combination (tank -mix) azimsulfuron+ bispyribac sodium recorded 2.2% and 1.9% only, increase in grain yield over its single application. No significant yield advantage of the tank mix application over its single application may be due to dominance of sedge weeds in the field. The ready mix application of bensulfuron methyl+pretilachlor recorded 47.7% increase in grain yield over control. Sequential application of flucetosulfuron at 5DAS followed by post emergence application of bispyribac sodium does not recordany significant increase in grain yield over its single application. This may be due to pytotoxicity of fluecetosufuron when applied at 5 DAS. However flucetosulfuron when applied at 20 DAS does not show any pytotoxicity to rice crop (data not presented).

Weed infestation in drum seeded W-DSR recorded grain yield reduction of 37.3% as compared to weed free plots. Manual weeding twice recorded lowest WI (3.2%) followed by application of azimsulfuron + bispyribac sodium at 22+25 g ha^{-1} (5.5%), bensulfuron methyl+pretilachlor (6.9%), flucetosulfuron (7.0%), bispyribac sodium (7.2%) and azimsulfuron (7.6%)(Table 5).

From the present study, it can be concluded that weed infestation cause grain yield reduction of 37.3% in drum seedred rice during early *ahu* season. The laborious, time consuming, costly hand weeding operation in W-DSR can be replaced by the low dose high efficacy early post emergence herbicides like flucetosulfuron 25 g ha^{-1} or azimsulfuron 35 g ha^{-1} or bispyribac sodium 30 g ha^{-1} or bensulfuron methyl+pretilachlor 60+600 g ha^{-1} for broad spectrum weed control and sustainable grain yield.

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Energy consumption, economics, yield and quality of rice (*Oryza sativa* L.) in different crop establishment methods

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ABSTRACT

A field experiment was conducted in clay loam soils of Jagtial, Telangana State during rainy season of 2015 to study the performance of rice under different establishment methods. The experiment was laid down in randomized block design with three replications. Eleven treatments were taken viz. dry and wet seeding with drum seeder at three row spacing i.e, 20, 25 and 30 cm, broadcasting of dry and sprouted seed in puddled soil, conventional transplanting, SRI and MSRI methods. The yield attributes viz. panicles m^{-2}, spikelets panicle^{-1} and per cent filled grains were found to be high in MSRI method with less chaffyness of grains. Highest grain yield was obtained with MSRI method (6848 kg ha^{-1}) followed by SRI method (6425 kg ha^{-1}) which was 31.5 and 23.4% higher than the conventional transplanting method, respectively. Drum seeding at 30 cm spacing with wet seed (pregerminated) and dry seed registered an increase of 22.1 and 17.8% in yield over conventional transplanting method, respectively. Rice crop matured 10-15 days earlier in drum seeding and 5-6 days in SRI and MSRI methods compared to conventional transplanting. The productivity day^{-1} was found to be maximum in drum seeding with pregerminated seed at 30 cm row spacing closely followed by MSRI and SRI methods and found to be superior to transplanting method. The labour requirement in transplanting was the highest and MSRI method registered the least followed by drum seeding. The energy consumption was less in drum seeding followed by SRI method compared to MSRI and broadcasting method. MSRI method fetched highest gross returns (169957 ha^{-1}), net returns (118657 ha^{-1}) and B:C ratio (2.3).

Key words: *Crop establishment, drum seeding, SRI, MSRI, labour requirement, productivity day^{-1}, energy consumption*

In India, rice is cultivated in an area of 43.95 mha with a production of 106.54 mt and productivity of 2424 kg ha^{-1}(Ministry of Agriculture 2014). Although conventional transplanting method is reported to be the best establishment method (Mankotia *et al*. 2009; Shan *et al*. 2012; Singh *et al*. 2013; SandhyaKanthi *et al*. 2014; Mohanty *et al*. 2017), some other alternate establishment methods such as dry and wet seeding are being explored in the recent past to reduce the cost of cultivation and to catch up with the season.

Due to global warming in the recent years, there has been a shift in the regular climate changes. Besides different anthropogenic activities, lowland paddy fields with standing water are major sources of methane emission which ultimately increases the global warming potential (Mahato 2014; Kumar *et al*. 2016). It had a commendable impact on rainfall pattern. There was either late onset of monsoon or early cessation of rains. The late onset of monsoon resulted in delay of sowing due to which there were unfavourable conditions at critical growth stages thereby reducing the yields. The early cessation of rains caused water deficit during peak period of water requirement which in turn had a great impact on the yields. This led to unavailability of water to raise the crop throughout its growth period.The scarcity of water triggered the unavailability of power due to which irrigation given to the crop was hampered.

Another exemplary problem associated with raising of rice crop in traditional method is the unavailability of labour. In India, labour migration is mostly influenced by social structures and pattern of development. Uneven development is the main reason of migration along with factors like poverty, landholding system, fragmentation of land, lack of employment opportunities, large family size and natural calamities. The high-land man ratio, caste system, lawlessness and exploitation at native place speed up the breakdown of traditional socio-economic relations in the rural areas and people decide to migrate to relatively prosperous areas in search of better employment and income (Kaur *et al*. 2011).

These problems forced the farmers to find alternate methods of raising rice crop which reduces the cost of cultivation on account of high labour and water requirement. These methods include dry and wet seeding, drum seeding, SRI and mechanised SRI methods. Keeping this in view, the present study was undertaken to investigate the effect of crop establishment methods on yield, energy consumption and economics of rice in *kharif* season.

MATERIALS AND METHODS

The field experiment was carried out at Regional Agricultural Research Station Farm, Agricultural College Jagtial, Telangana State during *kharif* season, 2015. The experimental soil was clay loam with neutral pH (7.3) and electrical conductivity (0.24 d Sm^{-1}), low in organic carbon (0.38%). The soil was low in available N (220.4 kg ha^{-1}) and medium in available P (35.3kg ha^{-1}) and K (312.2 kg ha^{-1}). The cultivar 'BPT 5204' was used as the test variety. The experiment was laid in randomized block design with three replications and eleven establishment methods *viz*., dry seeding with drum seeder at 20 cm spacing between the rows (T_1), wet (pre germinated) seeding with drum seeder at 20 cm spacing between the rows (T_2), dry seeding with drum seeder at 25 cm spacing between the rows (T_3), wet seeding with drum seeder at 25 cm spacing between the rows (T_4), dry seeding with drum seeder at 30 cm spacing between the rows (T_5), wet seeding with drum seeder at 30 cm spacing between the rows (T_6), broadcasting of dry seed in puddled soil (T_7), broadcasting of sprouted seed in puddled soil (T_8), conventional transplanting (farmer's practice) (T_9), SRI method (T_{10}) and MSRI method (T_{11}). A rainfall of 340.6 mm was received during the crop growth period spread in 19 rainy days.

Soil samples were collected before planting and after harvest of the crop and analyzed for available nitrogen, available phosphorus and available potassium. Available nitrogen was estimated by alkaline potassium permanganate method (Subbaiah and Asija 1956), available phosphorus by Olsen's method (Olsen *et al*. 1954) and available potassium by flame photometry (Muhr *et al.* 1963).

For sowing in treatments from T_1 to T_6, manually operated drum seeders consisting of two drums made of fibre with openings at different spacing were used. A 20 cm spacing drum seeder provided 20 cm spacing between the rows and 7 cm spacing between the plants of the rows (T_1 and T_2). Six rows could be laid when this drum seeder was drawn across a given area. The drum seeder with 25 (T_3 and T_4) and 30 cm (T_5 and T_6) spacing between the rows could lay 4 rows at a time in a given area with 7 cm spacing between the plants of a row. The drums were filled with the seed upto three fourth of their capacity. About 3-4 seeds can be placed in a hill with the help of the drum seeder at all the row spacing. For dry seeding in T_1, T_3 and T_5, dry seeds were directly taken in the drum and sown in the puddled field. For wet seeding in T_2, T_4 and T_6, the seeds were soaked in water for 24 hours, drained and incubated for 24 hours in gunny bags and filled in the drums for sowing. Thus, the seed rate worked out was 37.5 kg ha^{-1} at 20 cm and 35 kg ha^{-1} at 25 and 30 cm row spacing. For broadcasting of dry seed (T_7), the seeds @ 100 kg ha^{-1} were directly broadcasted into the puddled field uniformly. For broadcasting sprouted (pre germinated) seed (T_8), the seeds were soaked in water, drained and incubated for 24 hours before sowing. In case of conventional transplanting (T_9), nursery was raised by broadcasting the seed @ 75 kg ha^{-1}. Transplanting was done using thirty-day-old seedlings. Seedlings were uprooted and transplanted @ 2 seedlings $hill^{-1}$ about 2-3 cm deep in puddled soil at 20 x 15 cm spacing manually. In SRI (T_{10}) method, raised bed nursery was prepared and seeds were broadcasted. Seed rate adopted was 5 kg ha^{-1}. Twelve-day-old seedlings were transplanted manually at 25 x 25 cm in the puddled field. In MSRI (T_{11}) method, nursery was sown in plastic trays of 60 x 30 x 2.7 cm size, filled with well powdered soil. The soaked seeds were broadcasted in the trays and covered with soil. Water was sprinkled 3-4 times every day up to 6-7 days after sowing to keep the seed bed wet. From a week after sowing, water was applied through the water channel until transplanting. Machine transplanting was done using Kubota NSPU- 68C transplanter which planted 6 rows at one time with a spacing of 30 x 16 cm. Fifteen day old seedlings in mats were lifted from the plastic trays and placed directly in the trays of the transplanter. Seed rate adopted in MSRI was 25 kg ha^{-1}. The sowing/ transplanting in all the plots was done on 1^{st} August, 2015. The gross plot size in all the treatments was 9.3 x 4.5 m and net plot size was 8.7 x 3.5 m.

For control of weeds, pretilachlor was applied @ 7.5 ml in 150 gm soil per plot on 4 August, 2015. The left over weeds were removed by hand weeding at 15 days interval. Irrigation was applied as per requirement to the plots. No irrigation was applied on rainy days. It was withheld 15 days before harvesting of the crop. A fertilizer dose of 120, 60 and 40 kg N, P_2O_5 and K_2O ha^{-1} was applied. Phosphorus and potash were applied as basal dose in the form of SSP and MOP, respectively. Nitrogen was applied as 3 equal splits *viz*., as basal at the time of transplanting/sowing, maximum tillering and panicle initiation stage. Zinc was applied in the form of $ZnSO_4$ as foliar spray @ 2g lt^{-1} to all the plots at 30 DAS. Carbofuran 3G granules @ 25 kg ha^{-1} was applied at 27 DAS. Other plant protection measures were taken up as and when required. Harvesting in conventional transplanting and the rest of the methods was done on 144 DAS and 150DAS, respectively.

Data on the number of panicles in one m^2 area in net plot was counted and expressed as panicles m^{-2}. Ten panicles were selected at random from each plot to compute the number of spikelets $panicle^{-1}$, number of chaffy grains $panicle^{-1}$ and percentage of filled spikelets. For 1000-grain weight, five hand full of grain samples were collected at random from the net plot yield of each individual treatment. The grains were counted and weighed to arrive at test weight. The crop was harvested manually with the help of sickles when the grain almost matured and the straw had turned yellow and data on grain and straw yield was recorded (Kumar *et al*. 2017).

The hulling, milling and head rice recovery were also recorded as per Brar *et al*. (2007). Sample of one hundred grams of well dried paddy (12-14% moisture) from each treatment was dehulled in "Satake" dehuller and the weight of brown rice was recorded. Hulling percentage was computed using the following formula and expressed in percent.

Hulling (%)=(Weight of brown rice (g) X 100)/ (Weight of unhusked rice (g))

The brown rice obtained by dehulling was subjected to milling for 90 sec ***i.e.***, 5 percent milling in "Satake" polisher (Type-TM 05) and the weight of polished rice was recorded.

Milling (%)=(Weight of polished grain (g) X 100)/{Weight of rough rice (g)}

The milled samples were sieved to separate whole grains from the broken ones. Small proportion of whole grains, which passed along with broken grains were separated by hand. Full rice and three-fourth grains were taken as whole rice for computation. Head rice recovery (HRR) was calculated in percentage as

Head rice recovery (%)=(Weight of head rice recovered (g) X 100)/{Weight of sample used for milling (g)}

The duration of the crop from sowing to harvest was calculated and expressed as days. The grain yield obtained was divided by the crop duration to get the productivity day^{-1}. It was expressed as kg ha^{-1} day^{-1}. The number of labourers required during the entire crop period in different treatments from sowing to harvesting was calculated and expressed as the number of man days. The energy consumption during the entire crop period from sowing to harvest, taking into account the energy consumed by human labour, number of litres of diesel and fertilizers was recorded according to Patel *et al*. (1994), expressed as MJ ha^{-1} and presented in Table 1.

Table 1. Equivalent energy of the inputs used in the study

Particular	Unit	Equivalent energy (MJ)
Inputs		
Adult man	Man (hr)	1.96
Woman	Woman (hr)	1.57
Bullock	Pair (hr)	14.05
Diesel	litre	56.31
Chemical fertilizers		
Nitrogen	kg	60.60
P_2O_5	kg	11.10
K_2O	kg	6.70
Other chemicals	kg	120

Gross returns ha^{-1} were calculated by multiplying the grain and straw yield with their respective prevailing market price. The net return ha^{-1} was calculated by deducting the cost of cultivation from the gross returns. The benefit:cost ratio was also worked out by dividing the net returns from the cost of cultivation.

All the data were subjected to analysis of variance (ANOVA) as per the standard procedures. The comparison of treatment means was made by critical difference (CD) at P=0.05.

RESULTS AND DISCUSSION

Yield attributes and yield of rice were influenced by different crop establishment methods (Table 2). Highest number of panicles m^{-2} were recorded in MSRI method (T_{11}) which was at par with SRI method (T_{10}) and dry andwet seeding with drum seeder at 30 cm row to row spacing (T_5 and T_6) and was superior to rest of the treatments. Significantly higher number of spikelets were found in MSRI method (T_{11}) compared to broadcasting (T_7 and T_8), drum seeding with dry and wet seed at 20 cm spacing (T_1 and T_2) or dry seed at 25 cm spacing (T_3) and transplanting method (T_9) and at par with other methods. MSRI method (T_{11}) registered highest percentage of filled spikelets which was superior to broadcasting (T_7 and T_8) and drum seeding at 20 cm spacing (T_1 and T_2) and at par with other method. The number of chaffy spikelet $panicle^{-1}$ was in the reverse order and it was lower in MSRI method (T_{11}) which was at par with SRI method (T_{10}) and drum seeding with wet or dry seed at 30 cm row spacing (T_6 and T_5) or wet seed at 25 cm spacing (T_4). There was no significant difference among the establishment methods with respect to test weight of the grains.

In general, the scanty rainfall coupled with high mean temperature, relative humidity and wind velocity especially from vegetative to panicle initiation stage resulted in realisation of lower yields in the region. However, the grain yield and straw yield of rice was higher in MSRI method (T_{11}) and significantly superior to transplanting method (T_9) (Table 3). It was at par with SRI method (T_{10}) and drum seeding at 30 cm row spacing (T_6 and T_5) or wet seeding at 25 cm spacing (T_4). The grain yield recorded in MSRI method (T_{11}) and SRI method (T_{10}) was 31.5 and 23.4% higher than that in conventional transplanting (T_9), respectively. It is attributed to planting of young seedlings *i.e*., before third phyllochron at shallow depth of planting in wider spacing (25 x 25 cm), which leads to large root volume, profuse and strong tillers with large panicles, more and well filled spikelets with higher grain weight in SRI method (Satyanarayana and Babu 2004). Dass *et al*. (2015) in a review, concluded 50-100% increase in rice yield in India due to SRI method over conventional transplanting method. Pasha *et al.* (2014) and Ramana *et al*. (2015) also reported the superiority of MSRI or

Table 2. Yield attributes of rice as influenced by different crop establishment methods

Treatment	No. of panicles m^{-2}	No. of spikelets $panicle^{-1}$	% filled spikelets	No. of chaffy spikelets $panicle^{-1}$	Test weight (g)
T_1: Drum seeding (dry) at 20 cm row spacing	314	154	70.5	44	13.4
T_2: Drum seeding (wet) at 20 cm row spacing	327	174	75.7	41	13.5
T_3: Drum seeding (dry) at 25 cm row spacing	342	189	79.2	39	13.5
T_4: Drum seeding (wet) at 25 cm row spacing	351	196	82.0	34	13.6
T_5: Drum seeding (dry) at 30 cm row spacing	377	201	84.4	32	13.6
T_6: Drum seeding (wet) at 30 cm row spacing	394	208	85.2	30	13.6
T_7: Broadcasting dry seed	277	127	56.0	52	13.2
T_8: Broadcasting sprouted seed	317	164	71.3	44	13.2
T_9: Transplanting	332	179	76.8	39	13.3
T_{10}: SRI method	412	229	86.6	29	13.7
T_{11}: MSRI method	431	249	90.2	24	13.7
SEm $\pm$	34	28	6.6	6	0.2
CD (P=0.05)	71	59	13.8	13	NS

Table 3. Yield and quality parameters of rice as influenced by different crop establishment methods

Treatment	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Harvest index (%)	Hulling (%)	Milling (%)	Head rice recovery (%)
T_1: Drum seeding (dry) at 20 cm row spacing	4806	6268	43.4	82.6	71.7	61.7
T_2: Drum seeding (wet) at 20 cm row spacing	5108	6585	43.6	82.6	72.5	62.5
T_3: Drum seeding (dry) at 25 cm row spacing	5559	7032	44.1	83.1	73.1	62.9
T_4: Drum seeding (wet) at 25 cm row spacing	5771	7187	44.5	83.1	73.2	62.9
T_5: Drum seeding (dry) at 30 cm row spacing	6136	7527	44.9	83.1	73.4	63.2
T_6: Drum seeding (wet) at 30 cm row spacing	6358	7762	45.1	83.5	73.8	63.7
T_7: Broadcasting dry seed	4208	5705	42.4	80.4	70.4	61.4
T_8: Broadcasting sprouted seed	4888	6308	43.8	82.6	72.5	62.4
T_9: Transplanting	5207	6733	43.4	83.5	72.8	62.6
T_{10}: SRI method	6425	7871	45.0	84.0	74.0	64.1
T_{11}: MSRI method	6848	8313	45.0	87.1	77.5	67.0
SEm ±	563	595	2.2	2.6	1.7	1.6
CD (P=0.05)	1176	1243	NS	NS	NS	NS

SRI method over conventional transplanting method.

Drum seeding at 30 cm spacing with wet seed (T_6) and dry seed (T_5) also registered an increase of 22.1 and 17.8% in yield over conventional transplanting method, respectively. This can be attributed to more space, sunlight and nutrients available at wider spacing in drum seeding. Visalakshi and Sireesha (2014) evaluated drum seeder in farmers' fields and inferred that drum seeding was superior to conventional transplanting and broadcasting methods.

Harvest index remained unaffected by the different crop establishment methods. The hulling, milling and head rice recovery was also not influenced by different crop establishment methods. Similarly, physico-chemical, physical properties and nutrient status of the soil was unaltered by the crop establishment methods after the harvest of rice crop (Table 6 and 7). This is in contrast to Choudhury *et al*. (2007) and Mandal and Pramanik (2015) who found higher soil organic carbon, bulk density, available N, P, K with SRI method compared to conventional transplanting method.

The crop duration varied with different establishment methods (Table 4). Among them, the crop matured 10-15 days earlier in drum seeding treatments (T_1, T_2, T_3, T_4, T_5 and T_6) compared to transplanting method (T_9). MSRI method (T_{11}) and SRI method (T_{10}) also recorded less duration by 5-6 days compared to conventional transplanting method (T_9). The treatments under wet drum seeding (T_2, T_4 and T_6) had little less duration compared to the dry drum seeding treatments (T_1, T_3 and T_5). The duration of the crop was prolonged by 4-7 days in broadcasting method (T_7 and T_8) compared to drum seeding (T_1, T_2, T_3, T_4, T_5 and T_6). It might be due to competition among the plants. Conventional transplanting (T_9) recorded the longest

Table 4. Crop duration and productivity of rice as influenced by different crop establishment methods

Treatment	Crop duration	Productivity ha^{-1} day^{-1} (days)
T_1: Drum seeding (dry) at 20 cm row spacing	141	34.1
T_2: Drum seeding (wet) at 20 cm row spacing	138	37.0
T_3: Drum seeding (dry) at 25 cm row spacing	141	39.4
T_4: Drum seeding (wet) at 25 cm row spacing	139	41.5
T_5: Drum seeding (dry) at 30 cm row spacing	140	43.8
T_6: Drum seeding (wet) at 30 cm row spacing	138	46.1
T_7: Broadcasting dry seed	146	28.8
T_8: Broadcasting sprouted seed	143	34.2
T_9: Transplanting	155	33.6
T_{10}: SRI method	150	42.8
T_{11}: MSRI method	149	46.0
	SEm ±	3.9
	CD(P=0.05)	8.0

duration compared to all the other treatments. The reason attributed is the transplanting shock which might have prolonged the crop duration in this method. Gill *et al.* (2006) also reported that direct seeded rice matured 10 days earlier than transplanted crop.

Maximum productivity day^{-1} (Table 4) was observed in drum seeding with wet seed at 30 cm row spacing (T_6), which was closely followed by MSRI method (T_{11}). Both of them were at par with dry or wet seeding at 25 cm row spacing (T_3 and T_4), dry seeding at 30 cm spacing (T_5), SRI method (T_{10}) and significantly superior to rest of the methods including transplanting practice (T_9). Lowest productivity was recorded in broadcasting of dry seed in puddled soil (T_7). However, broadcasting either dry or sprouted seed (T_7 and T_8), drum seeding at 20 and 25 cm (T_1, T_2, T_3 and T_4) were found to be at par with transplanting method (T_9). Reduced duration with enhanced or at par yield might be responsible for increased daily production in the alternate establishment methods.

The labour requirement was found to be the highest in conventional transplanting (T_9) on account of nursery raising, puddling and transplanting (Table 5). Among the alternate methods, the number of labour days from sowing to harvesting was the highest in broadcasting of dry or sprouted seed in puddled soil (T_7 and T_8). MSRI method (T_{11}) registered the lower number of labour days over rest of the treatments.

Table 5. No. of man daysand energy consumption as influenced by different crop establishment methods in rice

Treatment	No. of man days	Energy consumption (MJ ha^{-1})
T_1: Drum seeding (dry) at 20 cm row spacing	154	14940
T_2: Drum seeding (wet) at 20 cm row spacing	155	14942
T_3: Drum seeding (dry) at 25 cm row spacing	154	14940
T_4: Drum seeding (wet) at 25 cm row spacing	155	14942
T_5: Drum seeding (dry) at 30 cm row spacing	154	14940
T_6: Drum seeding (wet) at 30 cm row spacing	155	14942
T_7: Broadcasting dry seed	202	15490
T_8: Broadcasting sprouted seed	203	15492
T_9: Transplanting	232	15043
T_{10}: SRI method	169	14945
T_{11}: MSRI method	149	15270

Table 6. Physical and physic-chemical properties of the soil after harvest as influenced by different crop establishment methods in rice

Treatment	pH	EC (d Sm^{-1})	OC (%)	Bulk density (g cc^{-1})
T_1: Drum seeding (dry) at 20 cm row spacing	7.63	0.87	43.4	1.32
T_2: Drum seeding (wet) at 20 cm row spacing	7.66	0.87	43.6	1.32
T_3: Drum seeding (dry) at 25 cm row spacing	7.68	0.88	44.1	1.33
T_4: Drum seeding (wet) at 25 cm row spacing	7.69	0.88	44.5	1.33
T_5: Drum seeding (dry) at 30 cm row spacing	7.70	0.88	44.9	1.33
T_6: Drum seeding (wet) at 30 cm row spacing	7.70	0.88	45.1	1.34
T_7: Broadcasting dry seed	7.60	0.87	42.4	1.32
T_8: Broadcasting sprouted seed	7.65	0.87	43.8	1.32
T_9: Transplanting	7.67	0.88	43.4	1.33
T_{10}: SRI method	7.70	0.89	45.0	1.34
T_{11}: MSRI method	7.70	0.89	45.0	1.34
SEm ±	0.05	0.01	2.2	0.01
CD (P=0.05)	NS	NS	NS	NS

Venkateswarlu *et al.* (2011) reported 50 percent reduction in labour requirement in nursery raising and transplanting in machine method compared to manual transplanting method. Compared to MSRI method (T_{11}), the labour requirement was higher in SRI method (T_{10}). Drum seeding also required considerably lesser labour compared to transplanting (T_9), broadcasting (T_7 and T_8) and SRI method (T_{10}).

The comparison of energy use pattern (Table 5) in different crop establishment methods in rice revealed that the highest input energy were consumed in broadcasting of dry seed in puddled soil (T_7) or sprouted seed in puddled soil (T_8) due to engagement of more labour on manual weeding. The lowest energy was consumed in drum seeding (T_1, T_2, T_3, T_4, T_5 and T_6) and SRI method (T_{10}) compared to other methods due to reduction in use of labour component. The energy consumed in MSRI method (T_{11}) was higher than SRI method (T_{10}) and transplanting method (T_9) due to consumption of diesel even though engagement of labour was reduced.

Significantly higher gross and net returns (Table 8) were realized in MSRI method (T_{11}) compared to transplanting (T_9), broadcasting (T_7 and T_8) and drum seeding at 20 and 25 cm row spacing (T_1, T_2, T_3 and T_4). It was at par with SRI method (T_{10}) and dry and

Table 7. Economics of rice as influenced by different crop establishment methods

Treatment	Cost of cultivation (Rs. ha^{-1})	Gross returns (Rs. ha^{-1})	Net returns (Rs. ha^{-1})	Benefit: Cost ratio
T_1: Drum seeding (dry) at 20 cm row spacing	51650	122960	71310	1.4
T_2: Drum seeding (wet) at 20 cm row spacing	51850	130039	78189	1.5
T_3: Drum seeding (dry) at 25 cm row spacing	51650	140380	88730	1.7
T_4: Drum seeding (wet) at 25 cm row spacing	51850	144767	92917	1.8
T_5: Drum seeding (dry) at 30 cm row spacing	51650	152944	101294	2.0
T_6: Drum seeding (wet) at 30 cm row spacing	51850	158554	106704	2.1
T_7: Broadcasting dry seed	60750	109504	48754	0.8
T_8: Broadcasting sprouted seed	60950	124497	63547	1.0
T_9: Transplanting	51750	132732	80982	1.6
T_{10}: SRI method	52150	159072	106922	2.1
T_{11}: MSRI method	51300	169957	118657	2.3
	SEm ±	11702	11702	0.2
	CD (P=0.05)	24458	24458	0.5

Price (kg^{-1}): Grain: 14.50 and Straw: 8.5

wet drum seeding at 30 cm row spacing (T_5 and T_6). Lowest gross and net returns were realized in broadcasting of dry followed by sprouted seed (T_7 and T_8). Venkateswarulu *et al*. (2011) found an additional net income of Rs. 13837/- ha^{-1} with machine planting which was 29% higher over transplanting in rice. However, gross and net returns obtained in drum seeding at 20 (T_1 and T_2) and 25 cm (T_3 and T_4) and 30 cm (dry seed) (T_5) and broadcasting methods (T_7 and T_8) were at par with transplanting method (T_9).

Similar to gross and net returns, highest benefit:cost ratio was recorded in MSRI method (T_{11}), followed by SRI (T_{10}) and drum seeding at 30 (T_5 and T_6) and 25 cm (T_3 and T_4) spacing which were superior to farmers' method of transplanting (T_9). The other methods were inferior to transplanting method. Higher returns and B:C ratio with MSRI (T_{11}), SRI (T_{10}) and drum seeding method (T_3, T_4, T_5 and T_6) was due to reduction in cost of cultivation on account of reduced labour requirement for planting and weeding coupled with realization of higher yields.

From this study it can be concluded that Mechanised SRI (MSRI) and SRI methods were found to be superior to conventional transplanting method in yield and profit but drum seeding emerged as an alternate method of rice establishment with higher daily productivity and less energy consumption with reduced duration.

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Yield, quality and economics of Basmati rice as influenced by different organic nutrient management practices

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ABSTRACT

A field study was conducted in Agronomy Research Farm, Central Research Station, Orissa University of Agriculture and Technology, Bhubaneswar during kharif 2012-13 to study the effect of organic source of nutrients on productivity, profitability and quality of Basmati rice. Application of Azospirillum (10 kg/ha)+ PSM (10 kg/ha) + EM Spray (twice) + neem cake along with 75% N (75% FYM + 25% vermicompost) resulted in the highest grain yield (3,570 kg/ha) and maximum net returns (Rs.39,654/ha) and was closely followed by Azospirillum (10 kg/ha)+ PSM (10 kg/ha) + EM Spray (twice) with 75% N (75% FYM + 25% vermicompost). Twenty five per cent organic nitrogen source could be saved by addition of Azospirillum + PSM + EM spray or Azospirillum+ PSM + EM spray + Neem cake without any yield reduction in Basmati rice. Application of 100% N (75% FYM + 25% vermicompost) increased the soil available N (253.48 kg/ha), P_2O_5 (45.46 kg/ha) and K_2O (181.62 kg/ha) content over the initial value of available N (246.21 kg/ha), P_2O_5 (43.38 kg/ha) and K_2O (167.84 kg/ha) after harvest of Basmati rice. It was closely followed by application of Azospirillum (10 kg/ha) + PSM (10 kg/ha) + EM Spray (twice) + neem cake along with 75% N (75% FYM + 25% vermicompost) and Azospirillum (10 kg/ha) + PSM (10 kg/ha) + EM Spray (twice) with 75% N (75% FYM + 25% vermicompost). Thus, both soil fertility and grain quality could be increased by these treatments.

Key words: *Basmati rice, Azospirillum, vermicompost, EM spray, economics*

Scented rice especially Basmati rice with organic tag has huge export potential. It commands a very high premium in domestic as well as international markets due to their long slender superfine grains, pleasant aroma, soft texture and extreme grain elongation. In India, area under Basmati rice is increasing due to attractive price. It is estimated that around 700 mt of agricultural waste is available in the country every year, but most of it is not properly used. This implies a theoretical availability of 5 tonnes of organic manures/ hectare arable land/year, which is equivalent to about 100 kg NPK/ha/year (Tandon 1997). A number of organic waste materials are available, which can supply a good amount of plant nutrients, NPK to produce comparable yield (Ghosh 2005). The complexing property of organic manures influence the availability and mobility of micronutrients. Inclusion of FYM in a fertilizer regime maintains micronutrients at non limiting levels for the rice-rice system. Improvement in soil physical and chemical as well as in biological activity through continuous application of chemical fertilizers along with FYM results in a greater soil quality index (SQI) and enhanced sustainability (Shahid *et al*. 2013). Long-term balanced fertilization in the form of FYM + NPK increases P availability to the plant leading to higher P uptake and yield maintenance (Bhattacharyya *et al*. 2015).

Rice produced by organic farming had better grain quality. Grain yield of Basmati rice improved with organic source of nutrients as compared to recommended level of N and untreated control (Mahajan *et al*. 2012). Biofertilizers, an alternate low cost resource have gained prime importance in recent decades. They are cost effective, ecofriendly and renewable source of plant nutrients to supplement fertilizers for sustainable agricultural development. Phosphate solubilizing bacteria alone or in combination with Azospirillum were able to establish in the rice rhizosphere and increased the grain yield of rice in autoclaved soil by 103-256% over control (Kannaiyan *et al*. 1982).

With respect to the effect of effective microorganisms (EM) on the growth and yield of rice, visual observations indicated that the EM treated plants performed better than those without EM (Myint 1994). In most areas, Basmati rice is grown under low input management condition resulting in lower productivity. The use of organic manures in augmenting soil fertility and crop productivity is well known. Thus, efforts need to be made for suitable combination of organic nutrients and management for maintaining productivity and profitability of Basmati rice.

MATERIALS AND METHODS

A field experiment was conducted to study the "effect of organic source of nutrients on productivity, profitability and quality of Basmati rice" during *kharif* season of 2012 at Agronomy Research Farm, Central Research Station, Orissa University of Agriculture and Technology, Bhubaneswar. The mean annual rainfall of the center was 1471.6 mm received in 100 days. Nearly 75 % of the annual rainfall is received between June to September. The soil was sandy loam and slightly acidic with pH 5.7, medium in organic carbon (0.51%), low in available N (246.21 kg/ha), high in available P_2O_5 (43.38 kg/ha) and medium in K_2O content (167.84 kg/ha). The experiment was conducted in randomised block design with three replications. The field experiment was laid out in a randomised block design with twelve treatments as T_1 : 100% N (75% FYM + 25% vermicompost), T_2 : 75% N (75% FYM + 25% vermicompost), T_3 : 50% N (75% FYM + 25% vermicompost), T_4 : T_2 + Azospirillum (10 kg/ha), T_5 : T_2 + Azospirillum + PSM (10 kg/ha), T_6 : T_2 + Azospirillum + PSM + EM Spray (two times), T_7 : T_2+ Azospirillum + PSM + EM Spray (two times) + Neem Cake (250kg/ha), T_8 : T_3 + Azospirillum, T_9 : T_3 + Azospirillum + PSM, T_{10} : T_3 + Azospirillum + PSM + EM spray (two times), T_{11} : T_3 + Azospirillum + PSM + EM spray (two times) + Neem Cake (250 kg/ha) and T_{12} : Control (no manure). The Basmati rice variety utilized in the experiment was Geetanjali aromatic. Seedlings were raised in wet nursery bed. Dhaincha seeds were sown @ 25 kg/ha and after 40 days ploughing was done to incorporate the plants into the soil for green manuring purpose. Sixteen days old seedlings were transplanted in the main field at a spacing of 20cm x 15cm. Biometric observations were recorded at fortnight interval starting from 15 days after transplanting upto the harvest on randomly selected and pegged five hills from every treatment plots (Kumar *et al*. 2017). After harvest, yield and quality parameters of rice were observed. The data collected from field observations and those recorded in laboratory were subjected to statistical analysis by standard analysis of variance techniques (Gomez and Gomez 1984).

Analysis of quality parameters

Kernel length and breadth (mm)

Ten milled kernels from each plot were taken at random and were placed on graph paper for their length breadth using a "Photo Enlarger" with a magnification of 3*. The mean length and breadth were expressed in mm.

Hulling per cent

Well dried rough rice sample from each plot weighing 100g were hulled in a mini "Satake Rice Medium" and the weight of brown rice was recorded. Hulling percentage was worked out as:

$$\text{Hulling}\ (\%) = \frac{\text{Weight of brown rice}\ (\text{g})}{\text{Weight of rough rice}\ (\text{g})}\ \text{x}\ 100$$

Milling per cent

The hulled brown rice samples were milled in a "Satake Rice Whitening and Caking Machine" for 5 minutes. The polished rice was weighed and milling percentage was calculated as:

$$\text{Milling}\ (\%) = \frac{\text{Weight of milled rice}\ (\text{g})}{\text{Weight of rough rice}\ (\text{g})}\ \text{x}\ 100$$

Head Rice Recovery (HRR)

The head rice yield was determined by separating whole grains and $3/4^{th}$ grains manually and percentage was expressed as:

$$\text{HRR}\ (\%) = \frac{\text{Weight of wholed milled rice}\ (\text{g})}{\text{Weight of rough rice}\ (\text{g})}\text{x}100$$

Amylose content

Representative samples containing 20-30 seeds for each sample were taken and graded to a very fine powder. Each sample was transferred in a commercial filter paper envelope. They were defatted for six hours with approximately 300 ml of hexane per balloon in a Soxhlet type continuous extractor. Samples were air dried so as to evaporate all hexane. Then extract solution and standard solutions were prepared by adding reagents following the procedure of Galicia *et al*. 2008.

Readings were taken at 620 nm in a spectrophotometer and standard curve was made. Percentage of amylose was calculated by using following formula. $Y_g = a(x) + b$

where, Y_g is the absorbance units at 620 nm, a is the slope, x is the amylose content and b is the intercept. % AM = (x.d) (100/f)

where, x is the amylose amount (mg), d is the dilution factor and f is the original weight of flours.

Volume expansion ratio (VER)

Volume expansion ratio was determined from the rate of cooked volume rice to that of the uncooked rice (Zhang and Shao 1999). 5g of rice was added to 15ml of water in a test tube and rise in volume (x ml) was noted. Rice was cooked and 15ml of water was added. Then rise in volume (y ml) was noted VER = y/x.

Elongation ratio (ER)

Kernel elongation is the linear expansion of the kernel after cooking which is being used in the quality evaluation. Elongation ratio was ratio of ten average length of cooked rice to the average length of raw rice (Prakash *et al*. 2002).

Kernel length after cooking (KLAC)

Ten average length of cooked rice in each treatment were taken where length of uncooked rice was calculated primarily.

Analysis of harvest index

The harvest index was calculated by the formula below and expressed in percentage.

$$HI = \frac{\text{Economic yield } (\text{kg / ha})}{\text{Biological yield } (\text{kg / ha})} x100$$

Analysis of Economics

Cost of production for all treatments were worked out on the basis of the prevailing market price of the input and the produce. The net return per hectare was calculated by deducting the cost of production from the gross return. The benefit-cost ratio was calculated treatment wise as per following formula to assess the economic impact of the treatments.

$$\text{Benift-cost ratio } (\text{B:C ratio}) = \frac{\text{Gross return } (\text{Rs.})}{\text{Cost of cultivation } (\text{Rs.})}$$

RESULTS AND DISCUSSION

Basmati rice grain yield obtained with 75% N (75% FYM + 25% vermicompost)+ Azospirillum (10 kg/ha)+ PSM (10 kg/ha) + EM spray (two times) + Neem cake (250 kg/ha) was maximum (3.57 t/ha) which was at par with 100% N (75% FYM + 25% vermicompost) *i.e.*, T_1 (3.32 t/ha), 75% N (75% FYM + 25% vermicompost)+ Azospirillum + PSM + EM spray *i.e.*, T_6 (3.31 t/ha), 75% N (75% FYM + 25% vermicompost) + Azospirillum + PSM *i.e.*, T_5 (3.24 t/ ha), 50% N (75% FYM + 25% vermicompost) + Azospirillum+ PSM+ EM spray + Neem cake *i.e.*, T_{11} (3.12 t/ha) and 75% N (75% FYM + 25% vermicompost) + Azospirillum *i.e.*, T_4 (3.11 t/ha).

There was significant reduction in grain yield due to decline in organic nitrogen nutrition from 100% N to 50% N through 75% N from 75% FYM + 25% N from vermicompost (Table 1). Application of biofertilizers in the form of either Azospirillum, Azospirillum + PSM or Azospirillum + PSM + EM spray increased the grain yield in all the treatments. The increases in grain yield were in the respective order of 1, 5 and 7 per cent at 75% N (75% FYM + 25% vermicompost) level and 0.4, 4 and 8 per cent at 50% N (75% FYM + 25% vermicompost) level. The increase in yield due to 75% N (75% FYM + 25% vermicompost) + Azospirillum + PSM + EM spray + Neem cake, 100% N (75% FYM + 25% vermicompost) and 75% N (75% FYM + 25% vermicompost) + Azospirillum + PSM + EM spray were 43.9, 33.8 and 33.5 per cent, respectively, over the control. These results are in close conformity with the findings of Singh *et al*. (2006), Davari and Sharma (2010), Singh *et al*. (2007), Yadav *et al*. (2009), Singh *et al*. (2011) and Moola Ram *et al*. (2011) where in they recorded significantly higher yield of rice under combined application of various organic source of nutrients.

In the present study with Basmati rice,

Table 1. Yield and economics of organic basmati rice as influenced by nutrient management.

Treat-ment	Grain (kg/ha)	Straw (kg/ha)	Har-vest index (%)	Cost of culti-vation (Rs./ha)	Gross return (Rs./ha)	Net return (Rs./ha)	B:C
T_1	3320	5850	36.20	38100	71080	32980	1.86
T_2	3080	5730	34.96	34950	66184	31234	1.89
T_3	2800	5720	32.86	31800	60576	28776	1.90
T_4	3110	5330	36.84	35450	66464	31014	1.87
T_5	3240	5840	35.68	35950	69472	33522	1.93
T_6	3310	6330	34.33	36250	71264	35014	1.96
T_7	3570	6380	35.87	36850	76504	39654	2.07
T_8	2810	5750	32.83	32300	60800	28500	1.88
T_9	2920	5930	32.99	32700	63144	30444	1.93
T_{10}	3030	5830	34.19	33200	65264	32064	1.96
T_{11}	3120	5290	37.09	33650	66632	32982	1.98
T_{12}	2480	4920	33.51	25550	53536	27986	2.09
SEm(±)	0.16	0.13	0.01	-	626	326	0.03
$CD_{(0.05)}$	0.48	0.39	0.03	-	1976	1976	0.08

significant response to biofertilizer application was noted. The result revealed that application of Azospirillum, Azospirillum + PSM, Azospirillum + PSM + EM spray with 75%N (75% N from FYM + 25% N from vermicompost) was at par with 100% N (75% FYM + 25% vermicompost) nutrition. Thus, addition of Azospirillum, Azospirillum + PSM, Azospirillum + PSM + EM spray could save 25% organic N source and combined application of Azospirillum + PSM + EM spray + Neem cake could save 50% organic N source without any considerable yield reduction in Basmati rice. Similar results were also reported by several researchers (Singh *et al*. 2011; Davari and Sharma, 2010 and Moola Ram *et al*. 2011) that combined application of two, three or four sources of organic nutrients with biofertilizers resulted in higher grain yield of Basmati rice.

Effect on quality parameters

Basmati rice variety "Geetanjali" with 75% N (75% FYM + 25% vermicompost) + Azospirillum + PSM + EM spray recorded the highest hulling per cent (84.2), milling per cent (76.1) and head rice recovery percent (60.2) which was at par with 100% N (75% FYM + 25% vermicompost) and 75% N (75% FYM + 25% vermicompost) + Azospirillum + PSM + EM spray + Neem cake with respective values of 83.1, 75.2, 59.2 per cent and 79.8, 72.6 and 58.3 per cent. There was no significant difference due to various treatments for kernel length, kernel breadth, kernel length-breadth ratio and elongation ratio (Table 2). However, application of 75% N (75% FYM + 25% vermicompost) + Azospirillum + PSM + EM spray + Neem cake recorded the highest value in each of the above characters and also it resulted in significantly higher values of kernel length after cooking, amylose content and protein content. This result is in conformity with the findings of Lognadhan and Rajeswari (2005), Davari and Sharma (2010), Singh *et al.* (2007) and Moola Ram *et al.* (2011) who observed that marketing oriented parameters like hulling, milling and head rice recovery were higher in organic manured plots compared to inorganic fertilizers applied plots. Higher contents of amylose and protein in organic Basmati rice was also reported by Murali and Setty (2001) and Hemalatha *et al.* (2000). Prakash *et al.* (2002) also reported that

Table 2. Quality of grains of organic Basmati rice as influenced by nutrient management practices.

Treatment	Hulling (%)	Milling (%)	Head rice recovery (%)	Kernel length (mm)	Kernel breadth (mm)	Length breadth ratio	Kernel length after cooking (mm)	Elongation ratio	Volume expansion ratio	Protein content (%)	Amylose content (%)
T_1	83.1	75.2	59.2	7.6	1.66	4.57	13.1	1.72	3.58	7.25	23.48
T_2	71.3	64.3	52.8	7.4	1.63	4.54	12.6	1.70	3.54	6.62	22.95
T_3	75.6	67.5	55.3	7.6	1.64	4.63	12.8	1.68	3.56	6.01	22.74
T_4	77.1	69.0	53.5	7.3	1.63	4.47	12.6	1.72	3.52	6.69	23.26
T_5	76.8	68.5	54.2	7.5	1.61	4.65	12.7	1.69	3.60	6.75	23.39
T_6	84.2	76.1	60.2	7.7	1.64	4.69	13.1	1.70	3.75	7.00	23.51
T_7	79.8	72.6	58.3	7.9	1.65	4.79	13.7	1.73	3.50	7.37	23.58
T_8	77.6	70.3	58.1	7.6	1.62	4.69	12.7	1.67	3.52	6.06	22.47
T_9	79.4	75.0	56.4	7.8	1.61	4.84	12.9	1.65	3.64	6.12	22.84
T_{10}	78.6	71.5	58.4	7.7	1.64	4.69	12.8	1.66	3.58	6.44	22.16
T_{11}	71.2	67.5	54.6	7.8	1.63	4.78	12.9	1.65	3.60	6.75	22.96
T_{12}	73.2	65.0	53.2	7.6	1.61	4.72	12.5	1.72	3.33	5.75	22.42
SEm (±)	1.55	1.18	0.75	0.20	0.03	0.15	0.10	0.04	0.02	0.15	0.11
$CD_{(0.05)}$	4.55	3.45	2.19	NS	NS	NS	0.28	NS	0.07	0.43	0.32

elongation ratio and breadth expansion ratio after cooking remained unattached by FYM and vermicompost application. Quality of the crop, being the varietal character (genetic), is also affected by crop and environment management including fertilizers and manures. The result of present findings with regard to quality of grains might be due to better soil environment including increased level of available nutrients (Table 3) which released nutrients for longer time resulting in enhanced level of N, P and K uptake (Table 4), thereby better growth and development with supply of two or more sources of organic nutrients.

Economics

Gross returns from Basmati rice as influenced by organic source of nutrients were in the order of T_7> T_6>T_1> T_5> T_{11}> T_4> T_2> T_{10}> T_9> T_8> T_3> T_{12} (Table 1), the highest being Rs. 76,504/ha. The net returns obtained were in order of T_7> T_6> T_5>T_{11}> T_1>T_{10}> T_2>T_4> T_9> T_3> T_8> T_{12}, the highest being Rs. 39,654/ ha and lowest being Rs. 27,986/ha. The highest gross returns and net returns in T_7 were due to more grain yield and less cost of cultivation in comparison to T_1. Similar trend was also observed for other organic source of nutrients. Benefit-cost ratio was maximum (2.09) in T_{12} followed by T_7 (2.07). This might be due to the lower cost of cultivation in T_{12} and more grain production in T_7. There were marginal reduction of benefit-cost ratios due to various organic nutrient management practices. The lowest benefit-cost ratio of 1.86 was estimated with T_1 which might be due to higher cost of production towards FYM and vermicompost. Similar results were also reported by Davari and Sharma (2010) who observed that application of FYM increased cost of cultivation by 34-38%, vermicompost by 61-71%, FYM + wheat residues (WR) by 50-60% and Vermicompost + WR + biofertilizer by 83-94%. But all the above organic manure treatments recorded higher net return over control. Singh *et al*. (2009) also reported that green manuring along with biofertilizers (Azospirillum + PSB + BGA) coupled with FYM (5 t/ha) or vermicompost (2.5 t/ha) recorded higher average net return and benefit-cost ratio followed by application of biofertilizers (Azospirillum + PSB + BGA) along with green manuring. Niru kumari *et al*. (2010) also observed that scented rice receiving dhaincha green manure @ 5 t/ ha + FYM @ 10 t/ha was found to be the most appropriate organic nutrient management system for higher productivity and profitability. Similar results were also reported by Barik *et al*. (2011).

Table 3. Soil fertility status after harvesting of organic Basmati rice as influenced by nutrient management

Treat-ment	Soil pH	Soil EC (dS/m)	OC (%)	Available nutrients (kg/ha) N	P_2O_5	K_2O
T_1	6.26	0.045	0.61	253.48	45.46	181.62
T_2	6.13	0.042	0.57	250.88	44.38	175.29
T_3	6.01	0.041	0.55	248.25	43.98	169.28
T_4	6.16	0.042	0.57	250.97	44.39	176.28
T_5	6.18	0.043	0.58	251.06	44.50	179.74
T_6	6.20	0.044	0.59	252.16	43.78	180.68
T_7	6.27	0.045	0.63	252.51	44.72	181.40
T_8	6.09	0.042	0.56	248.97	43.73	170.88
T_9	6.12	0.043	0.57	249.88	43.98	171.89
T_{10}	6.18	0.044	0.58	250.06	44.15	173.37
T_{11}	6.21	0.044	0.59	250.60	44.81	175.54
T_{12}	6.06	0.041	0.51	225.79	42.94	156.62
SEm (±)	0.04	0.00	0.02	2.55	0.98	1.33
$CD_{(0.05)}$	0.12	0.01	0.06	7.47	2.88	3.90
Initial	5.70	0.040	0.51	246.21	43.38	167.84

Table 4. Nutrient uptake at harvest of organic Basmati rice as influenced by nutrient management

Treat-ment	Nitrogen uptake (kg/ha)		Phosphorus uptake (kg/ha)		Potassium uptake (kg/ha)	
	Grain	Straw	Grain	Straw	Grain	Straw
T_1	38.51	26.91	8.39	3.45	12.52	76.11
T_2	32.65	24.07	7.64	3.04	11.21	76.90
T_3	26.88	23.45	6.83	2.80	10.02	76.30
T_4	33.28	22.39	7.81	2.72	10.95	70.41
T_5	34.99	25.11	8.06	3.09	11.53	78.02
T_6	37.07	27.85	8.57	3.61	11.95	85.58
T_7	42.13	30.62	9.38	3.25	13.53	85.87
T_8	27.26	23.00	7.19	2.99	10.26	76.59
T_9	28.62	24.31	7.38	3.32	10.92	80.29
T_{10}	31.21	24.49	7.51	2.86	10.97	78.47
T_{11}	33.69	22.75	7.86	2.85	11.76	72.37
T_{12}	22.82	18.70	5.65	2.11	8.56	65.14
SEm(±)	1.56	1.63	0.33	0.12	0.97	3.52
$CD_{(0.05)}$	4.57	4.77	0.97	0.35	2.85	10.32

Increasing cost of fertilizers, growing ecological concern and conservation of energy have created considerable interest for the use of organics as a source of plant nutrients. A combination of organic nutrients can be used for maintaining productivity and profitability of Basmati rice thus benefiting a larger section of farming community.

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Major nutritional differences among selected local, foreign and diabetic rice varieties consumed in South East Nigeria

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ABSTRACT

Price differences among different categories of rice consumed in Nigerian have necessitated the need to investigate some useful parameters that could prove their nutritional worth. Three each of Nigerian local varieties (Abaomege Kpurukpuru, Izzi 306 and Ikwo Adaigbo), foreign processed types (Sarina, Super Eagle and Mama Africa) and very expensive special imported types (assumed as diabetic rice) (Golden Penny, Brown Basmati and White Basmati) were analyzed for some important constituents including protein, amylose and vitamin A using standard methods. Brown Basmati, Izzi 306, Ikwo Adaigbo and Mama Africa had comparatively high protein contents with the mean values of 9.13 ± 0.014, 8.80 ± 0.008, 8.62 ± 0.049 and 8.49 ± 0.007, respectively. Incidentally, the three assumed diabetic rice types showed extraordinarily high percentage amylose contents (28.7 ±0.141 to 31.7± 9.141) relative to others. On the other hand, only Ikwo Adaigbo showed a presence of vitamin A with mean value 0.015 ± 0.000 (µg/g). In the same vein, significant variations (p <0.05) were also observed between the varieties in other important components. The inexplicably high amylose contents in the assumed diabetic rice varieties and the presence of Vitamin A in Ikwo Adaigbo were the major distinguishing factors between the rice types.

Key words: *Rice, amylose content, Vitamin A, proximate compositions, minerals*

Rice (*Oryza sativa* L.) grain plays a very important dietary role in the nutrition of human beings amongst the cereal crops. It ranks 2nd after wheat and maize (Akintola 1998) and constitutes the most important staple food for about half of the world's population (Thakur and Gupta 2006; Osaretin and Abosede 2007). Rice plays a very important role in the diets of many people in developing countries where it provides 27% of the dietary energy supply and 20% of dietary protein intake. Rice is an excellent source of complex carbohydrates, protein and minerals (Yadav and Jundal 2007). In addition to varietal differences, protein content of rice is affected by environmental conditions, such as soil and nitrogenous fertilizer application. Protein is mainly distributed in the bran and periphery of endosperm. The central part of rice grain contains only a small proportion of rice's protein. Rice is unique in the richness of alkali-soluble proteins or glutellin (about 70 %), whereas other cereals are rich in alcohol- soluble proteins, include 4-9 % water soluble proteins or albumin and 10 % salt soluble protein or globulins (Sanni *et al*. 2005). According to USA Rice Federation (2002), rice contains 7.1 - 8.3 % protein for brown rice, 6.3-7.1 % for milled rice, 11.3-14.9 % for rice bran, 14.1-20.6 % for rice embryo.

More than 90% of the energy in rice comes from carbohydrate. Rice contains both simple and complex carbohydrates. Simple carbohydrates in rice are starch and fiber. About 85 % of the rice grain weight is starch. Amylose is the linear fraction of the rice starch, and is negatively correlated with the cohesiveness, tenderness, colour and the gloss of the cooked rice.

Acceding to the USDA nutrient database, rice contains about 70 g carbohydrates/100 g. Also a report on USA Rice Federation (2002) revealed that rice contains 67.9 -75.5 % for brown rice and 76.7-78.4 % for milled rice. Eggum *et al*. (1982) as supported by Ibukun, (2008) stated that milled rice contains 0.7-25% fat. Rice is considered as a health food because it contains linoleic acid but not cholesterol. The moisture content of rice is not constant, but varies as ambient changes. Rice under storage condition with high moisture content in dry condition creates the problem of losing weight due to loss of moisture. Other proximate contents such as crude fiber, already stated in the literature as component of carbohydrate though indigestible made up of 0.6-10 % in brown rice, 0.2-0.5 % in milled rice, 7.0-11.4 % in rice bran and 2.3-3.2 % in polished rice. For ash content, brown rice contains 1.0-1.5 %, milled rice contains 0.3-0.5% while in polished rice is 5.2-7.3 % (USA Rice Federation 2002).

Rice contains several types of vitamins in trace amounts including vitamin E that protects vitamin A and essential fatty acid from oxidation. Recently, scientist in Switzerland and Germany developed "Golden Rice" through genetic engineering and substantially increased vitamin A content in rice due to its importance. In terms of mineral compositions, potassium (K) is the most abundant mineral found in rice (brown, parboiled brown, milled and parboiled milled rice) followed by magnesium (Mg) and calcium (Ca). Among microelements, the presence of copper (Cu), iron (Fe), molybdenum (Mo), manganese (Mn), sodium (Na) and Zinc (Zn) in rice is outstanding. Rice mainly consists of starch made up of two ingredients, amylose which is a linear combination of glucose by means of -1, 4 linkage and amylopectin that has a cluster (fan-shaped) of -1, 6 linkage branching out from the linear chain of -1, 4 linkage. Rice is divided into non-glutinous and glutinous rice based upon its starch characteristics. Glutinous rice consists solely of amylopectin, while non-glutinous rice comprises both amylose and amylopectin. In general, rice with high amylose content is not sticky when cooked, while rice with low amylose content becomes soft and sticky when cooked.

There is a structural increase in rice consumption in the world and this increase would continue in the nearest future. Rice bran is a valuable commodity in Asia and is used for many daily needs. It is a moist, oily outer layer which produces oil upon heating (Dutta *et al*. 1998). Raw rice may be ground into flour for many uses, including making many kinds of beverages, such as amazake, harchata, rice milk and rice wine. Rice may also be made into various types of noodles. Raw, wild or brown rice may also be consumed by raw-foodists or fruitarians if soaked and sprouted (usually a week to 30 days- gaba rice) (Wasserman and Calderwood 1972). Processed rice seeds may be boiled or steamed before eating. Boiled rice may be further fried in cooking oil or butter (known as fried rice) or beaten in a tub to make mochi.

Rice is grown in all the ecological zones of Nigeria with different varieties, processing and adaptation traits for each ecology (Sanni *et al*. 2005). Ebonyi State is a major rice producer in Nigeria and rice production in the state has witnessed a spectacular increase in the recent time. The ideal vegetation for rice production in Ebonyi State has given rise to different varieties that have adapted to specific local environment, and these varieties bear names reflecting the towns in which they are grown (Alaka and Okaka 2011). Rice is an economic crop which is important in household food security, ceremonies, nutritional diversification, income generation and employment (Perez *et al*. 1987). It is utilized mostly at the household level where it is consumed as boiled, fried or ground rice with stew or soup (Osaretin and Abosede 2007).

Consumer demand for good quality rice is high resulting in high patronage for imported rice types. Since rice production is the major occupation of most farmers in Ebonyi State and to ensure that locally processed rice varieties remain vital and relevant to rural economy and agricultural production, there is need to evaluate their quality so as to compare them with their imported counterparts, hence the decision to compare the proximate, mineral compositions, amylose and vitamin A contents of some selected local, foreign and diabetic rice varieties consumed in South Eastern Nigeria.

MATERIALS AND METHODS

Sample collection

A total of nine rice samples; three each of local varieties (Abaomege Kpurukpuru Izzi 306 and Ikwo Adaigbo), foreign varieties (Sarina, Super Eagle and Mama

Africa) and diabetic rice varieties (Golden Penny, brown Basmati and White Basmati) were bought from Ebonyi State, Enugu State and their environs (all in South Eastern Nigeria).

Analytical method

The biochemical analysis was carried out at the Global Technology Institute (GTI) in Akwa Ibom State of Nigeria and the Biochemistry laboratory of the National Rice Research Institute (NRRI), Cuttack, India. The rice varieties were ground with a plate mill, and dried in a hot air oven at 400C for 12 hours to reduce the moisture content up to 14%. The ground samples were used for biochemical analysis.

Estimation of crude protein, fat and ash

The crude protein (N x 5.95), fat and Ash for all rice varieties were determined using approved methods 46-11A, 30-10 and 08-01 (AACC, 2000).

Minerals composition measurement

The minerals composition were determined using AAS model 305B (Osaretin and Abosede 2007). The base line of the instrument was set to zero with the Boerhinger commercial control as per manufacturer's instruction. Rice grain (50g) was taken with moisture content 12-13% and dehulled and milled (10%). From milled sample, 2g flour was taken in 100 ml volumetric flask and 15ml of conc. HNO_3 was added. The mixture was kept overnight. Next day, 2ml of $HClO_4$ was added and heated at 60ºC till brown fumes of HNO_3 stops. Then sample was allowed to digest at 90ºC till white fumes come out. The mixture was cooled down and made volume up to 50ml using double distilled water. Then filtrate was obtained through Whatman No. 41 filter paper into 125ml bottles for taking readings in AAS.

Estimation of vitamin A

The estimation of beta carotene was followed as described by Santra *et al*. (2006) with some modifications. Ten gram flour was taken in 250 ml volumetric flask and volume made up to 100 ml with water saturated n-butanol (WSB). For complete extraction of β-carotene, the contents of the flasks were mixed vigorously for 5 min. and kept overnight (16-18 hrs) at room temperature under dark condition. Next day, the contents were shaken again for 10 min. and filtered completely through the Whatman No.1 filter paper into a 100 ml volumetric flask. The absorbance was measured at 440 nm and the calibration curve was established. Pure WSB was used as blank during measurement. The β-carotene content was calculated from calibration curve from known amount of β-carotene as discussed below and expressed as parts per million (µg/g). For making standard solution of β-carotene (Sigma), WSB was used to make the concentration of 5 **µ**g/ml (WSB was prepared by mixing n-butanol with distilled water at ratio 8:2). With the proper dilution of the standard solution in 10 ml volumetric flasks the calibration curve was prepared. The absorbance of each dilution was measured and the calibration curve was established. Content of β-carotene of unknown samples was calculated from standard curve expressed as µg/g of sample flour.

Amylose measurement methods

Amylose and amylopectin contents were estimated by using amylose/amylopectin assay kit Megazyme kit (Megazyme Ireland International, Ltd., Bray Ireland) followed according to the manufacturer's recommendation. Exactly 20 mg rice flour sample was taken into a 10 ml falcon tube and dispersed by heating with 1 ml dimethyl sulphoxide (DMSO) and lipids were removed by precipitating the starch in ethanol (6 ml). Precipitated starch of the sample was dissolved in an acetate/salt solution where amylopectin is precipitated by the addition of concanavalin A (4 ml) followed by centrifugation. The amylose in the supernatant was then enzymetically hydrolysed to D-glucose and analyzed using glucose oxidase/peroxidase reagent. The total starch was also measured in a separate aliquot of the acetate/salt solution using same treatment. The concentration of amylose in the starch sample was estimated as the ratio of GOPOD absorbance at 510 nm of the supernatant of the concanavalin A precipitated sample to that of the total starch sample.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) according to (Snedecor and Cochran 1969) to detect any difference in mean values from triplicate runs of each treatment.

RESULTS AND DISCUSSION

Carbohydrate was the highest % proximate component as usual among selected rice varieties (Table 1). White

Table 1. Proximate composition of the nine rice samples (%)

Samples	Moisture	Crude protein	Crude fiber	Ash	Fat	Carbohydrate
Abaomege Kpurukpuru	13.30±0.141[b]	7.95±0.042[g]	1.32±0.014[c]	0.81±0.014[i]	2.21±0.021[b]	74.41±0.092[e]
Izzi 306	13.15±0.071[b]	8.80±0.008[f]	1.50±0.021[f]	1.04±0.056[h]	2.10±0.028[cd]	73.41±0.021[e]
Ikwo Adaigbo	14.10±0.141[a]	8.62±0.049[b]	1.62±0.007[b]	1.38±0.007[c]	2.48±0.014[a]	71.80±0.177[f]
Sarina	13.20±0.282[c]	8.14±0.056[e]	1.61±0.006[d]	1.42±0.021[f]	2.08+0.008[d]	73.55±0.346[d]
Super Eagle	13.45±0.071[e]	8.25±0.014[d]	1.60±0.008[e]	1.22±0.007[g]	2.13±0.014[c]	73.35±0.049[a]
Mama Africa	13.60±0.006[d]	8.49±0.007[c]	1.63±0.014[c]	1.58±0.007[e]	2.03±0.014[e]	72.67±0.014[d]
Golden penny	13.65±0.071[e]	8.24±0.008[d]	1.68±0.006[b]	1.07±0.014[d]	2.11±0.014[cd]	73.25±0.099[c]
Brown Basmati	13.20±0.007[f]	9.13±0.014[a]	1.56±0.007[a]	1.80±0.008[a]	2.42±0.028[e]	71.89±0.042[e]
White Basmati	12.60±0.005[g]	8.24±0.028[c]	1.01±0.014[d]	0.72±0.014[b]	2.02±0.006[e]	75.41±0.014[b]

Means with different superscripts are statistically significant difference ($p < 0.05$)

basmati had the highest % of carbohydrate followed by Abaomege Kpurukpuru, Sarina, Izzi 306 and Super Eagle with mean values of 75.41 ± 0.014, 74.41 ± 0.092, 73.55 ± 0.346, 73.41 ± 0.021 and 73.35 ± 0.049, respectively. Ikwo Adaigbo had the least carbohydrate content with mean values of 71.80 ± 0.177. Brown Basmati had the highest ash content with a mean value of 1.8 ± 0.008. Ash content is taken to be the measure for mineral composition of a sample. Abaomege Kpurupuru had the least protein content with a mean value of 7.95 ± 0.042 while Brown Basmati, Izzi 306 and Ikwo Adaigbo had the highest protein contents with mean values 9.13 ± 0.014, 8.80 ± 0.008 and 8.62 ± 0.049, respectively.

The results of the mean amylose content showed that Abaomege Kpurukpuru had the least amylose content (%) and the highest amylopectin content (%) with mean value of 18.4 ± 0.283 and 81.60 ± 0.283, respectively (Table 2). Brown Basmati and Golden Penny had the highest mean amylose content with values of 31.70 ± 9.141 and 30.2 ± 0.636, respectively. The results of the mineral composition (Table 3) shows that Golden Penny had the highest potassium content (mg/kg) while Mama Africa had the highest magnesium content with mean values of 486.32 ± 0.000 and 170.14 ± 0.000, respectively. Brown Basmati had the highest iron content (mg/kg) with the mean value of 18.60 ± 0.007 followed by Golden Penny with the mean value of 16.44 ± 0.000. Among the rice varieties, Brown Basmati had the highest zinc content (mg/kg) with the mean value of 36.3 ± 0.141. Ikwo Adigbo was the only variety that showed a quantifiable value for vitamin A with mean value of 0.015±000a (Table 4).

Table 2. Percentage amylose and amylopectin contents of the nine rice samples (%)

Sample	**Amylose**	**Amylopectin**
Abaomege Kpurukpuru	18.40±0.283[g]	81.60±0.283[a]
Izzi 306	25.60±1.131[d]	74.40±1.31[d]
Ikwo Adaigbo	20.65±0.071[f]	79.35±0.071[b]
Sarina	20.15±0.071[f]	79.85±0.71[b]
Super Eagle	24.50±9.141[e]	75.50±0.141[c]
Mama Africa	27.70±0.141[c]	72.30±0.141[e]
Golden Penny	30.20±0.636[b]	69.80±0.636[f]
Brown Basmati	31.70±9.141[a]	68.30±0.141[g]
White Basmati	28.70±0.141[a]	71.30±0.141[g]

Means with different superscripts are statistically significant difference ($p < 0.05$)

The results for the nine rice varieties characterized for their proximate composition, amylose content, mineral and vitamin A contents show some marked differences in the inter groups as well as similarities in the intra groups varieties. For moisture content, all the values generated for each of the varieties are almost similar and below 14% optimal values for bag storage of grains (Juliano and Villareal 1993), except Ikwo Adigbo that had a value of 14.1%. Low moisture content is known to enhance keeping quality of rice under storage.

The fat contents (%) did not vary significantly ($p<0.05$) with the different varieties except Ikwo Adigbo that had a high value of 2.48 ±0.014. The results were much higher than the values of 1.10-1.50% for some milled rice varieties earlier reported by (Juliano and Villareal 1993). Since fat is more on the bran layer, the more this layer is removed during milling, the less the fat content of the milled rice (Okaka 2005). This could be the reason for the high fat content of Ikwo Adigbo. Higher fat content exposes the grains to spoilage during storage due to oxidation. For the nine rice varieties studied, Brown Basmati had the highest percentage protein content followed by Izzi 306, Ikwo Adaigbo and Mama Africa. All the varieties studied contained sufficient amount of protein which are above the reported values of 7% (Dipti *et al*. 2002; Dutta *et al*.

Table 3. Mineral composition of the nine rice samples (mg/kg)

Sample	Potassium	Magnesium	Sodium	Calcium	Iron	Zinc
Abaomege Kpurukpuru	278.16±0.014[c]	156.55±0.007[cd]	56.13±0.00[b]	73.5±0.007[g]	8.98±0.000[g]	21.98±0.007
Izzi 306	276.21±0.000[c]	154.125±0.007[d]	56.12±0.000[b]	78±0.000[f]	7.955±0.007[h]	24.2±0.000[b]
Ikwo Adaigbo	374.20±0.000[cd]	162.15±0.007[c]	58.14±0.000[b]	66±0.028[e]	12.18±0.000[f]	24.2±0.000[b]
Sarina	368.19±0.028[e]	158.14±0.000[cd]	55.13±0.000[b]	75.5±0.007[b]	13.24±0.000[e]	26.3±0.000[ab]
Super Eagle	382.95±0.000[b]	160.165±0.007[cd]	56.12±0.000[b]	67.09±0.014[d]	13.24±0.000[e]	24.2±0.000[b]
Mama Africa	367.90±0.007[d]	170.14±0.000[c]	60.14±0.028[b]	72.43±0.028[bc]	14.27±0.014[d]	20.5±0.141[a]
Golden Penny	486.32±0.000[a]	166.17±0.085[a]	55.13±0.014[b]	71.5±0.007[c]	16.44±0.000	20.5±0.141[a]
Brown Basmati	476.32±0.000[a]	164.12±0.000[a]	55.13±0.014[b]	91.05±0.014[a]	18.605±0.007[a]	36.3±0.141[ab]
White Basmati	428.34±0.000[a]	112.05±0.014[b]	63.17±0.014[a]	75.32±0.014[bc]	7.58±0.000[b]	28.5±0.141[a]

Means with different superscripts are statistically significant difference (p <0.05)

1998). Protein helps in body growth; repairing and maintaining of body tissues. Prolonged parboiling and other environment/edaphic factors lower the protein content of rice. Brown Basmati had the highest value for protein. Crude fiber reduces the risk of bowel disorders and fights constipation. All the studied varieties showed high ash content which did not differ significantly (P<0.05) from each other and were within the acceptable ash value of 0.50-2.50 (%) reported by Edeogu *et al*. (2007). Ash residual generally taken to be a measure of the mineral content in milled rice is an indication of a good quantity of mineral content in the rice sample (Dipti *et al*. 2003).

Amylose content of rice is considered to be one of the most important factors influencing the cooking and processing characteristics of rice (Delwiche *et al*. 1995). The percentage amylose content of the assumed three diabetic rice; Brown Basmati, white Basmati and Golden Penny were high for natural rice (Oko *et al*. 2012; Panlasigui *et al*. 1991) and could be suspected to have been artificially constructed/reconstituted to meet this standard. Although Pete Vegas (Sage V Foods) in an online article claimed that Basmati rice (from India and Pakistan) has high amylose content and a firm dry texture when properly cooked, he failed to give the range of the percentage amylose to serve as a guide. Takeda *et al*. (1987) had calculated true amylose content of rice to be in the range of 15-19% for non-waxy rice. Rice varieties with a greater proportion of starch in the form of amylose tend to have a lower glycemic index. Amylose content of milled rice are said to correlate positively with hardness values of cooked rice and negatively with stickiness values (Perez and Juliano 1987). Low amylose levels are associated with cohesive, tender, and glossy cooked rice. On the other hand, high levels of amylose cause rice to absorb more water and consequently expand more during cooking, and the grains tend to cook dry, fluffy, and separate (Juliano 1971). Rice starch with high amylose starch show higher degree of retrogradation and lower increase in consistency index, shear stress and plastic viscosities than rice starch of lower amylose content (Tukomane and Varavinit 2008). Denardin *et al*. (2012) reported that high amylose feeds results in longer satiation, gain in body weight and apparent increases in digestibility, fecal water content and nitrogen excretion, reduced fecal pH, lower postprandial blood glucose response, serum total cholesterol, triglycerides levels, pancreas weight, and higher fasting serum glucose concentration as well as increased liver weight. Panlasigui *et al*. (1991) reported that digestibilities and glycemic responses are significantly different among rice varieties with similar amylose concentrations, arguing that amylose and amylopectin ratio is not the sole determining factor in rate of starch digestion and postprandial glycemic responses. However, the report of Fitzgerald *et al*. (2011) which stated that amylose was the only grain constituent that affected glycemic index, might be the basis on which the rice with high amylose contents in our report are used as diabetic specialty foods.

Table 4. Vitamin A (β -carotene) composition of the nine rice samples (µg)

Sample	Vitamin A (β -carotene)
Abaomege Kpurukpuru	0±- [b]
Izzi 306	0±-[b]
Ikwo Adaigbo	0.015±000[a]
Sarina	0± - [b]
Super Eagle	0± - [b]
Mama Africa	0± - [b]
Golden Penny	0± - [b]
Brown Basmati	0± - [b]
White Basmati	0±- [b]

Means with different superscripts are statistically significant difference (p <0.05)

Micronutrient deficiencies in the rice are caused mainly due to low dietary intake of iron, vitamin A and zinc. Among vitamins, vitamin A deficiency (VAD) is prevalent among the people whose diets are based mainly on rice. Rice does not contain any β-carotene (apart from the golden rice), which the human body could convert into vitamin A. Major rice consuming population in the world therefore, having threat of VAD which affecting small children and pregnant women. Vitamin A can be obtained from food, either as preformed vitamin A in animal products or as provitamin A carotenoids, mainly β-carotene in plant products (*e.g*., dark-green leafy vegetables and fruit). The intake of vitamin A provides humans with an important nutrient for vision, growth, reproduction, cellular differentiation and proliferation, and integrity of the immune system. Therefore, the presence of β-carotene (vitamin A) in Ikwo Adaigbo is a very desirable trait which could be improved for better rice nutrition in the area.

The mineral contents of the varieties were within range expected (Shabbir *et al*. 2008). The zinc content of all the tested varieties were found satisfactory, as it was within the range reported by Kennedy *et al*. (1975). The calcium content was high when compared to the range reported by Kennedy *et al*. (1975). Presence of calcium in rice is a clear indication that when taken, will aid normal development and maintenance of bones and teeth, clotting of the blood and nerve irritability in the blood.

In conclusion, the values for percentage amylose recorded for the three so-called diabetic rice types does not present them as natural rice types as no literature supports such value of amylose for any cereal crop. On the other, the presence of vitamin A (β-carotene) in Ikwo Adaigbo is a desirable trait that could be exploited by both breeders and plant biotechnologists to develop an alternative to the called Golden rice.

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Characterization of red and purple-pericarp rice (*Oryza sativa* L.) based on physico-chemical and antioxidative properties of grains

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ABSTRACT

In the preset study range of variations in physico-chemical, cooking characteristics and antioxidant properties of six pigmented rice (four purple and two red) cultivars from north east India were evaluated. Significant variation (P<0.05) was detected among the cultivars for all the traits evaluated except for volume expansion ratio (VER). All the genotypes had long slender grain. Hulling and milling % for all the genotypes were more than 74 and 61%, respectively. Head rice recovery (HRR) was more than 50% in Manipuriblack, Kalobhat and Assambiroin. The range of amylose content (AC) varied from 2.19 to 24.87% where as Mornodoiga was found with highest AC. All the genotypes except Manipuriblack elongated more than 9mm after cooking. Most of the genotypes were found with soft gel consistency (GC). Similarly, all the genotypes except Assambiroin had water uptake (WU) value ≤ 100ml/100g rice. The concentration of total anthocyanin content (TAC), total phenolic content (TPC) and antioxidant activity (ABTS) differed significantly among the genotypes with highest concentration of these parameters were observed for the purple grain (Mamihunger) whereas no significant difference between the colour groups (red and purple) was observed for total flavonoid content (TFC), gamma-oryzanol and phytic acid content which envisages that value of these parameters depends on genotypes and not on kernel colour. A high correlation of TAC with TPC and ABTS suggest that the major phytochemicals responsible for the tested antioxidant activities are phenolic acids and anthocyanin.

Key words: *Pigmented rice, antioxidants, grain quality, physico-chemical characteristics*

Rice (*Oryza sativa* L.) is consumed as a staple food by more than half of the world's population with approximately 95% of production in Asia (Bhattacharjee *et al*. 2002). It is the only cereal crop cooked and consumed mainly as whole grains, and quality considerations are much more important than for any other food crops (Hossain *et al*. 2009). Grain quality has always been an important consideration in rice variety selection and development. It is the primary determinant for market place and consumer acceptability. The Kernel appearance, size, shape, nutritional value and cooking characteristics are important for judging the quality and preference of rice from one group of consumer to another (Kanchana *et al*. 2012). In particular, the cooking and eating qualities are very important determinants of cooked rice grain quality (Ge *et al*. 2005). The content of amylose in rice is considered the principal determinant of rice quality. However, rice varieties with similar amylose content have shown to possess different rice characteristics on cooking which indicated that secondary differences exist among varieties with similar amylose content.

Most of the rice crops grown and consumed throughout the world have the white pericarp but there are many special cultivars of rice known as pigmented rice characterized by red, black and purple pericarp. This pigmentation depends on the kinds of deposition of phenolic compounds such as anthocyanin and proanthocyanidin in the aleurone layer of the grain

(Finocchiaro *et al*. 2010; Pereira- Caro *et al*. 2013a, 2013b). Great interest has been shown in the polyphenols in rice for their multiple biological activities. These phenolic compounds include ferulic acid and diferulates, anthocyanins, anthocyanidins and polymeric proanthocyanidins (condensed tannins) (Chun *et al*. 2005). Phenolics have free radical scavanging activity which protect cell against oxidative damage.

Free radicals have been claimed to play an important role in affecting human health by causing many diseases (*e.g.*, heart diseases, cancer, hypertension, diabetes and atherosclerosis). In the past decade, antioxidants have shown their relevance in the prevention of various diseases, in which free radicals are implicated. This coloured rice are known source of antioxidant compounds including flavonoid, anthocyanin, phytic acid, proanthocyanidin, tocopherols, tocotrienols, γ-oryzanol, and phenolic compounds (Butsat and Siriamornpun 2010; Goufo and Trindade 2014) which can decrease oxidative stress in vivo, highly effective in reducing cholesterol levels in the human body and exert beneficial effects on human health (Santos-Buelga and Scalbert 2000; Ghie and Walton 2007; Lee *et al*. 2008). Major anthocyanins such as peonidin, peonidin 3-glucoside and cyanidin 3-glucoside extracted from black rice, also reported to exert an inhibitory effect of cell invasion on various cancer cells (Chen *et al*. 2006).

Considering health protecting and promoting effect of pigmented rice, the objective of this study was to compare physico-chemical, cooking properties and antioxidant potentials of the six pigmented rice cultivars native to the North-east India *viz*., Mamihunger, Manipuri black, Chakhao, Kalobhat, Mornodoiga and Assambiroin.

MATERIALS AND METHODS

Pigmented paddy varieties were collected from the sub-station of NRRI, Gerua, Assam and multiplied in the NRRI experimental field, Cuttack, India. All the paddy samples were from the recent harvest of *Kharif*, 2015. The agronomic data were collected from the field time to time. The paddy samples were sun-dried (moisture up to 12-13%) and cleaned for foreign materials, packed in polyethylene bags and kept inside cloth bags, and stored at 4°C. The samples were dehulled through laboratory rice huller, Satake, Japan make. The kernels were ground by a grinding machine (Glen mini grinder) and sheaved through 100 mesh size and then stored at 4°C for further experiments.

Anthocyanin content of rice samples was measured in UV-V is spectrophotometrically according to Swain and Hillis (1959) with alcoholic extract.

Gamma-oryzanol content (GOC) was determined with RP-HPLC. γ-oryzanols extraction by HPLC was performed according to Chen *et al*. (2005) with simplification. Briefly, 0.5 grams of samples (Brown rice flour) were mixed with 5 ml of HPLC-grade isopropanol, vortexed for 2 min at 25°C, centrifuged at 4500 g for 10 min and the supernatant was collected. After 2-3 times repetition, supernatant fractions were evaporated under hot water bath and then extracts were dissolved in 5 ml of HPLC-grade isopropanol. After filtration through a 0.45 μm membrane, 20 μl aliquots were injected into the column (C18- Phenomenex Column). It was separated by an analytical Shimadzu High Performance Liquid Chromatography (RP-HPLC) system equipped with an LC-20AT pump and PDA detector (Shimadzu, Kyoto, Japan). The composition of the mobile phase was 35% acetonitrile, 55% methanol and 10% isopropanol and operated in low pressure gradient mode.

Total phenolic content (TPC) was determined by modified protocol of Zilic *et al*. 2011. About 0.3 g of brown rice flour sample and 10 ml 70% acetone were mixed thoroughly in a centrifuge tube at room temperature. After centrifugation for 20 minutes at 15000g, aliquots (0.2 ml) of aqueous acetone extracts were transferred into test tubes and their volumes made up to 0.5 ml with distilled water. After addition of the Folin-Ciocalteu reagent (0.25 ml) and 20% aqueous sodium carbonate solution (1.25 ml), tubes were vortexed. After 40 min, the absorbance was recorded at 725 nm against a reagent blank. The total phenolic content of each sample was determined by means of a calibration curve prepared using catechol and expressed as mg catechol equivalents (CE) per gram of brown rice flour.

Total flavonoid content was determined according to Eberhardt *et al*. (2000). One gram rice grain was extracted in 10 ml of 40% (v/v) ethanol for 30 min at room temperature. The supernatant, after centrifugation for 20 min at 15,000g was used in experiments. Briefly, 0.075 ml of 5% $NaNO_2$ was mixed with 0.5 ml of the sample (ethanolic extract diluted with 1 ml of water). After 6 min, 0.15 ml of a 10% $AlCl_3$ solution was added, and the mixture was allowed to stand for another 5 min. Then, 0.5 ml of 1 M NaOH was added, and the volume was made up to 2.5 ml with distilled water. The absorbance was measured at 510 nm immediately after mixing, against the blank containing the extraction solvent instead of a sample. The results are expressed as mg CE (catechine equivalent) per 100g of dry matter.

ABTS radical scavenging was assayed by modified protocol of Serpen *et al*. 2008. In this methodology, both soluble and insoluble fraction of antioxidant compounds come into contact with the ABTS radical. The ABTS+ reagent was prepared by reacting a 7 mmol/l aqueous solution of ABTS with 2.45 mmol l^{-1} potassium persulfate and further dissolved in the mixture of ethanol: water (50:50, v/v) so that 1: 5: 28 (V/V) ratio of ABTS, potassium persulphate and ethanol-water was maintained and final OD at 734 nm will be 0.70 + .02. Six milliliters of ABTS+ reagent was added to 10 mg of brown rice flour and the mixture was vortexed for 1.5 min to perform the surface reaction. Following centrifugation at 9200 g for 2 min, the absorbance of the optically clear supernatant was measured at 734 nm exactly 30 min and 60 min of sample mixing with the ABTS reagent. The antioxidant capacity was expressed as percent inhibition.

Phytic acid content was determined by modified protocol of Gao *et al*. 2007. Extraction of PA was done by taking 1g brown rice flour sample in 10ml 2.4% HCl in the 100ml conical flask and the flasks containing the samples were shaken at 220 rpm for 16h in an incubator Shaker at 50°C (Rivotek, India) and centrifuged at 10,062 g in a table-top centrifuge (Remi, India) at 25°C for 20 min. The supernatant was then collected and after adding 1g NaCl, it was shaken for 20 min at 350 rpm. After keeping for 20 min at -20°C, these tubes were again centrifuged at 3000 g for 20 min. After 25 times dilution of supernatant with double distilled water, 3 ml sample and 1 ml wade reagent (0.03% $FeCl_3$, $6H_20$+0.3% Sulpho-salicylic acid) were mixed thoroughly by vortexing and after keeping it for 1 hour OD was measured in 500nm. Standard curve was prepared by sodium phytate so that the blank OD will be 0.453+ 0.002. PA concentration was determined by using the following formula: PA% = {(0.463-OD) x 25 V} / (22.05 x M). Here OD is absorbance; V=final volume (ml); M=weight of sample (g).

For physical traits, 100 g of rice seeds were de-hulled and milled using a standard de-husker and miller, respectively and the milling, head recovery ratio (HRR), kernel length (KL), kernel breadth (KB) and length/breadth (L/B) were calculated. For the gel consistency (GC), 100 mg of rice flour was taken in test tube (13 x100 mm), 0.2 ml of ethanol containing 0.03% thymol blue and 2.0 ml of 0.2 N of KOH were added and kept in boiling water-bath for 8 min, cooled, mixed well and kept in ice bath for 20 min. Later, the test tubes were laid horizontally on the flat base, graph paper for one hour and length of gel spreading of those tubes were measured (mm).

For chemical traits, six number milled rice grains were taken in petriplates and 10 ml of 1.7% of KOH was added and kept in an incubator at 27-30 °C for 23 hours to measure alkali spreading value (ASV). Whereas, the amylose content (AC) was measured using a spectrophotometer (Thermo spectronic USA) as per Juliano *et al*. (2009).

Cooking properties were determined by taking 15 ml of water in 50 ml graduated centrifuge tubes and 5 g of rice samples were added in it. Rice samples were cooked for 20 min in a water bath. Length of ten cooked rice kernels was measured using graph paper for computing the kernel length after cooking (KLAC). Volume expansion ratio and elongation ratio were calculated after cooking as per established methods.

Statistical analysis

All experiments were carried out in triplicates and presented as mean±standard deviation of mean using SAS version 9.2. The data were statistically analysed by Duncan's multiple range tests at 5% significance level.

RESULTS AND DISCUSSIONS

Wide variation in the physico-chemical and cooking properties were observed in the pigmented rice (Table 1). The hulling percentage was more than 74% in all the pigmented rice with higher value (78%) observed

Table 1. Physico-chemical and cooking characteristics of pigmented rice.

	HUL(%)**	MIL(%)**	HRR(%)**	L/B**	AC(%)**	ASV*	KLA**	VER**	GC**	WU**
Mamihunger (purple grain)	^{A}78.00±2.0	^{B}69.67±1.50	^{D}49.50±1.0	^{B}4.22±0.04	^{B}15.60 ±0.20	^{A}6.00	^{B}9.27±0.11	4.00±0.50	^{D}43.00±1.0	^{E}104.67±0.57
Manipuriblack (purple grain)	^{B}75.00±2.0	^{C}61.33±1.15	^{A}59.50±1.00	^{C}3.30±0.15	^{F}2.19±0.10	^{A}6.00	^{C}8.47±0.12	3.75±0.05	^{A}75.00±1.0	^{B}195.33±0.55
Chakhao (purple grain)	^{B}74.50±1.0	^{C}62.50±1.00	^{D}48.83±0.57	^{A}4.36±0.10	^{D}5.84 ±0.05	^{B}5.00	^{AB}9.67±0.28	3.77±0.07	^{A}75.00±1.0	^{C}134.67±0.57
Kalobhat (purple grain)	^{B}75.00±2.0	^{A}73.00±1.32	^{B}56.83±1.15	^{D}3.15±0.05	^{E}4.57±0.11	^{B}5.00	^{AB}9.50±0.50	3.75±0.25	^{B}65.33±0.57	^{D}110.50±0.51
Mornodoiga (red grain)	^{B}75.33±0.57	^{C}61.83±1.15	^{E}35.50±1.0	^{D}3.04±0.07	^{A}24.87± 0.57	^{D}3.00	^{B}9.33±0.26	3.75±0.25	^{B}66.33±1.15	^{F}82.33±0.28
Assambiroin (red grain)	^{B}74.50±1.0	^{C}62.50±1.00	^{C}55.00±1.0	^{D}3.08±0.06	^{C}11.13±0.33	^{C}4.00	^{A}9.87±0.30	3.75±0.25	^{C}48.33±1.15	^{A}332.50±0.50

Note- Values are presented as mean ± Standard daviation (n=3), Mean with different letters (A-F) within the same column are significantly different ($P < 0.05$), * Standard daviation in ASV is zero, ** no significance difference among genotypes for VER, ASV-alkali spreading value, AC-amylose content(%), GC-gel consistency in mm, HRR=Head rice recovery(%), HUL-hulling(%), KLA-kernel length after cooking, L/B-length/breadth ratio, MIL-Milling(%), VER-volume

in Mamihunger. The milling percentage was in the range of 61.33 (Manipuri black) to 73.00 (Kalobhat). Head rice recovery, one of the major criteria that determine the grain quality varied from 35.50 (Mornodoiga) to 59.50% (Manipuri black). This important grain quality varies depending on variety, grain type, cultural practices and post harvest conditions (Razavi and Farahmandfar 2008; Emadzade *et al*. 2009). HRR of more than 55% was found in Manipuri black, Kalobhat and Assambiroin. In this study, HRR was found positively correlated (r=0.819;Table 3) with ASV, L/B ratio or axial ratio which provide information about the grain type. All the pigmented rice were found to have long slender grain as the axial ratio was more than 3.0 and varied from 3.04 to 4.36.

Cooking quality of rice mainly depends on amylose content and gelatinization temperature. Amylose content (AC), an important grain quality character which determines the texture of cooked rice varied from 2.19 to 24.87%. Manipuriblack was nonwaxy type (>2%), very low amylose content was observed in Chakhao and Kalobhat (within 2- 9%) and low amylase content (within 10-20%) was observed in Mamihunger and Assambiroin where as Mornodoiga had moderate level (within 20-25%) and preferred amylose content. Cultivar with high amylose level are associated with lower blood glucose level and slower emptying of the human gastrointestinal tract compared to those with low levels of this macromolecule (Frei and Becker 2003). In this study, high significant negative correlation of AC with ASV (-0.961) and HRR (- 0.887) was observed (Table 3).

The alkali spreading value was in the range of 3-6 for all the genotypes. Mornodoiga had high intermediate ASV (3) and Assambiroin, chakhao and kalobhat had intermediate ASV (4-5) where as Mamihunger and Manipuri black had low ASV (6). It signifies that the intermediate group requires high gelatinization temperature (70-74^{0}C) and low gelatinization temperature (<70^{0}C) is required for mamihunger and manipuriblack. It was reported that the gelatinization temperature affects water uptake, volume expansion ratio and kernel elongation (Vanaja and Babu 2003).

Length-wise expansion without a corresponding increase in girth is considered a highly had desirable long grain after cooking (> 9mm). There was no significant difference among genotypes for VER which was 3.75 for all the genotypes except Mamihunger (4.0). VER was found significant positive correlation with HUL (r=0.958; Table 3).

Gel consistency (GC) ranged from 43.00 to 75.00. Based on GC classification, moderate GC was observed in Mamihunger and Assambiroin (within 41-60) where as other genotypes has soft and desirable GC (within 61-100). When cooked, rice types with hard gel consistency harden faster than those with a soft gel consistency. Rice with soft gel consistency cook more tenderly and remain soft even upon cooling (Oko *et al*. 2012). The correlation between percentage amylose and gel consistency was negative in direction though not significant (Table 3), suggesting the unlikelihood of correlated responses in selecting for these traits.

The WU value was in the range of 82.33 to 332.50. Mornodoiga required less than 100ml water / 100g rice where as for rest of the genotypes it was more than 100. Highest water uptake was observed in Assambiroin (332.50ml/100g rice). The appearance in the quality of cooked rice grains is associated with the amount of water uptake during cooking process (Tan *et al.* 2000). It is worthy to note that high water uptake ratio affects the palatability of the cooked rice negatively (Oko *et al*. 2012).

Different antioxidant parameters like, total anthocyanin content, flavonoid, gammaoryzanol, and total phenolics, phytic acid and ABTS antioxidant assay are shown in Table 2. The phenolics, flavonoids and anthocyanins, are known to act as electron donors that are capable of reacting with free radicals and convert them to stable compounds, and thus the radical chain reaction is terminated (Laokuldilok *et al*. 2011). The concentration of TACs, TPC and ABTS differed significantly among the genotypes. Highest concentrations of TACs were observed for the grains with purple pericarp color (Mamihunger-96.71 and Manipuri black-96.22 mg/100g), which were more than 25 times higher than the TACs concentrations of grains with red pericarp color (Assambiroin and Mornodoiga). Similar results were also obtained by other researchers (Abdel-Aal *et al*. 2006; Laokuldilok *et al*. 2011) who reported that purple-pigmented rice having a higher level of anthocyanin than red-pigmented rice. The higher concentration of TAC in purple rice may be due to the deposition of both anthocyanin and proanthocyanidin pigment in the aleurone layer where as only proanthocyanidin present in the red rice. Highest concentration of TPC was detected in the purple grain (955.28mg/100g) and lowest in the red grain (191.35mg/100g). These results is in agreements with findings of Choi *et al*. 2007, Shen *et al*. 2009, Goffman and Bergman 2004 for red and black rice varieties. Significant correlation of TAC with TPC (r=0.971) envisages its higher content in the purple pericarp (Table 3). The higher total phenolics contents of purple pericarp appear to be attributable to their higher anti-oxidative activities. The evaluation of the antioxidant properties of cereals is getting more importance because of their free radical scavenging activity. Highest ABTS assay

Table 2. Antioxidant properties and ABTS assay in pigmented rice.

	TAC (mg/100g)	TFC (mg CE/100g)	Gammaoryzanol (mg/100g)	TPC (mg /100g)	Phyticacid (g/100g)	Antioxidant (AAE/g)
Mamihunger (purple grain)	^{A} 96.71±0.98	^{F}146.34±0.58	^{A} 98.10±0.74	^{A} 955.28±0.92	^{C}0.11±0.01	^{A}3187.24±0.39
Manipuriblack (black grain)	^{B} 96.22±0.98	^{D}223.76±1.16	^{D} 65.21±1.0	^{B}621.25±0.85	^{B}0.23 ±0.02	^{B}3127.45±0.38
Chakhao (purple grain)	^{C} 88.55±1.24	^{A}289.20±1.11	^{E}52.65±0.86	^{C}555.80±0.93	^{B}0.20 ±0.02	^{C}3119.34±0.72
Kalobhat (purple grain)	^{C} 89.21±0.98	^{B}284.21±0.83	^{C}67.46±0.98	^{D}491.50±0.51	^{B}0.23 ±0.01	E3061.91±0.27
Mornodoiga (red grain)	^{D} 5.10±0.66	^{C} 272.51±0.63	^{B}80.71±1.01	^{E}355.27±0.97	^{A} 0.32±0.03	^{F}3044.22±0.60
Assambiroin (red grain)	^{D} 3.33±0.63	^{E}211.22±0.96	^{E}52.92±0.68	^{F}191.35±1.05	^{B} 0.20±0.01	^{D}3105.23±0.38

Note- Values are presented as mean ± Standard daviation (n=3); Mean with different letters (A-F) within the same column are significantly different (P < 0.05); CE- Catechine Equivalent; AAE-Ascorbic Acid Equivalent; TAC= Total anthocyanin content; TFC= Total flavonoids content; TPC= Total phenolics content

Table 3. Correlation of physico-chemical and antioxidant properties of pigmented rice.

	WU	VER	KLA	AC	ASV	GC	HUL	MIL	HRR	LB	TAC	GAM	TPC	TFC	PHY	ABTS
WU	1.000															
VER	-0.304	1.000														
KLA	0.215	-0.064	1.000													
AC	-0.298	0.268	0.225	1.000												
ASV	0.083	-0.209	-0.445	**-0.961**	1.000											
GC	-0.267	-0.670	-0.378	-0.405	0.512	1.000										
HUL	-0.433	**0.958**	-0.210	0.387	-0.272	-0.623	1.000									
MIL	-0.395	0.437	0.169	-0.138	0.170	-0.365	0.465	1.000								
HRR	0.512	-0.086	-0.234	**-0.887**	**0.819**	0.015	-0.181	0.251	1.000							
LB	-0.313	0.619	0.088	-0.137	0.149	-0.064	0.436	0.108	-0.047	1.000						
TAC	-0.421	**0.814**	-0.336	-0.251	0.364	-0.236	0.763	0.548	0.278	0.705	1.000					
GAM	-0.608	0.779	-0.307	0.572	-0.397	-0.465	**0.922**	0.420	-0.393	0.180	0.567	1.000				
TPC	-0.530	**0.819**	-0.454	-0.109	0.268	-0.160	0.805	0.419	0.108	0.700	**0.971**	0.665	1.000			
TFC	-0.246	-0.790	0.216	-0.164	0.167	0.773	-0.755	-0.129	-0.197	-0.242	-0.537	-0.557	-0.526	1.000		
PHY	-0.158	-0.752	-0.114	0.322	-0.251	0.577	-0.567	-0.436	-0.473	-0.669	-0.769	-0.213	-0.641	0.688	1.000	
ABTS	0.117	0.782	-0.222	-0.211	0.210	-0.434	0.634	0.088	0.333	0.717	0.781	0.318	0.751	-0.797	**-0.882**	1.000

Number in bold indicates significance at p=0.05level. ASV-alkali spreading value, AC-amylose content (%), GC-gel consistency, HRR=Head rice recovery (%), HUL-hulling (%), KLA-kernel length after cooking, L/B-length/breadth ratio, MIL-Milling (%), VER-volume expansion ratio, WU-water uptake (ml/100g rice). ANTH-anthocyanin (mg/100g), GAM-gammaoryzanol (mg/100g), PHE-phenol (mg/100g), TFC- total flavonoid content (mgCE/100g), PHY-phytic acid (g/100g), ABTS-2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt

was observed for purple rice (Mamihunger-3187.24 AAEg-1foll) and lowest ABTS value was observed for red grain (Assambiroin-3105.23 $AAEg^{-1}$). Positive correlation of ABTS with TAC (r = 0.781) and TPC (r = 0.751) clarifies that higher ABTS scavanging assay in purple grain may be due to higher TAC and TPC content (Table 3). These results suggest that the major phytochemicals responsible for the tested antioxidant activities are phenolic acids and anthocyanin.

Gamma-oryzanol is another one of the phytochemicals that found at high concentration in rice bran has been reported to exhibit more antioxidant activity than vitamin E as six fold and their important bioactivities include anti- and inflammatory activity, enhancement of the immune system, heart disease, cardiovascular disease, glycemic control, diabetes and inhibit tumor promotion (Saenjum *et al.* 2012). Recently, polyphenolic compounds including flavonoids is known as safe and non-toxic antioxidants. Many studies have shown that a high dietary intake of natural phenolics is strongly associated with longer life expectancy, reduced risk of developing some chronic diseases, various types of cancer, diabetes, obesity, improved endothelial function and reduced blood pressure (Halliwell 2007; Yan and Asmah 2010; Jonathan and Kevin 2006). Furthermore, phytic acid which complexes with iron, zinc considered as another antioxidant as it bring about a favorable reduction in the formation of hydroxyl radicals in the colon (Graf and Eaton 1993). Total flavonoid content among the pigemted rice ranged from 146.34 to 289.20 mg/100g and the gamma-oryzanol content ranged from 52.65 to 98.10 mg/100g. Higher flavonoid and gamma-oryzanol content was observed in purple grain (Chakhao and Kalobhat; > 200 mg/100g and Mamihunger; 98.10 mg/100g, respectively. Gammaoryzanol content was found positively correlated with TAC (r=0.567) and TPC (r=0.665) though correlation is not significant (Table 3). Similarly, lowest phytic acid content was found in purple grain Mamihunger (0.11%). Though significant differences were observed among the genotypes for flavonoid, gammaoryzanol and phytic acid content but no significant difference was observed between the colours. It envisages that content of gammaoryzanol and flavonoid content accumulated in bran parts of pigmented rice grain depends on rice genotype and not on the kernel colour.

Traditional rice breeding has been mainly focused on improving agronomic traits, such as yield and disease and insect-resistance, as well as improvement of grain quality, such as milling quality, grain appearance, and cooking quality. Recently, there has been an interest in developing rice varieties rich in one or more phytochemical fractions to potentially contribute to improved human health and develop new market opportunities. In this study, purple rice was found with higher antioxidant properties compared to red rice and most of the pigmented rice was observed with desirable grain quality. The knowledge generated could be used in future breeding programmes for the development of a variety with high antioxidant properties combined with valuable grain quality traits. Also the correlation of variables studied will help in carefully selecting the variables.

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Effect of drought on morpho-physiological, yield and yield traits of chromosome segment substitution lines (CSSLs) derived from wild species of rice

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ABSTRACT

Eighty chromosome segment substitution lines (CSSL) developed in the background of Curinga x O. rufipogon and Curinga x O. meridionalis along with four checks (tolerant and susceptible) were subjected to vegetative and reproductive stage drought stress. At vegetative stage, drought stress significantly reduced total chlorophyll content, relative leaf water content with an increase in proline content. RUF-44, MER-13 and MER-20 were found promising with consistent performances in various morpho-physiological observations. The higher accumulation of proline, more chlorophyll retention and more relative leaf water content at vegetative stage during moisture stress were major criteria for stable yield production of drought tolerant CSSLs. At reproductive stage stress, the CSSLs with high grain yield, minimal relative yield reduction (RYR) and lowest susceptibility index (DSI) were considered as drought tolerant and the reverse as susceptible line. RYR and DSI along with high grain yield under moisture stress was observed in MER-20 and MER-13 with 81.84% and 8.35% RYR and 0.83 and 0.11 DSI values in dry and wet seasons, respectively. However, the extent of RYR was maximum with high DSI in IR 20 and Curinga in both the seasons.

Key words: *CSSL, physiological traits, yield attributes, drought stress*

In India, the total area under irrigated, rainfed lowland and upland rice is 22.0, 14.4 and 6.3 million ha, respectively (Singh 2009). Out of the total 20.7 million ha of rainfed rice area reported in India, approximately 16.2 million ha lie in eastern India (Singh *et al*. 2000), of which 6.3 million ha of upland area and 7.3 million ha of lowland area are highly drought-prone (Pandey and Bhandari 2009). As the global climate changes continue, water shortage and drought have become an increasingly serious constraint limiting rice production worldwide (Wassmann *et al*. 2009 a, b). Among the abiotic stresses in the rainfed systems, drought is the most important factor limiting rice productivity (Ali *et al*. 2008; Venuprasad *et al*. 2008). Rice is particularly sensitive to drought stress and even mild drought stress can result in significant yield reduction in rice (Centritto *et al*. 2009).

Wild species of rice (genus Oryza) contain many useful genes but a vast majority of these genes remain untapped to date. The wild rice relatives serve as a rich reservoir of novel genes or alleles that can be used for the improvement of existing rice cultivars. Chromosome segment substitution lines (CSSLs), which carry a specific donor chromosome segment in the genetic background of a recurrent cultivar, are powerful tools for enhancing the potential of genetic analysis and identifying naturally occurring favorable alleles in unadapted germplasm. To date, CSSLs derived from distant relatives of rice including *O. meridionalis, O. glumepatula, O. rufipogon and O. glaberrima* have been constructed (Hirabayashi *et al*. 2010, Shim *et al*. 2010, Yoshimura *et al*. 2010) in different institutions.

Cornell University, USA is pioneering in using wild genetic resources to improve the performance of elite rice cultivars for drought stress. Eighty chromosomal segment substitution lines developed from crosses between the tropical japonica elite cultivar, Curinga, and two wild relatives, OR44 (*O. meridionalis*) and IRGC105491 (*O. rufipogon*) have been received by National Rice Research Institute (NRRI), Cuttack from Cornell University, USA for testing under field condition to identify the best drought tolerant lines for reproductive stage stress which can be rapidly introgressed further into multiple commercial cultivars.

MATERIALS AND METHODS

The plant material consisted of two sets of CSSLs (total eighty CSSL lines) and four checks including IR20 (drought sensitive), CR143-2-2, Azucena (drought tolerant), and Curinga (parent). These were collected from McCouch laboratory, Cornell University, USA. The two sets of CSSL was developed by backcrossing two different wild donor parents with recurrent parent Curinga (*O. sativa* ssp. tropical *japonica*) (CUR), a commercial rice variety released in 2005, developed at Brazil (de Morais *et al.* 2005), respectively, using marker assisted selection. The recurrent parent Curinga is a semi-early maturing, drought-tolerant cultivar with an average yield under upland conditions of 4,465 kg/ha. In the first set the donor was *O. meridionalis* Ng, acc. W2112 (Oryzabase :http://www.shigen.nig.ac.jp/rice/oryzabaseV4/), and in the second set, the donor was *O. rufipogon* Griff. acc. IRGC 105491 (International Rice Research Institute, IRRI; http://www.irgcis.irri.org:81/grc/IRGCISHome.html) (Arbelaez *et al*, 2015). The *O. meridionalis*/*Curinga* CSSL (32 in No) is hereafter referred as MER, whereas O.rufipogon/Curinga CSSLs (48 in No) referred as RUF. The objective was to phenotype these CSSLs under field condition to identify the best drought tolerant lines for reproductive stage stress which could be rapidly introgressed further into multiple commercial cultivars.

The experiment was conducted at National Rice Research Institute Cuttack, (NRRI) Odisha during two seasons *i.e.*, dry and wet seasons of 2014 under rain out shelter condition for stress and nearby field for non stress (irrigated) trial. The plant material was consisting of eighty CSSLines and four checks IR20 (drought sensitive), CR143-2-2, Azucena (drought tolerant), and Curinga (parent).

Seeds of each CSSlines were dry direct seeded in two replications following randomized block design (RBD) under both rain out shelter (for stress) and control field conditions. Seeds were dibbled to a depth of 2 cm with 20 cm row spacing and 10 cm spacing between hills. To avoid differences in flowering time and impose uniform stress at the time of flowering in each line, staggered sowing was done in 10 days interval. Based on flowering durations, the lines were grouped into two groups: Group -1 (86-95 DFF) and Group -2 (75-85 DFF). Recommended dosage of fertilizers (N: P_2O_5: K_2O @ 40:20:20 kg/ha) were applied basally. The crop in rainout shelter was irrigated after sowing and then in three days interval after germination for good crop stand, while the crop grown under field condition was frequently irrigated. Weeds were controlled manually until full canopy was achieved.

Stress treatment

Stress treatment was imposed in two cycles; one in vegetative stage and other one in reproductive stage. For vegetative stage, the stress was imposed on 21 days old seedlings for 14 days and then re-irrigated for recovery. For reproductive stage stress, when the crop attained panicle initiation stage (65 days after germination in Group-1 and 55 days after germination in Group-2), irrigation was withdrawn about 25-30 days till the soil moisture tension (SMT) reaches upto -50kPa at 30cm and -70kPa at 15cm depth with 13% and 15% soil moisture content.

Observations

Morpho-physiological traits like early vegetative vigor (EVV), relative leaf water content (RLWC), total chlorophyll content and proline content were measured at 0, 7 and 14 days after stress for vegetative stage stress. EVV was scored following standard evaluation system (IRRI 2002), the total chlorophyll content was estimated according to method of Arnon (1949), proline content was measured by the method of Bates *et al*. (1973) and RLWC by Barrs and Weatherley (1962).

For reproductive stage stress, days to 50% flowering, plant height, effective tiller number, panicle number, stem weight, panicle weight, no. of fertile grains, no. of chaffs, total number of spikelets, grain filling percentage and maturity dates were recorded precisely.

Drought Susceptibility Index (DSI) for grain yield and Relative Yield Reduction (RYR) were calculated as follows:

1. Drought susceptibility index (DSI) was used as per Fischer and Maurer (1978):

DSI = (1- Ys/Yc)/D

Where Ys = Grain yield of the CSSLs under stress condition

Yc = Grain yield of the CSSLs under control condition

D = 1-(Mean yield of all CSSLs under stress / Mean yield of all CSSLs under control)

2. Relative yield reduction (RYR) was estimated by the equation of Kumar *et al*. 2008: RYR% = 100 X [1-(Grain yield under moisture stress / Grain yield under control)]

All hydrological observations during stress period like soil moisture content (SMC) at 15cm and 30cm depth by gravimetric method and soil moisture tension by installing tensiometer tubes at 15cm and 30cm depth were recorded in weekly intervals. During peak stress period, low soil moisture content (SMC %) of 12.13 to 14.72% and high soil moisture tension (SMT) of -30kPa to -40 kPa for vegetative stage and SMC of 9.13 to11.12%, SMT of -50kPa to -55kPa for reproductive stage at 30cm soil depth was maintained.

RESULTS AND DISCUSSION

(i) Morpho-physiological traits during vegetative stage stress

Identification of a drought tolerant CSSLs of rice is a difficult job for several reasons. Several attributes are related to drought tolerance. It is highly impossible to have a genotype possessing all these characters responsible for drought tolerance. For the selection of such genotypes, the studies on morpho-physiological characters related to plant parts are essential (Deshmukh *et al*. 2004). Eighty CSSLs and four checks under rainout shelter condition were evaluated for drought tolerance in two seasons (Dry and wet season) of 2014.

Early vegetative vigor (EVV) of 21 days old seedlings was scored following standard evaluation system (IRRI 2002) before imposing stress. The scoring values were 1,3,5,7 and 9, which specifies extra vigorous, vigorous, normal, weak and very weak growth conditions respectively at seedling stage. This scale was used for evaluating genetic material and varieties under stress and control conditions. Among 84 lines, 32 lines had SES score '1', 23 lines had '3', 15 lines had '5', 11 lines had '7' and 3 lines had '9' score in both the seasons.

Moisture stress significantly reduced the relative leaf water content (RLWC) and total chlorophyll content with the increase in proline accumulation over the seasons. Among the lines, 14 days after moisture stress during dry and wet season, RLWC ranged from 46.04% to 75.53% and 46.67%- 70.75%, respectively. Among the best 10 lines RUF-44 (71.19% and 70.70%) and MER-20 (73.30% and 70.42%) were found promising and consistent with higher RLWC over the seasons. According to Almeselmani *et al*. (2011; 2006) RLWC indicates the water status of the cells and has significant association with yield and stress tolerance. This is a very important trait that indicates drought tolerance and varieties which exhibit restricted changes in relative water content per unit reduction of water potential are often considered to be relatively drought tolerant (Vurayai *et al*. 2011).

In the present study, plants showed a tendency to accumulate proline under severe moisture stress. In dry season MER-6 (32.62 µmol g fr wt^{-1}) followed by MER-13 (32.20 µmol g fr wt^{-1}) and RUF-27 (29.65 µmol g fr wt^{-1}) recorded highest proline accumulation at 14 days after stress while in wet season RUF-44 (21.03 µmol g fr wt^{-1}) followed by RUF-13 (20.92 µmol g fr wt^{-1}) and MER-16 (20.89 µmol g fr wt^{-1}) had higher values. Among the best 10 lines RUF-19 and MER-13 were observed to have highest accumulation of proline in both the seasons. The role of proline in adaptation and survival of plants had been observed by Watanabe *et al*. 2000 and Saruhan *et al*. 2006. The resistant varieties accumulate high proline content and tolerate stress for longer time than susceptible varieties (Saruhan *et al*. 2006).

Photosynthetic pigments plays important role in harvesting light. The content of both chlorophyll 'a', 'b' and total chlorophyll content changes under drought

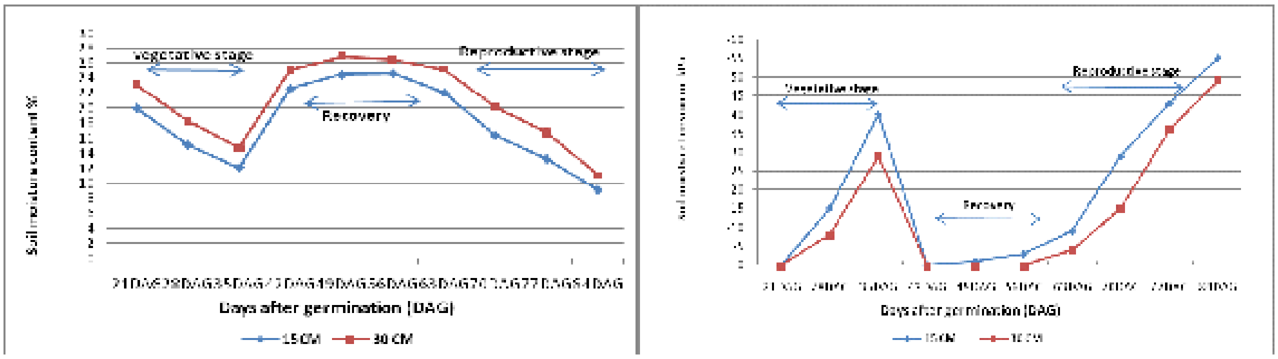

Fig. 1. Soil moisture content (SMC%) and soil moisture tension (SMT) at 15cm and 30 cm depth during moisture stress period starting from vegetative to reproductive stage

stress (Farooq *et al*. 2009). Total Chlorophyll content decreased with the increase in moisture stress. In dry season highest content was recorded in RUF-32 (1.79 mg g fr wt^{-1}), RUF-30 (1.73 mg g fr wt^{-1}) and MER-13 (1.64 mg g fr wt^{-1}), while in wet season RUF-5 recorded highest chlorophyll content of 2.43 mg g fr wt^{-1} followed by RUF-32 (2.37 mg g fr wt^{-1}) and MER-10 (2.14 mg g fr wt^{-1}). RUF-32, MER-13 and MER-4 were found common in both the seasons among the best 10 lines under moisture stress. LI Rong-hua *et al*. (2006) reported that in barley the chlorophyll content was decreased in different genotypes with different levels under drought stress and the decrease was more prominent in sensitive genotypes than tolerant genotypes.

(ii) Yield and yield attributes under reproductive stage stress

Significant differences were observed in grain yield of CSSLs under moisture stress and control conditions. Moisture stress reduced the grain yield irrespective of rice lines. In dry season, out of 84 lines only 13 lines produced grain yield in the range of 0.22-36.96 g m^{-2} whereas, other lines could not produce grain yield under drought condition. Among the other promising lines, highest grain yield was obtained in MER-20 (36.96 g m^{-2}) with minimal relative yield reduction (RYR %) of 81.84% and low DSI of 0.83 followed by CR 143-2-2

Table 1. Performance of promising CSSLines for yield and yield traits under reproductive stage drought during dry season.

Sl no.	CSSLs	Days to 50% flowering (DFF)		Plant height (cm)		Grain Yield (g m^{-2})		Relative Yield Reduction (RYR %)	Drought Suscept-ibility Index(DSI)	Total dry matter (g m^{-2})		Grain filling (%)	
		C	S	C	S	C	S			C	S	C	S
1	MER-20	72	77	103.6	96.9	203.5	36.96	81.84	0.83	815.15	592.40	92.69	19.95
2	RUF-27	75	77	70.10	67.4	95.5	4.00	95.81	0.97	667.50	443.85	82.64	9.54
3	RUF-32	76	86	97.70	86.4	423.0	3.70	99.12	1.00	769.35	645.00	96.84	7.49
4	MER-14	69	78	62.8	64.2	77.5	1.19	98.46	0.99	676.50	414.50	80.00	4.91
5	MER-30	76	81	75.80	61.9	162.8	1.18	99.28	1.00	817.75	645.00	85.62	4.88
6	RUF-7	69	76	79.60	78.3	189.0	0.53	99.72	1.01	653.00	654.00	89.13	2.57
7	RUF-16	71	74	73.40	79.5	73.0	0.38	99.48	1.00	621.50	684.50	53.64	1.21
8	RUF-19	76	79	77.40	70.8	121.0	0.34	99.72	1.01	736.00	422.00	89.09	1.46
9	RUF-1	77	78	84.30	83.9	101.0	0.24	99.76	1.01	777.50	366.00	87.66	0.86
10	MER-32	78	79	79.20	75.7	45.0	0.23	99.49	1.00	741.50	399.50	72.46	1.83
11	RUF-10	73	82	73.70	64.2	223.5	0.22	99.90	1.01	752.50	747.00	90.53	0.67
12	IR 20(CH)	76	83	87.10	81.5	195.0	0.00	100	1.01	812.50	616.45	80.32	0.00
13	AZUCENA (CH)	84	89	93.2	86.3	114.0	0.58	99.49	1.00	595.50	586.00	84.13	0.52
14	CR 143-2-2 (CH)	70	75	89.5	80.8	288.5	30.96	89.27	0.90	797.50	739.85	82.81	17.08
15	CURINGA (CH)	80	82	77.0	88.6	195.0	0.00	100	1.01	792.50	424.50	84.89	0.00
LSD (84 lines) at 5%		4.19	6.62	12.68	9.28	84.0	3.44			122.32	105.97	9.45	2.24

C = Control, S = Stress

Table 2. Performance of promising CSSLines during wet season for yield and yield traits under reproductive stage drought.

Sl no.	CSSLs	Days to 50% flowering (DFF)		Plant height (cm)		Grain Yield (g m^{-2})		Relative Yield Reduction (RYR%)	Drought Suscepti-bility Index (DSI)	Total dry matter (g m^{-2})		Grain filling (%)	
		C	S	C	S	C	S			C	S	C	S
1	RUF-44	75	85	71.75	88.45	169.0	147.68	12.51	0.17	709.25	727.2	78.50	60.85
2	RUF-14	79	81	79.90	80.18	162.0	101.83	37.11	0.51	719.80	529.8	62.00	54.32
3	MER-10	61	73	80.55	78.70	122.7	82.33	32.90	0.45	604.40	588.8	77.90	50.68
4	MER-13	78	82	79.45	89.63	86.6	79.38	8.35	0.11	596.00	717.5	61.75	59.69
5	RUF-47	69	74	82.80	82.38	139.0	74.80	46.34	0.63	665.50	563.5	58.50	49.89
6	RUF-13	68	73	72.45	83.68	125.0	73.58	40.95	0.56	770.95	547.5	61.75	44.63
7	RUF-38	68	75	81.90	86.88	98.4	68.75	30.13	0.41	683.20	535.8	55.50	51.75
8	RUF-2	64	72	94.05	81.38	134.0	67.45	50.94	0.68	852.70	531.5	86.50	45.75
9	RUF-5	83	91	70.70	78.25	78.9	61.65	21.85	0.30	791.35	447.75	73.00	55.08
10	RUF-33	65	72	94.5	107.25	261.4	51.28	80.38	1.10	610.85	580.05	95.00	63.99
11	RUF-30	80	72	76.35	61.00	85.37	47.63	44.21	0.61	799.40	430.60	69.00	45.14
12	IR 20(CH)	78	88	88.40	88.3	188.0	0.00	100.0	1.37	763.6	564.18	75.00	0.00
13	AZUCENA(CH)	85	91	99.35	97.18	246.8	60.80	75.37	1.03	788.60	628.5	91.00	30.18
14	CR 143-2-2(CH)	73	75	88.05	71.08	193.0	128.6	33.40	0.46	797.00	583.5	84.00	45.35
15	CURINGA (CH)	75	82	75.40	77.00	92.0	0.00	100.0	1.37	765.45	413.18	73.45	0.00
16	RUF-10	74	78	80.90	77.75	67.3	49.93	25.84	0.35	684.85	480.25	73.50	44.54
17	RUF-32	69	75	98.50	73.95	245.6	47.18	80.80	1.11	784.80	431.45	94.00	39.60
18	RUF-16	72	75	78.65	73.63	246.8	46.85	81.02	1.11	740.05	491.10	91.00	58.90
19	RUF-1	68	77	81.95	78.00	81.10	43.20	46.73	0.64	798.10	576.45	73.50	46.37
20	MER-20	73	77	73.60	85.70	102.0	42.88	57.97	0.79	794.45	437.13	66.95	53.51
21	MER-32	68	77	80.90	76.95	62.50	42.45	32.08	0.44	740.90	493.65	57.25	36.81
22	RUF-7	74	74	77.95	66.50	74.74	40.83	45.37	0.62	741.45	432.26	72.00	48.25
LSD (84 lines) at 5%		3.73	2.71	8.86	7.60	57.8	19.89			101.62	75.87	5.34	11.83

C = Control, S = Stress

(30.96 g m^{-2}) and RUF-27 (4.00 g m^{-2}) with 89.27%, 95.81% of RYR and 0.90, 0.97 DSI respectively. The RYR and DSI value ranged from 81.84%-100% and 0.83-1.01, respectively. Ahmad *et al*. (2003) have reported that drought susceptible varieties had higher values (DSI >1), while resistant varieties had lower values (DSI<1). The yield stability in resistant varieties was due to specific adaptive feature that make it able to produce stable grain yield even in stress condition (Van Heerden and Lune 2008). Grain filling % was highest in MER-20 (19.95%) followed by, CR 143-2-2 (17.08%), RUF-27 (9.55%), and RUF- 32 (7.49 %) (Table 1).

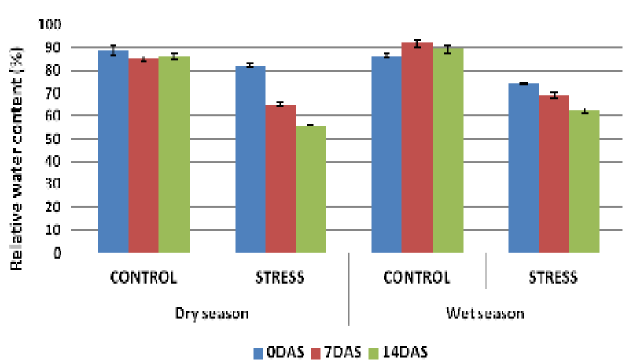

Fig. 2. Mean performance of relative leaf water content (RLWC) of 84 CSSLs under moisture stress and control conditions at vegetative stage in both dry and wet seasons

There are some reports indicated that lower soil moisture inhibit photosynthesis and decrease translocation of assimilates to the grain which lowered grain weight (Van Heerden *et al*. 2008 and Liu *et al*. 2008). Wild relatives of rice typically have long awns, severe shattering for seed dispersal, higher dormancy, coloured pericarp, smaller grain size and open panicle (Sweeney and Mc Couch 2007). Common wild rice (*O. rufipogon*) is the wild ancestor of cultivated rice (Second 1982; Oka 1988; Wang *et al*. 1992). During the course of domestication from wild rice to cultivated rice, only 60% of the numbers of alleles of wild rice were remained in cultivated rice (Sun *et al*. 2001). To broaden the genetic variation and overcome the yield plateaus, exploitation and utilization of the favorable

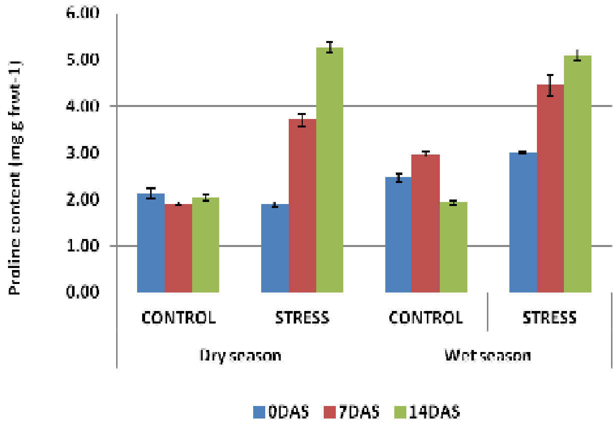

Fig. 3. Mean performance of proline content of 84 CSSLs measured under moisture stress and control condition at vegetative stage in both dry and wet seasons

alleles of wild rice which have been lost or weakened in cultivated rice has become more important and urgent in modern breeding programs. Grain yield of wild rice genotypes is normally less compared to other cultivated rice varieties. As these CSSLs are derived from wild ancestors yield of *O. rufipogon* and *O. meridionalis* is less under favorable condition and after getting exposed to moisture stress in dry season it became more less. However, the temperature inside the rainout shelter was little higher than outside (4% during dry season, which might have played a role to reduce the grain yield more during dry season compared to wet season).

Among the 84 lines tested during wet season, highest grain yield was obtained in CSSL line RUF-44 (147.68 g m^{-2}) with highest grain filling of 60.85% followed by the tolerant check CR 143-2-2 (128.63 g m^{-2}), RUF-14 (101.83 g m^{-2}), MER-10 (82.32 g m^{-2}) and MER-13 (79.38 g m^{-2}) under moisture stress. MER-13 recorded with minimal RYR of 8.35% and low DSI of 0.11 followed by RUF-44, RUF-5 and RUF-10 with 12.51%, 21.85% and 25.85% RYR and 0.17, 0.30 and 0.35 of DSI respectively (Table 2).

The mean value of DSI close to or below 1.0 for any trait indicates its relative tolerance to drought. However, high values for DSI represent drought susceptibility (Winter *et al*. 1988). The attributes like DSI and RLWC have a direct bearing on the ability of a genotype to withstand against water stress (Singh 2003). This is reflected in relative values of percent reduction in yield due to water stress in comparison with control condition in both the seasons. The data on these attributes in rain out shelter and controlled condition are presented in Table 1 and Table 2.

Moisture stress is a complex mechanism. Various adaptive features of plant make it able to produce stable yield under stress regimes. Resistant/ tolerant varieties showed stable growth and grain yield due to high accumulation of osmolytes and better scavenging system. Based on the vegetative and reproductive stage stress performances, the chromosome segment substitution lines RUF-44 and MER-13 in wet season and MER-20 and RUF-32 in dry season are identified to be best drought tolerant lines among 84 lines tested. In dry season though the yield of RUF-32 (3.70 g m^{-2}) was little less than RUF-27 (4.00 g m^{-2}) and in wet season the yield of MER-13 (79.38 g m^{-2}) was less than RUF-14 (101.83 g m^{-2}) and MER-10 (82.33 g m^{-2}), for morpho-physiological traits during vegetative stage, MER-20 and RUF-44 had high water retention capacity (RLWC), MER-13 had high chlorophyll and proline content, and RUF-32 had high chlorophyll content in both the seasons. However, RUF-10, RUF-32, RUF-16, RUF-1, MER-20, MER-32 and RUF-7 lines commonly had better drought tolerance in both the seasons though they had poor yield compared to best lines. Therefore, from the data of moisture stress (under rain out shelter) and control conditions at both the stages, it can be concluded that the tolerant lines with high RLWC, more proline accumulation, high

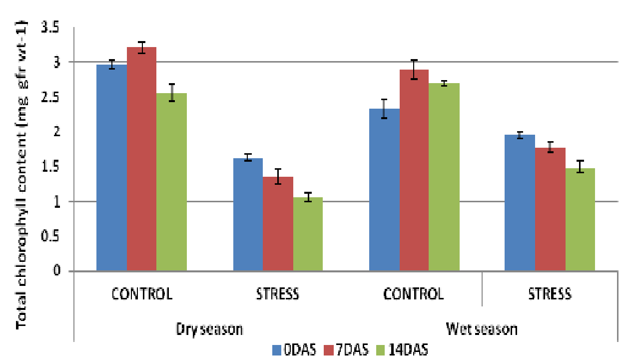

Fig. 4. Mean performance of total chlorophyll content of 84 CSSLs measured under moisture stress and control condition at vegetative stage in both dry and wet seasons

chlorophyll content and high grain yield, minimal RYR and low DSI can be used further for agronomy and breeding programmes aiming at management practices under drought and variety development for drought prone areas.

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Evaluation of pre-mixture of flubendiamide and buprofezin for management of major insect-pests of rice

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ABSTRACT

Experiment was conducted for two seasons at the Central Research Farm, Gayeshpur, BCKV, Nadia, West Bengal to manage the rice leaf folder (Cnaphalocrosis medinalis Guenee*), stem borer (Scirpophaga incertulas* Walker*) and brown plant hopper (Nilaparvata lugens* Stål*) in rice (cv. Satabdi) by using pre-mixture of two new molecules, flubendiamide 4% + buprofezin 20% SC. Result of the experiments revealed that the combined product was highly effective against the mentioned pests and found superior and harvested highest grain yield 43.13q and 41.79q per hectare during kharif and rabi seasons, respectively.*

***Key words:** Brown plant hopper, flubendiamide + buprofezin, leaf folder, stem borer*

Rice (*Oryza sativa* L.) is the staple food for more than half of the world population. According to the United States, Department of Agriculture (USDA) the world rice production is 465.03mtin 2011-12, where, India ranked second (104.32mt) in rice production after China (USDA 2012). India contributes 45% total food grain and continues to play a vital role in national food security. A number of insect pests are reported to ravage rice fields in tropics. Total global potential loss in rice due to pests is about 37% (Oerke 2006). Among the insect pests of rice, yellow stem borer, leaf folder and brown planthopper are the most important. About 3-95% losses caused by stem borer (Ghose *et al*. 1960), 50% by leaf folder (Balasubramaniam *et al*. 1973) and 10-70% by brown plant hoppers (BPH) infestation (Kulshreshtha *et al*. 1974). Control strategies for rice pests are extensively dependent on the use of synthetic chemical insecticides. However, recognition of detrimental effect of insecticide such as resistance to insecticide, secondary pest outbreak, non-target effects, environmental pollution etc. have prompted the development of alternative control strategies and use of environmentally safer chemicals. Therefore, the experiment was conducted to evaluate the combined effect of flubendiamide 4% + buprofezin 20% SC for management of major insect-pests of rice.

MATERIAL AND METHODS

The field experiments were conducted at Centran Research Farm, Gayeshpur, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal during *kharif*, 2012 and *rabi*, 2013 to evaluate the bio-efficacy of a pre mixture insecticide, flubendiamide 4% + buprofezin 20% SC against rice leaf folder (*Cnaphalocrosis medinalis* Guenee), stem borer (*Scirpophaga incertulas* Walker) and brown planthopper (*Nilaparvata lugens* Stal). The experiments were carried out in Randomized Complete Block Design (block size 5m x 5m) consisting of ten treatments with four replicates. In the experiment, 30 days old seedlings (cv. *Satabdi*) were transplanted and the different treatments were applied first at tillering stage and second at panicle initiation stage. The observations on dead heart/white ear head, folded leaves and number of brown plant hopper were recorded from randomly selected 10 hills in each plot. Observations of dead heart were taken before spraying, 10 days and 20 days after first spraying whereas for white ear head only after 20 days of second spraying. The percentage of infestation was calculated by using the following formula:

$$\%\text{ of dead heart} = \frac{\text{No. of dead hearts}}{\text{No. of total tillers}} \text{x100}$$

$$\%\text{ of white ear} = \frac{\text{No. of white ears}}{\text{No. of total productive tillers}} \text{x100}$$

(Heinrichs *et al*. 1985)

Observations on leaf folder were taken before spraying, 7 days and 15 days after each spray. The percentage of infestation was calculated by using the following formula:

$$\%\text{ leaf folder} = \frac{\text{No. of folded leaf per hill}}{\text{No. of tillers per hill}} \text{x100}$$

The population of brown plant hoppers (BPH) were counted on the date of spraying and subsequently after 10, 15 and 20 days after second spraying. The grain yield was recorded in each plot was subsequently converted to per hectare basis as quintal per hectare (q/ha). Yield and yield attributing characters were recorded following standard procedures (Kumar *et al.* 2017).

Treatments Details:

Treatments	Dosage (Formulation/ha)
T_1-Flubendiamide 4% +buprofezin 20% SC	750ml
T_2 - Flubendiamide 4% + buprofezin 20% SC	875ml
T_3 - Flubendiamide 20 % WG	125g
T_4 - Flubendiamide 20 % WG	150g
T_5 - Flubendiamide 20 % WG	175g
T_6 - Buprofezin 25 % SC	600ml
T_7 - Buprofezin 25 % SC	700ml
T_8-Buprofezin 25 % SC	800ml
T_9- Buprofezin 5%+ deltamethrin 0.625% EC	1500ml
T_{10}- Control	-

RESULTS AND DISCUSSION

Leaf folder

Performance of the insecticide during *kharif*, 2012 against leaf folders has been presented in Table 1 and Table 2. The treatment T_2 *i.e.*, flubendiamide 4% + buprofezin 20% SC @ 875ml/ha, was found best at each time of observations and recorded 2.27%, 2.13% leaf infestation after 7 days of 1st and 2nd spray, respectively and 2.37%, 2.01% after 15 days of 1st and 2nd spray, respectively. This was followed by the treatments, T_5- flubendiamide 20 % WG @ 35g a.i./ha and T_4- flubendiamide 20 % WG @ 30g a.i./ha. During the second season (*rabi*-2013) the pest appeared late but assumed a substantial size during the second spray. However, observations revealed that after 15 days of 1st and 2nd spraying only 0.24%, 0.26% leaf damage were recorded in treatment T_2- flubendiamide 4% + buprofezin 20% SC @ 875ml/ha followed by the T_5- flubendiamide 20 % WG @ 35g a.i./ha and T_4, flubendiamide 20 % WG @ 30g a.i./ha, respectively (Table 1). Present findings are in agreement with the findings of Kartikeyan *et al*. (2012) who reported that the lowest percent of leaf folder was recorded in combined product of flubendiamide 4% + buprofezin 20% SC @ 875 ml/ha. While the superiority of flubendiamide against leaf folder on rice was reported by Bhanu and Reddy (2008), Kulagod *et al*. (2011), Girish *et al*. (2012).

Rice stem borer

During *kharif* 2012, population of the rice stem borers were initially low but afterwards it ravaged the crop in numbers and reaching ETL very soon with a steady increase in population as evidenced by the data in the untreated plots (Table 3). Similar to leaf folder the treatment T_2- flubendiamide 4% + buprofezin 20% SC @ 875ml/ha again found to be the best in reducing the insect population after 20 days of spraying in both first and second sprays (0.47% and 0.86%). However, during *rabi*, 2013 (Table 3) it clearly showed that this pest was quite abundant in the summer paddy where per cent white ear head in untreated plots reached to 30.32%. The treatment T_2 sustained only 2.06% white ear head and emerged as the best performer in reduction of rice stem borer population significantly. The results are in agreement with the findings of Kartikeyan *et al*. (2012), Rath (2011) and Anonymous

Table 1. Effect of different combinations and pre-mixture of flubendiamide and buprofezinagainst leaf folder (*Cnaphalocrosis medinalis* Guenee) population in rice

Treatments	Per cent of leaf folder infestation											
	First Season (*kharif*, 2012)						Second Season (*rabi*, 2013)					
	First spray			Second spray			First spray			Second spray		
	1DBS	7DAS	15DAS	1DBS	7DAS	15DAS	1DBS	7DAS	15DAS	1DBS	7DAS	15DAS
T_1	7.00	5.99	6.08	6.79	3.33	3.17	0.00	0.00	0.47	1.39	0.66	0.50
	(15.34)	(14.17)	(14.28)	(15.11)	(10.51)	(10.26)		(0.00)	(3.92)	(6.76)	(4.66)	(4.05)
T_2	7.09	2.27	2.37	5.09	2.13	2.01	0.00	0.00	0.24	0.68	0.48	0.26
	(15.44)	(8.67)	(8.85)	(13.04)	(8.40)	(8.16)		(0.00)	(2.79)	(4.74)	(3.97)	(2.90)
T_3	6.89	6.37	6.41	6.98	3.67	3.47	0.00	0.00	0.59	1.91	1.21	0.63
	(15.21)	(14.62)	(14.67)	(15.32)	(11.05)	(10.74)		(0.00)	(4.42)	(7.94)	(6.32)	(4.54)
T_4	7.11	4.89	4.04	6.44	2.85	2.64	0.00	0.00	0.55	1.43	0.95	0.59
	(15.46)	(12.78)	(11.59)	(14.70)	(9.72)	(9.35)		(0.00)	(4.24)	(6.86)	(5.58)	(4.41)
T_5	6.77	2.91	3.24	5.65	2.45	2.26	0.00	0.00	0.30	0.94	0.59	0.33
	(15.08)	(9.83)	(10.38)	(13.75)	(9.01)	(8.65)		(0.00)	(3.16)	(5.56)	(4.41)	(3.31)
T_6	7.19	9.04	10.34	9.50	7.92	6.18	0.00	0.32	1.71	2.52	1.80	1.05
	(15.55)	(17.50)	(18.76)	(17.96)	(16.35)	(14.39)		(3.23)	(7.51)	(9.14)	(7.72)	(5.89)
T_7	6.92	9.18	9.84	9.13	7.56	6.20	0.00	0.00	1.13	2.64	1.61	0.79
	(15.26)	(17.64)	(18.28)	(17.59)	(15.96)	(14.42)		(0.00)	(6.09)	(9.36)	(7.29)	(5.11)
T_8	6.18	8.41	10.04	9.01	7.49	6.16	0.00	0.51	1.26	2.29	1.85	0.80
	(14.40)	(16.85)	(18.48)	(17.47)	(15.88)	(14.37)		(4.11)	(6.45)	(8.71)	(7.82)	(5.12)
T_9	6.84	6.59	6.44	7.56	3.68	3.42	0.00	0.26	0.48	1.51	1.06	0.70
	(15.16)	(14.87)	(14.70)	(15.96)	(11.06)	(10.66)		(2.90)	(3.97)	(7.06)	(5.90)	(4.80)
T_{10}	6.87	10.65	12.56	9.70	8.28	6.75	0.00	0.50	1.87	3.00	2.05	1.11
	(15.19)	(19.04)	(20.76)	(18.14)	(16.72)	(15.06)		(4.05)	(7.86)	(9.97)	(8.23)	(6.05)
SEm±	0.78	0.44	0.41	0.48	0.28	0.49	-	1.10	1.37	1.13	1.03	1.53
CD (0.05)	NS	1.31	1.22	1.43	0.83	1.45	-	NS	4.08	3.37	3.06	NS

DBS=Days before spraying, DAS= Days after spraying, **figures in the parentheses indicate the angular transformed [Sin $^{-1}$ $\sqrt{\{x/100\}}$] values

Table 2. Protection over control of leaf folder *Cnaphalocrosis medinalis* Guenee) after application of pre-mixture of flubendiamide and buprofezin in rice

Treatments	Per cent protection of leaf folder infestation over control							
	First Season (*kharif*, 2012)				Second Season (*rabi*, 2013)			
	First spray		Second spray		First spray		Second spray	
	7DAS	15DAS	7DAS	15DAS	7DAS	15DAS	7DAS	15DAS
T_1	43.76	51.59	59.78	53.04	100.00	74.87	67.80	54.95
T_2	78.69	81.13	74.28	70.22	100.00	87.17	76.59	76.58
T_3	40.19	48.96	55.68	48.59	100.00	68.45	40.98	43.24
T_4	54.08	67.83	65.58	60.89	100.00	70.59	53.66	46.85
T_5	72.68	74.20	70.41	66.52	100.00	83.96	71.22	70.27
T_6	15.12	17.68	4.35	8.44	36.00	8.56	12.20	5.41
T_7	13.80	21.66	8.70	8.15	100.00	39.57	21.46	28.83
T_8	21.03	20.06	9.54	8.74	-2.00	32.62	9.76	27.93
T_9	38.12	48.73	55.56	49.33	48.00	74.33	48.29	36.94
T_{10}	-	-	-	-	-	-	-	-

DBS=Days before spraying, DAS= Days after spraying

(2011) where they reported that the lowest percent of dead heart and white ear head was recorded in pre-mixture product of flubendiamide 4% + buprofezin 20%SC @ 875 ml/ha. Bhanu and Reddy (2008), Kulagod *et al.* (2011) observed significantly lower stem borer damage in flubendamide treatment over untreated check.

Brown plant hopper (BPH)

This insect appeared at late growth stage of rice. In our experiment too, it was observed in profuse number starting from the early panicle initiation stage and were

Table 3. Effect of different combinations and pre-mixture of flubendiamide and buprofezin against yellow stem borer (*Scirpophaga incertulas* Walker) population in rice

Treatments	First Season (*kharif*, 2012)				Second Season (*rabi*, 2013)			
	% DH before & after first spray			% WE after second spray	% DH before & after first spray			% WE after second spray
	1DBS	10DAS	20DAS	20DAS	1DBS	10DAS	20DAS	20DAS
T_1	0.00	0.00 (0.00)	0.56 (4.28)	1.07 (5.95)	0.00	0.00 (0.00)	0.56 (4.28)	4.46 (12.19)
T_2	0.00	0.00 (0.00)	0.47 (3.92)	0.86 (5.32)	0.00	0.00 (0.00)	0.47 (3.92)	2.06 (8.25)
T_3	0.00	0.24 (2.79)	0.67 (4.71)	3.22 (10.34)	0.00	0.24 (2.79)	0.67 (4.71)	5.82 (13.96)
T_4	0.00	0.00 (0.00)	0.51 (4.11)	1.47 (6.97)	0.00	0.00 (0.00)	0.51 (4.11)	3.67 (11.04)
T_5	0.00	0.00 (0.00)	0.46 (3.90)	0.93 (5.52)	0.00	0.00 (0.00)	0.46 (3.90)	2.78 (9.60)
T_6	0.00	0.00 (0.00)	0.94 (5.55)	8.12 (16.56)	0.00	0.00 (0.00)	0.94 (5.55)	27.37 (31.55)
T_7	0.00	0.29 (3.09)	1.06 (5.92)	8.05 (16.48)	0.00	0.29 (3.09)	1.06 (5.92)	26.59 (31.04)
T_8	0.00	0.00 (0.00)	1.73 (7.56)	7.41 (15.79)	0.00	0.00 (0.00)	1.73 (7.56)	26.06 (30.70)
T_9	0.00	0.25 (2.85)	0.83 (5.23)	3.28 (10.43)	0.00	0.25 (2.85)	0.83 (5.23)	5.97 (14.14)
T_{10}	0.00	0.48 (3.96)	2.55 (9.19)	9.23 (17.69)	0.00	0.48 (3.96)	2.55 (9.19)	30.32 (33.41)
SE(m±)	-	-	1.97	1.20	-	-	1.97	1.16
CD (0.05)	-	-	NS	3.57	-	-	NS	3.43

DBS=Days before spraying, DAS= Days after spraying, **figures in the parentheses indicate the angular transformed [$\sin^{-1}\sqrt{\{x/100\}}$] values

Table 4. Per cent protection over control of yellow stem borer (*Scirpophaga incertulas* Walker) population after application of pre-mixture of flubendiamide and buprofezin in rice

Treatments	Per cent protection of yellow stem borer population over control					
	First Season (*kharif*, 2012)			Second Season (*rabi*, 2013)		
	% of DH and WE* after first and second spray			% of DH and WE* after first and second spray		
	10DAS	15DAS	20DAS*	10DAS	15DAS	20DAS*
T_1	100.00	78.04	88.41	100.00	78.04	85.29
T_2	100.00	81.57	90.68	100.00	81.57	93.21
T_3	50.00	73.73	65.11	50.00	73.73	80.80
T_4	100.00	80.00	84.07	100.00	80.00	87.90
T_5	100.00	81.96	89.92	100.00	81.96	90.83
T_6	100.00	63.14	12.03	100.00	63.14	9.73
T_7	39.58	58.43	12.78	39.58	58.43	12.30
T_8	100.00	32.16	19.72	100.00	32.16	14.05
T_9	47.92	67.45	64.46	47.92	67.45	80.31
T_{10}	-	-	-	-	-	-

DBS=Days before spraying, DAS= Days after spraying

subjected to only second spray. Treatments with buprofezin as a component, solo or in combination, were found significantly superior to the rest. However, its pre-mixture with flubendamide in the treatment T_2, *i.e.*, flubendiamide 4% + buprofezin 20% SC @ 875ml/ha was recorded most efficacious than the other treatments in reducing the pest population over the control (Table 5). The results are at par with the findings of Kartikeyan *et al*. (2012) wherein the lowest BPH population were recoreded in flubendiamide 4% + buprofezin 20%SC @ 875 ml/ha while Hegde and Nidagundi (2009)

Table 5. Effect of combinations and pre-mixture of flubendiamide and buprofezinag ainst Brown plant hoppers (*Nilaparvata lugens* Stål) population in rice

Treatments	First Season (*kharif*, 2012)				Second Season (*rabi*, 2013)			
	Numbers of brown planthoppers / hill before and after second spray				Numbers of brown planthoppers / hill before and after second spray			
	0DBS	10DAS	15DAS	20DAS	0DBS	10DAS	15DAS	20DAS
T_1	12.33 (3.58)	5.87 (2.52)	6.27 (2.60)	7.40 (2.81)	7.33 (2.80)	3.93 (2.11)	4.13 (2.15)	5.20 (2.39)
T_2	10.60 (3.32)	3.60 (2.02)	4.20 (2.17)	5.53 (2.46)	6.87 (2.71)	1.93 (1.55)	2.00 (1.58)	3.47 (1.99)
T_3	15.80 (4.04)	23.40 (4.88)	21.20 (4.66)	14.40 (3.86)	10.00 (3.24)	14.27 (3.84)	14.27 (3.84)	8.67 (3.02)
T_4	14.93 (3.92)	23.27 (4.87)	21.93 (4.73)	15.40 (3.99)	10.27 (3.28)	13.27 (3.71)	14.53 (3.88)	8.67 (3.02)
T_5	15.13 (3.95)	22.87 (4.83)	20.07 (4.53)	14.47 (3.86)	9.67 (3.19)	13.40 (3.73)	13.73 (3.77)	8.40 (2.98)
T_6	12.60 (3.62)	5.80 (2.51)	6.47 (2.64)	7.53 (2.83)	7.27 (2.79)	4.00 (2.12)	4.20 (2.17)	5.13 (2.37)
T_7	12.13 (3.55)	5.07 (2.36)	5.60 (2.47)	6.73 (2.69)	7.40 (2.81)	3.27 (1.94)	3.47 (1.99)	4.47 (2.23)
T_8	11.33 (3.44)	4.20 (2.17)	4.73 (2.29)	5.80 (2.51)	7.13 (2.76)	2.60 (1.75)	2.80 (1.81)	4.00 (2.12)
T_9	12.40 (3.59)	5.47 (2.44)	6.20 (2.59)	7.27 (2.78)	7.33 (2.80)	4.07 (2.13)	4.20 (2.17)	5.00 (2.34)
T_{10}	16.27 (4.09)	24.73 (5.02)	21.80 (4.72)	15.47 (3.99)	10.13 (3.26)	14.47 (3.87)	12.53 (3.60)	8.80 (3.05)
SEm±	0.11	0.09	0.07	0.09	0.06	0.09	0.08	0.08
CD (0.05)	0.33	0.25	0.20	0.27	0.18	0.26	0.25	0.24

DBS=Days before spraying, DAS= Days after spraying**figures in the parentheses indicate the root transformed $\left\{\sqrt{(x+0.5)}\right\}$ values

Table 6. Per cent protection over control of Brown plant hoppers (*Nilaparvata lugens* Stål)population after application of pre-mixture of flubendiamide and buprofezin in rice

Treatments	Per cent protection of Brown plant hoppers population over control					
	First Season (*kharif*, 2012) After second spray			Second Season (*rabi*, 2013) After second spray		
	10DAS	15DAS	20DAS	10DAS	15DAS	20DAS
T_1	76.26	71.24	52.17	72.84	67.04	40.91
T_2	85.44	80.73	64.25	86.66	84.04	60.57
T_3	5.38	2.75	6.92	1.38	-13.89	1.48
T_4	5.90	-0.60	0.45	8.29	-15.96	1.48
T_5	7.52	7.94	6.46	7.39	-9.58	4.55
T_6	76.55	70.32	51.33	72.36	66.48	41.70
T_7	79.50	74.31	56.50	77.40	72.31	49.20
T_8	83.02	78.30	62.51	82.03	77.65	54.55
T_9	77.88	71.56	53.01	71.87	66.48	43.18
T_{10}	-	-	-	-	-	-

reported buprofezin 25% SC significantly reduced the BPH population.

Yield and incremental cost benefit ratio

The incremental cost benefit ratio and yield of rice/ha as presented in Tables 7 and 8 for the seasons *kharif* - 2012 and *rabi* - 2013, respectively. It was found that in both the seasons, yield of rice was highest in treatment T_2 (43.13 and 41.79 q/ha, respectively) which corroborates the finding of Kartikeyan *el al*. 2012; Rath 2011; CRRI Annual Report (2010-11). The highest ICBR; 1: 6.48 and 1: 5.72 were recorded in the *kharif*

Table 7. Incremental cost benefit ratio [first season (*kharif*, 2012)]

Treatments	Cost of insecticide for two spray per hectare(Rs.)	Labour cost per hectare for two spray(Rs.)	Total cost per hectare (Rs.)	Total Yield per hectare (quintal)	Additional yield per hectare (quintal)	Additional gross income per hectare (Rs.)	Additional net income per hectare (Rs.)	Incremental cost benefit ratio
T_1	1500	1670	3170	32.25	8.81	11456.25	8286.25	1: 2.61
T_2	1750	1670	3420	43.13	19.69	25593.75	22173.75	1: 6.48
T_3	1625	1670	3295	28.13	4.69	6093.75	2798.75	1: 0.85
T_4	1950	1670	3620	30.62	7.19	9340.50	5720.50	1: 1.58
T_5	2275	1670	3945	31.82	8.38	10890.75	6945.75	1: 1.76
T_6	1344	1670	3014	24.38	0.94	1218.75	-1795.25	1: -0.60
T_7	1568	1670	3238	24.62	1.19	1540.50	-1697.50	1: -0.52
T_8	1792	1670	3462	24.88	1.44	1872.00	-1590.00	1: -0.46
T_9	1860	1670	3530	30.32	6.88	8940.75	5410.75	1: 1.53
T_{10}	-	-	-	23.44	-	-	-	-
SEm (±)	-	-	-	3.22	-	-	-	-
CD (0.05)	-	-	-	9.57	-	-	-	-

T_1- Flubendiamide 4% + Buprofezin 20% SC @ 750ml/ha (Rs.1000/lit.) , T_2 - Flubendiamide 4% + Buprofezin 20% SC @ 875ml/ha (Rs.1000/lit.), T_3 - Flubendiamide 20 % WG @ 25g a.i./ha (Rs. 6500/kg), T_4 - Flubendiamide 20 % WG @ 30g a.i./ha (Rs. 6500/kg), T_5 - Flubendiamide 20 % WG @ 35g a.i./ha (Rs. 6500/kg), T_6 - Buprofezin 25 % SC @ 150 g a.i./ha (Rs. 1120/Litre), T_7 - Buprofezin 25 % SC @ 175 g a.i./ha (Rs. 1120/Litre), T_8-Buprofezin 25 % SC @ 200 g a.i./ha (Rs. 1120/Litre), T_9- Buprofezin 5%+ Deltamethrin 0.625% EC @1500ml/ha (Rs. 620/lit.), T_{10}- Control (Untreated check) Labour charge Rs. 167 per day per man, Price

Table 8. Incremental cost benefit ratio [second season (*rabi*, 2013)]

Treatments	Cost of insecticide for two spray per hectare(Rs.)	Labour cost per hectare for two spray(Rs.)	Total cost per hectare (Rs.)	Total Yield per hectare (quintal)	Additional yield per hectare (quintal)	Additional gross income per hectare (Rs.)	Additional net income per hectare (Rs.)	Incremental cost benefit ratio
T_1	1500	1670	3170	40.33	14.96	20937.00	17767.00	1: 5.60
T_2	1750	1670	3420	41.79	16.42	22984.50	19564.50	1: 5.72
T_3	1625	1670	3295	35.20	9.83	13755.00	10460.00	1: 3.17
T_4	1950	1670	3620	35.50	10.13	14175.00	10555.00	1: 2.92
T_5	2275	1670	3945	36.80	11.43	16002.00	12057.00	1: 3.06
T_6	1344	1670	3014	26.72	1.35	1890.00	-1124.00	1: -0.37
T_7	1568	1670	3238	27.70	2.33	3255.00	17.00	1: 0.01
T_8	1792	1670	3462	28.00	2.63	3675.00	213.00	1: 0.06
T_9	1860	1670	3530	30.40	5.03	7035.00	3505.00	1: 0.99
T_{10}	-	-	-	25.37	-	-	-	-
SEm (±)	-	-	-	2.80	-	-	-	-
CD (0.05)	-	-	-	8.31	-	-	-	-

T_1- Flubendiamide 4% + Buprofezin 20% SC @ 750ml/ha (Rs.1000/lit.) , T_2 - Flubendiamide 4% + Buprofezin 20% SC @ 875ml/ha (Rs.1000/lit.), T_3 - Flubendiamide 20 % WG @ 25g a.i./ha (Rs. 6500/kg), T_4 - Flubendiamide 20 % WG @ 30g a.i./ha (Rs. 6500/kg), T_5 - Flubendiamide 20 % WG @ 35g a.i./ha (Rs. 6500/kg), T_6 - Buprofezin 25 % SC @ 150 g a.i./ha (Rs. 1120/Litre), T_7 - Buprofezin 25 % SC @ 175 g a.i./ha (Rs. 1120/Litre), T_8-Buprofezin 25 % SC @ 200 g a.i./ha (Rs. 1120/Litre), T_9- Buprofezin 5%+ Deltamethrin 0.625% EC @1500ml/ha (Rs. 620/lit.), T_{10}- Control (Untreated check). Labour charge Rs. 167 per day per man, Price of paddy grain Rs. 1400/quintal.

- 2012 and *rabi*- 2013, respectively from the treatment T_2- flubendiamide 4% + buprofezin 20% SC @ 875ml/ha.

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Farmers' participatory approach using indigenous rice (*Oryza sativa* L.) crop diversity in mountain agriculture towards improvement of farm income

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ABSTRACT

A number of rice landraces known from Kashmir valley (India) mostly belong to short grained japonica types and some of them like MushkBudgi, Kamad, Nun Bouel, etc.are highly aromatic and the cooked rice is tasty with desirable texture. After successful revival programme of some elite rice landraces in 2013, the participatory trials were conducted during 2014 and continued during 2015 in association with farmers of the niche areas through a unique way of multi-varietal trials (Mother trials) and a single variety trials (Baby trials). Paired 't' comparisons illustrated significant yield advantage of improved Mushkbudji (IMB) and improved Kamad (IKD) of 17% and 22.8% over respective farmer's variety during 2014,whereas, the yield superiority of 31.6% and 26.5% was recorded during 2015. The preference for IMB was significantly high with respect to most of the traits. The economics of cultivation showed that IMB is the best commercial and competitive choice for the farmers providing a net benefit of Rs. 2.50 lacs /ha which is around 150% more than any non-aromatic variety. The study also revealed role of farmer's participation and market links in conserving and maintaining the rice biodiversity in the niche areas for future.

Key words: *Biodiversity conservation, farmers' evaluation, participatory varietal selection, rice landraces, temperate rice*

Farmers in marginal environments conserve the crop diversity just to sustain production under adverse environmental changes and to reduce the threat of crop loss during unfavourable cropping seasons. The cultivation of landraces at the cost of modern varieties reveals the farmers' eagerness to pay for the genetic diversity. The other reasons may be the unsuitability of modern varieties for such environments or existing variability is delivering higher monetary returns to farmers compared to modern varieties and sometimes cultural factors play the pivotal role in continuous cultivation of these landraces. Some of them are very much competitive to modern cultivars in certain niches and through simple breeding efforts and other research interventions such lines could broadly be promoted for similar agro-ecological areas for improving farmers' income. Such important cultivars could be sustainably conserved through market links because market growth and participation of farmers is directly linked to the economic development of villages (Bellen 2004; Brush 2004; Barrett 2008). Small land holding farmers dealing with poorly functioning markets get the low price virtually received by them as producers (Barrett 2008). In a well-functioning market, landrace can be competitive and perform well under improved management and can provide commercial opportunities to farmers.

More than 100 indigenous rice landraces have been documented from Kashmir valley of India suited to different agro-ecological niches possessed with combined adaptive traits for the temperate climate

(Najeeb *et al*. 2016). These landraces mostly belong to short grained *japonica* types and some of them are highly aromatic and cooked rice is tasty with desirable texture. Some aromatic rices like MushkBudgi, Kamad, Nun Bouel, etc. enjoy huge demand in local markets due to high consumer preference, and are consumed especially during matrimonial occasions and festivals. Subsequent to revival programme of elite rice landraces of Kashmir valley in 2013 as comprehensively discussed in earlier publication (Najeeb *et al*. 2016), farmers participatory varietal selection (PVS) programme was undertaken to study farmers preferences for the improved versions of some elite landraces and to assess their adoption rate, value of production and market links. Participatory rice improvement and utilizing biodiversity has been carried out by earlier workers (Wicombe *et al*. 2001; Wicombe *et al*. 1996; Gyawali *et al*. 2010). High indigenous genetic diversity of aromatic short grain rice cultivars is widely distributed in specific pockets of different states of India and are known for their specific adaptation and cultivation, however on the promotional facet very less attention has been paid towards the development of these types (Rani *et al*. 2006). Pure line approach combined with the farmer participation in the selection process resulted in selection of relatively high yielding pure lines of Kalajeera, Haladichudi, and Machhakanta after four cycles of selection (Pritesh *et al*. 2017). In this context, we describe here the participatory techniques used in on-farm testing of improved versions of elite rice landraces with farmers and same is described in the detail.

MATERIALS AND METHODS

Location of the study site

The study sites are located in tehsil Kokernag of district Anantnag (South province) of Kashmir valley (latitude 33.58 N^0, 75.30 E^0 longitude) at an altitude of 1980 m. The location is situated in the intermediate altitude range which is relatively colder than the lower belts but comparatively favourable compared to high altitude regions of Kashmir valley. The cold springs fed by the melting glaciers are the source of irrigation water in such ecologies. The farmers have generally small to marginal land holdings. The farming community in this region have conserved a substantial amount of rice crop diversity at community level.

Field trials

The participatory trials were conducted by the farmers in a system of multi-varietal trials (Mother trials) and a single variety trials (Baby trials) after raising the seedlings at central place of area of adoption of study. The vocabulary of the mother and the baby trials follow Snapp (1999).

The Mother trials

The mother trial was composed of some genetically improved landraces as test varieties (improvement made at Mountain Research Centre for Field Crops, Khudwani) and two farmer's varieties as checks and was planted as a single replicate and replication was provided by repeating the trial with different farmers in the same village. The plot size of each entry in each mother trial was 10 m^2. The mother trials were conducted during 2014 and repeated during 2015. These trials were planted and managed by the farmers under the supervision of researchers. Summary of the trials is described in Table 1.

The baby trials

These trials were conducted during *Kharif* 2014 and continued during *kharif* 2015. Each baby trial consisted of one test variety *viz.*, improved Mushkbudji (IMB) or improved Kamad (IKD) and compared with the farmer's claimed Mushkbudji (FMB) or farmer's Kamad (FKD). One Kanal of land (500 m^2) was allocated to each baby trial for making comparison with farmers own variety laid on the same area of land and were grown as paired plots. The area was demarcated by the researchers but planted and managed by the farmers using their own management and fertilizer inputs out of their available resources. The details of the trials are illustrated in Table 1.

Farmers cultivated the improved variety alongside farmer's own claimed variety in paired plots in the same field under their management. Due to the fact of more resources required for supervision only few mother trials were conducted compared to more number of baby trials. The methods as described by Joshi and Witcombe (1996) were followed in the participatory evaluation. About 18 out of 25 (72%) and 27 out of 43 (63%) baby trials of IMB were conducted along with FMB as the check during 2014 and 2015, respectively and rest of the trials carried out during the

Table 1. Summary of farmer participatory trials (mother and baby trials) conducted during *kharif* 2014 and 2015

Mother trials

District Anantnag	2014 Villages	No. of trials conducted	Varieties tested	2015 Villages	No. of trials conducted	Varieties tested	Trials supervised by	Trials managed by
	Sagam	3	1. IMB	Sagam	3	1. IMB	Researchers	Farmers
	Nagam	3	2. IK)	Nagam	3	2. IKD		
	Khalhar	3	3.Nun-Bouel	Khalhar	3	3.Nun Bouel		
	Danwtpora	3	4.Lul Anzul	Danwetpora	3	4.FMB(Check)		
	Tangpowa	3	5.Tangdhar Zag	Tangpowa	3	5.FKD(Check)		
	Hiller Bhai	3	6. FMB (check)					
			7. FKD)(Check)					
	Total trials	18	Total varieties including checks=07	Total trials	15	Total varieties including checks=05		

Plot size allotted for each entry=10 m^2 (Plot size of all mother trials were demarcated by the researchers) and net area of the trial 70 m^2 and 50 m^2 during 2014 and 2015, respectively.

Table 1 Conttd.

Baby trials

District Anantnag	2014 Village	Variety tested	No. of trials laid	Comparison	2015 villages	Variety tested	No. of trials laid	Comparison	Planned by	Managed by
	Sagam	IMB	3	IMB vs FMB	Nagam	IMB	3	IMB vs FMB	Researchers	Farmers
		IKD	3	IKD vs FKD		IKD	3	IKD vs FKD		
	Nagam	IMB	3		Khalhar	IMB	3			
		IKD	2			IKD	2			
	Nagam	IMB	3		Danwt-	IMB	5			
		IKD	0		pora	IKD	2			
	Khalhar	IMB	2		Lisser	IMB	3			
		IKD	1			IKD	2			
	Danwtpora	IMB	2		Tang-	IMB	6			
		IKD	2		powa	IKD	3			
	Lisser	IMB	1		Upper-	IMB	2			
		IKD	0		Hiller	IKD	1			
	Tangpowa	IMB	3		Hiller-	IMB	2			
		IKD	2		Bhai	IKD	0			
	Upper Hiller	IMB	1		Pirtakia	IMB	2			
		IKD	0			IKD	2			
	Hiller Bhai	IMB	1		Sof	IMB	1			
		IKD	0			IKD	1			
	Pirtakia	IMB	1							
		IKD	0							
	Sof	IMB	1							
		IKD	0							
		Total trials	25 (paired trials)			Total trials	43 (paired trials)			

Paired plots of one Kanal each (500 m^2); one for improved variety and other for check (for making comparison) were demarcated by the researchers at all trial sites

two years were of IKD with FKD as check.

Statistical analysis

In mother trials, three number of trials in each village were treated as blocks/replications. The grain yield (kg/plot) and maturity data of mother trials were subjected to ANOVA as an RBD. Pooled analysis was done using statistical software "Plant Breeding tools" version 1.4 (IRRI, 2014). Pooled least significant differences (LSD) were computed at different probability levels for comparing the mean yield of variation over all mother trials (Snedecor and Cochran 1973). Paired t-test comparisons were computed for baby trials to test the performance of improved variety in comparison to the farmer's variety as check (Snedecor and Cochran 1973). Preferential/qualitative assessment of IMB in comparison to FMB for eight mostly preferred traits under mountain agro-ecologieswere recorded as scores *viz*., "highly preferred", "equally preferred" and "not preferred"and the data was analysed by χ^2 analysis.

RESULTS AND DISCUSSIONS

Variance analysis for both the years were carried out and significant differences were noticed for the genotypes (germplasm lines) and test locations *viz*., different farmer's fields used in the present experiment (Table 2). Further the mean square due to interaction effect was also observed to be significant. Year-wise analysed data of mother trials illustrated that IMB and IKD were significantly higher yielders than their respective checks. The grain yield advantage of IMB was around 50% and 44% over FMB during 2014 and 2015, respectively. IKD showed a similar trend with a yield advantage of around 45 and 27% over FKD, respectively for the two years (Table 3). The lower yields in 2014 were the results of low temperature stress that prevailed for one week when the crop was in flowering to milking stage that significantly affected crop growth and yield. Further, both the improved varieties showed significant yield superiority over important landraces used as other test varieties in the study. Since the mother trials were under farmers levels of inputs over a wide range of land types and management regimes, so all these responses were the representative of entire niche area. In addition, both the improved varieties showed the maturity duration of 143-144 and 137-139 days in 2014 and 2015, respectively, however the durations were at par with the farmer's cultivars (FMB and FKD).

Quantitative data of baby trials

Paired 't' comparisons demonstrated significant yield advantage of IMB of 17% and 31.6% and of IKD it was 22.8% and 26.5% over their respective checks FMB and FKD, respectively during 2014 and 2015. However no significant yield superiority was observed for IMB over IKD during both the years (Table 4). The mother and baby trials methodology have been followed by earlier workers to popularize the varieties developed through participatory plant breeding (Virk *et al*. 2003; Gyawali *et al*. 2007; Joshi *et al*. 2007; Gyawali *et al*. 2010; Rafiq *et al*. 2016). Under participatory varietal selection farmers identify and ascertain the performance of varieties using knowledge and proven agronomic management in their own field conditions (Tiwari *et al*. 2004) and the end result of the idea is the faster identification and adoption of the varieties suiting farmers' situations (Tiwari *et al*. 2009). Ghislain *et al*. (2016) identified the best upland rice varieties that meet farmers' criteria using participatory varietal selection method in order to speed up their early adoption and therefore increase upland rice diversity. Similarly three genotypes were observed to be drought tolerant during participatory varietal selection (Mall *et al*. 2015).

Qualitative data of baby trials

Opinions were sought for farmer preferred traits from different number of farmers belonging to different villages of the study site. These were the farmers who

Table 2. Analysis of variance for grain yield and days to maturity recorded infarmer participatory trials (Mother trials) conducted during *kharif* 2014 and 2015.

Kharif 2014						*Kharif* 2015					
Source of variation	d.f.	Economic traits	M.S	F-value	Probability	Source of variation	d.f.	Economic traits	M.S.	F-value	Probability
Replications within locations	10	Grain yield	0.02	0.02	N.S.	Replications within locations	5	Grain yield	7.19	1.89	N.S.
		Maturity	1.86	2.77	<0.01			Maturity	7.62	13.1	<0.05
Locations	4	Grain yield	15.62	19.5	<0.01	Locations	4	Grain yield	32.5	8.57	<0.01
		Maturity	52.6	78.5	<0.01			Maturity	11.4	19.6	<0.01
Varieties	6	Grain yield	30.95	38.6	<0.01	Varieties	4	Grain yield	43.9	11.6	<0.01
		Maturity	19.7	29.4	<0.01			Maturity	15.1	26.0	<0.01
Location x Varieties	24	Grain yield	3.32	4.15	<0.05	Location x Varieties	16	Grain yield	22.8	6.01	<0.01
		Maturity	7.52	11.22	<0.05			Maturity	3.07	5.29	<0.01
Pooled error	60	Grain yield	0.80			Pooled error	20	Grain yield	3.79		
		Maturity	0.58					Maturity	0.67		

MS=mean squares; N.S.=non-significant; d.f. =degree of freedom

Table 3. Grain yield (kg/plot) and maturity duration of improved varieties along with other important landraces and farmer's varieties (as control) in mother trials conducted during *kharif* 2014 and 2015

	Year 2014				Year 2015				
Variety	GY/plot(kg) (Maturity DAS)	Superiority (%)	Superiority[c]	Rank	GY/plot(Kg) (Maturity DAS)	% Superiority	Superiority[c]	Rank	Mean Ranking
IMB	4.22±0.86 (144.7±2.0	44.1[a]	N.S.	1	6.06±0.54 (137.6±1.46)*	50.3[a]	N.S.	1	1
IKD	4.04±0.55 (143.5±2.55)	27.6[b]	N.S.	2	6.00±0.51 (139.2±1.80)	45.6[b]	N.S.	2	2
NunBouel	3.23±0.87 (150.9±2.02)	-	-	3	4.52±0.47 (146.2±1.02)	-	-	3	3
LulAnzyl	2.57±0.57 (151.1±1.80)	-	- -	6 -	-	-	-	-	-
Karnahi zag	2.52±0.45 (150.7±2.03)	-	-	7	-	-	-	-	-
Tandhar Zag	2.49±0.25 (150.2±1.90)	- -		8	-	-	-		
FMB	2.93±0.29 (142.6±1.95)	-	-	5	4.03±0.43 (137.3±1.0)	-	-	5	5
FKD	3.15±0.32 (143.2±1.53)	-	-	4	4.12±0.36 (140.1±1.50)	-	-	4	4
lsd (0.05 level)	0.30 (3.5)	-	-	-	0.43 (4.2)	-	-	-	-
CV (%)	14.5 (12.4)	-	-	-	12.1 (5.3)	-	-	-	-
Mean over all Trial	2.89±0.57 (147.1±3.22)	-	-	-	4.9±0.72 (138.5±2.98)	-	-	-	-

± represents standard deviation: *Figures in bracekets represent maturity duration, DAS=days after sowing; GY=grain yield IMB=improved Mushkibudji; IKD=improved Kamad FMB = Farmers' Mushkibudji, FKD=Farmers'Kamad a=Superiority over Farmers' Mushkibudji: b= superiority over Farmers' Kamad:c superiorityof IMB over IKD:

Table 4. Relative comparison for grain yield (q ha^{-1}) of improved landraces in on-farm trials (baby trials) conducted during *Kharif* 2014 and 2015.

Year	No. of baby trials*	Comparison	Mean grain yield (qa)	t-value	Prob.	Superiority (%)
2014	18	IMB Vs FMB	2.59±0.38 2.20±0.23[x]	4.6	<0.05	17.2
2015	27	IMB Vs F MB	2.83±0.40 2.15±0.25 [x]	5.8	<0.01	31.6
2014	7	IKD Vs FKD	2.58±0.20 2.10±0.15 [x]	8.3	<0.01	22.8
2015	16	IKD Vs FKD	2.72±0.45 2.15±0.32 [x]	9.2	<0.01	26.5
2014	4	IMB Vs IKD	2.59±0.38 2.58±0.20	1.05	0.30	N.S
2015	8	IMB Vs IKD	2.75±0.35 2.52±0.50	1.85	0.18	N.S

*Each paired trial were conducted by the same farmer qa refers to number of quintals recorded per Kanal (500m ^{2}) of land allotted for the trial x refers to yield data of farmer's variety conducted on the same area in the form of paired plot for comparison

participated in the varietal evaluation including the host farmers. Due to mono-cropping in temperate ecologies; the higher yield, good grain quality and early maturity besides aroma are being considered as most valuable traits in the rice variety by the farmers for faster adoption (Rafiq *et al*. 2016). After evaluation of IMB in comparison to FMB the responses from the participant farmers at selected baby trial site were recorded as highly preferred", "equally preferred" and "not preferred". The analysis was done by merging "equally preferred" and "not preferred responses into one class because of least response for latter class. The results demonstrated that the preference for IMB was significantly high with respect to most of the traits,

Table 5. Farmers' perception for IMB obtained during 2014 and 2015 from a sample of evaluating sites (baby trial planting sites)

S.No.	Trait	Year	No. of evaluative sites(baby trials)	n*	Improved variety preferred	Equally preferred	Check preferred	Percentage preference for the improved variety	x^2 value	Probability
1	Early maturity	2014	10	180	96	43	41	N.S.	2.43	0.15
		2015	12	136	72	58	6	N.S.	12.6	0.25
2	Tillering ability	2014	10	180	131	38	11	72.7	41.5	<0.01
		2015	12	136	98	25	13	72.0	32.7	<0.01
3	Plant height	2014	10	180	139	33	7	77.2	60.3	<0.01
		2015	12	136	85	41	10	62.5	22.8	<0.05
4	General phenotypic acceptability	2014	10	180	128	42	10	71.1	42.2	<0.01
		2015	12	136	102	28	6	75.0	39.4	<0.01
5	Straw yield	2014	7	33	30	3	0	90.9	22.9	<0.01
		2015	10	36	26	8	2	72.2	18.1	<0.05
6	Aroma	2014	5	10	10	0	0	100.0	-	-
		2015	7	16	16	0	0	100.0		
7	HRR	2014	5	10	10	0	0	100.0	-	-
		2015	7	16	16	0	0	100.0		
8	General cooking quality	2014	5	10	10	0	0	100.0	-	-
		2015	7	16	16	0	0	100.0		
9	Intention for growing the variety for next year	2014	5	10	10	0	0	100.0	-	-
		2015	7	16	16	0	0	100.0	-	-

*Total number of farmers including host farmers at all evaluation sites (selected baby trials) who participated in the performance assessment of IMB in comparison to FMB

however in case of maturity duration; response for IMB did not differed significantly from FMB (Table 5). For post harvest traits like head rice recovery (HRR), general cooking quality and aroma, not a single non-preferential scoring was observed against IMB. The 100 % response for intention to grow IMB for the next year can be imagined based on the fact that farmers purchased IMB seed at unsubsidized rates. No modern rice variety has so far completely substituted the local landrace possessing speciality traits like aroma together with good grain and straw yield (Rijal *et al*. 1998) in spite of this fact the value and utility of agro-biodiversity is emerging a growing concern at national and international levels. In fact less attention has been given to evaluate some important landraces with farmers (Sperling *et al*.1993; Joshi and Witcombe 1996; Witcombe *et al*. 2005; Gyawali *et al*. 2007) and there is lack of literature support on the use of them. The threat of extinction strongly advocates the need to understand the value of landraces in terms of market links and consumer awareness.

Economics of cultivation of IMB and IKD (improved landraces) was worked out in comparison to growing K-39, which is the most popular but non aromatic cultivar in the adopted area of the study. The cost of cultivation was estimated by considering prevalent market rates for land preparation, for labour hiring for all operations, cost of fertilizers, other inputs etc. Similarly returns were also estimated based on prevailing market rates of paddy and straw. After estimating the returns, it was found that the best commercial choice to the farmers in the study niche area is to grow IMB which provide a net benefit of Rs. 2.50lacs/ha which is around 150% more if variety like K39 is being grown (Table 6). The estimated benefit and B: C ratio was the highest by cultivating IMB (B: C 3.92:1) followed by IKD (B:C 2.69) and commercially a poor choice to farmers was to grow K 39 (B:C ratio 1.44). Before the launch of the revival programme of elite rice landraces, rough rice (paddy) of FMB and FKD were sold directly to the local millers at Rs. 2000/ q (approximately double than normal rices) or after getting milled from the local miller, the milled rice was being sold to the middlemen/brokers at Rs. 3000-3500/ q. Subsequent to revival programme and farmers awareness regarding the value of conserving agro-biodiversity, the improvement in the livelihood of the

Table 6. Economics of cultivating indigenous rice landraces in the identified niche areas

	Name of the variety		
	K- 39	***Mushkbudgi***	***Kamad***
Special trait	A non aromatic but most popular variety in mid altitudes of Kashmir valley (1800-1950 m amsl)	Highly priced aromatic rice landrace of Kashmir	Medium priced and moderately aromatic rice cultivar of Kashmir
Estimated cost of cultivation/ha(Rs. '000)	65.0	65.0	65.0
Returns			
Average grain yield (t/ha)	5.5	4.5	4.5
Average rice recovery (t/ha)	3.3	2.7	2.7
Price of paddy /ton (Rs. '000)	13.0	40.0	30.0
Income from paddy /ha (Rs. '000))	71.5	180.0	135.0
Averagestraw yield/ha (in bundles)	3000	2500	2500
Straw income/ha@Rs.20.00/bundle (Rs '000)	60.0	50.0	50.0
Total returns/ha if the produce *i.e.,* paddy is directly sold to rice miller (Rs.'000)	131.5	230.0	185.0
Net benefit/ha (Rs. **'000)**	**66.5**	**165.0**	**120.0**
B:C ratio	2.03	3.53	2.85
Total returns/ha (Rs. '000) if milled rice is being sold by the farmer directly in the local market .	99.0+60.0=159.0(@ Rs.3000.0/q milled rice)	270.0+ 50.0 =320.0 (@Rs.10000/q milled rice)	190.0+50.0 = 240.0 (@Rs.7000.0/q milled rice)
Net benefit/ha(Rs. **'000)**	**94.0**	**255.0**	**175.0**
B:C ratio	1.44 :1	3.92:1	2.69:1

farmers growing IMB was assessed through change in income levels. In this regard the feedback from the study site were received during 2015 and more than 100 IMB growing farmers from different villages of niche were asked to give their opinion regarding the price offered to IMB by different stakeholders and its marketing with respect to rough rice and milled rice (Fig. 1). The feedback showed that 45% farmers sold the rough rice to rice exporter at Rs. 4000/q on cash just at the time of threshing of crop. Furthermore, 31 % of the farmers sold the milled rice directly to their permanent customers in the urban areas of the valley on an average of Rs. 11350/q. Among the surveyed farmers 12.5% were found to be seed producers of IMB and had sold their produce as processed seed to University at Rs. 6600/q. Also 10% of the farmers reported that their produce was procured by State Department of Agriculture as processed seed at Rs. 6000/q. Less than 2% of the farmers informed that their produce was rejected only because of admixture. While making visits to participating farmers in the adoption area of the study, it was observed that area expansion under IMB from the farm saved seed had considerably increased because of high preferences from millers and customers and good market price for the variety compared to other rice varieties under cultivation. Before PVS study there was low uptake of IMB by the farmers to replace FMB and after the successful conduction of PVS programme, farmers were able to identify and select the better alternative and overcome the various limitations which made them to replace their unimproved versions. Cleveland and Soleri (2007) and Sperling *et al*. 2008 also reported that seed selection, saving from farm produce, acquiring healthy and quality seeds and seed production to

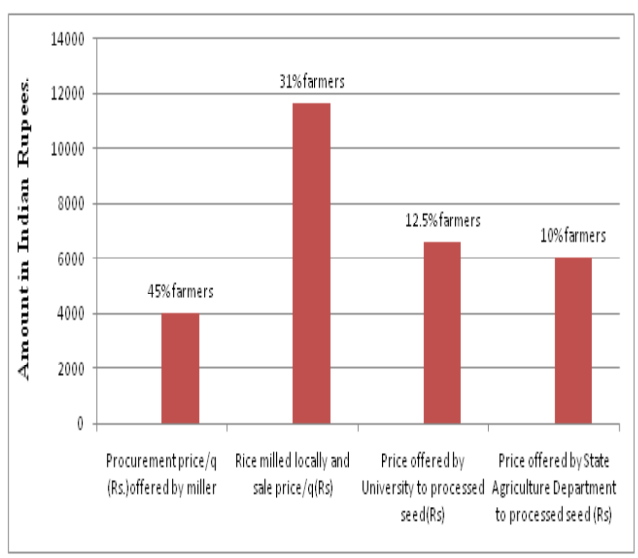

Fig. 1. Price offered to IMB bydifferent stakeholders

exchange besides, dissemination and popularization of the varieties are the outcome of farmer participatory approach. The undertaken programme in the adoption site has not only minimised the resources but has provides farmers in the cluster of villages of the niche an acceptable alternative. It has thus helped farmers not only to raise their farm income but has helped in conservation and enhancing the genetic diversity by adding the important and useful allelic resources to already existing rice germplasm base.

The present study reveals the role and importance of farmers' participation in evaluation and assessment of the variety in comparison to his own variety as check which in turn can play an effective and a significant role in faster adoption and popularization of improved variety/landrace. Further any kind of test material need to be evaluated both qualitatively and quantitatively for a number of traits from farmers' point of view. The study also demonstrated that cultivation of improved versions of aromatic rices are the best commercial choices for farmers in the identified niche areas to increase farm income and livelihood as compared to modern varieties.

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SHORT COMMUNICATION

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Characterization of rice (*Oryza sativa* L.) landraces and cultivars using agro morphological traits

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ABSTRACT

A set of 152 rice genotypes comprising of landraces, traditional varieties, exotic lines, cultures and released varieties collected from nine different states of India as well as from nine countries were evaluated for 12 agromorphological traits by principal component analysis for determining the pattern of genetic diversity and relationship among individuals. The largest variation was observed for the trait single plant yield with CV of 39.52% followed by single panicle sterile seed (35.41%) and single panicle fertile seed (29.34%). The trait panicle length exhibited the least variation with the CV of 14.86%. Principal component analysis revealed four significant principal components and accounted for a cumulative variation of 74.90%. The first principal component accounted for 34.97 %, second for 19.56%, third for 10.55% and fourth for 9.81% of total variation. Biplot exhibited convex of the hull which was occupied by the genotypes namely TKM3, ADT 47, CO 39, Nootripathu, Veeradangan, Cult 3000 and T1035 as these genotypes were exhibited highest level of variation. They can be used as diverse parents in crossing programme. Traits namely culm thickness, panicle length, days to heading, flag leaf length, plant height, width of flag leaf were discriminated the genotypes in higher degree. Cluster anlaysis identified five distinct groups among 152 genotypes which can be utilized for the trait improvement breeding programme.

Key words: *Rice, principal component analysis, genetic diversity, clustering, characterization*

Rice (*Oryza sativa* L.) is the major staple food for the most of the people in South East Asia in order to cater the need of burgeoning population, production level has to be increased tremendously. Local landraces are best adapted to their native environment and harbour many agronomical genes that can be utilized in the development of high yielding cultivars with tolerance to biotic and abiotic stresses (Lisa *et al*. 2011; Zhao *et al*. 2011). Landraces are considered as intermediate between cultivated varieties and their wild progenitors (Pusadee *et al*. 2009). These landraces are in extinction stage due to lack of adequate research attention. Characterization of landraces for the desired traits can lead to identification of new sources of tolerance to biotic and abiotic stresses in rice (Wang *et al*. 2007).

The principal component analysis (PCA) is one of the most successful techniques that have been used in germplasm characterization which involves cumbersome data. The purpose of PCA is to reduce the large dimensionality of the data space (*i.e*., observed variables) to the smaller intrinsic dimensionality of feature space (*i.e*., independent variables), which are needed to describe the data economically when there is a strong correlation between observed variables. Principal component analysis (PCA) is a classical statistical method based on linear transform which has been widely used in data analysis and comparison. Principal component analysis is based on the statistical representation of a random variable which involves the calculation of the eigen value decomposition of a data covariance matrix or singular value decomposition of a data matrix, usually after mean-centering the data for each attribute. It is the simplest of the true eigenvector based multivariate analyses. Often, its operation can be thought of as revealing the internal structure of the data in a way which best explains the variance in the data (Lenord and Peter 2009). The present study was aimed to understand the diversity present among the rice accessions using different agro morphological traits which will lead to understand relationship among the different genetic groups.

The germplasm collection of 152 rice accessions used in this study involving landraces and varieties collected from nine different states of India as well as from nine countries namely China, Columbia, India, Indonesia, Japan, Philippines, Srilanka, Taiwan and USA. It consisted of 38 landraces, 19 cultures, 12 exotic collections and 83 varieties. A set of 152 genotypes were grown in Paddy Breeding Station, Department of Rice, Tamil Nadu Agricultural University, Coimbatore, India during *Rabi* 2013. This area is situated at latitude of 11ºN and longitude of 77ºE with clay soil of pH 7.8. These genotypes were transplanted 20 days after sowing as two seedlings per hill in augmented design with a spacing of 20 x 20 cm. Each plot per accession consisted of five rows each of 2 m long and at a distance of 40 cm between the plots. Normal cultural practices were followed as per standard recommendation.

Principal component analyses (PCA) based on twelve quantitative traits was performed to find out the relative importance of different traits in capturing the genetic variation. The factors of these traits were used to determine the contribution of each factor towards variation. The standardized values were used to perform PCA using PAST 3 (Hammer *et al*. 2001). A scree plot was drawn from the eigen values associated with a component or factor in descending order versus the number of the component or factor. Scree plot used for visually assess which components or factors explain most of the variability in the data.

The genetic diversity maintained in a species is considered as a function of its ecological and evolutionary history (Hamrick and Godt 1996). The high genetic diversity among rice landraces and cultivars have been described in relation to agro-morphological traits (Singh and Singh 2009; Borkakati *et al*. 2000; Jegadeeswaran *et al*. 2014), morpho-physiological characters (Vairavan *et al*. 1973), enzymatic characters (Glaszmann *et al*. 1989), and molecular markers (Nachimuthu *et al*. 2015; Sarma and Bahar 2005; Bhuyan *et al*. 2007). The high genetic diversity among rice varieties in India is due to combined effect of wide eco-geographical conditions, diverse agro-ecosystems associated with various rice farming practices and diverse human cultural preferences.

In the present study, 152 accessions exhibited significantly varying performance for the twelve agro morphological traits (Table 1, Fig 1a & 1b). These traits namely culm thickness, heading date, plant height, flag leaf length, flag leaf width, panicle length, productive tillers, single panicle fertile seed, single panicle sterile seed, single panicle total seed, 1000 seed weight and single plant yield were observed. The largest variation was observed for the trait single plant yield with CV of 39.52% followed by single panicle sterile seed (35.41%) and single panicle fertile seed (29.34%). The trait panicle length exhibited the least variation with the CV of 14.86%. The genotype HKR47 recorded highest yield with mean of 31.71g and Cult14106 observed with lowest low (3.69g). The overall mean of germplasm accessions was 16.95g. The skewness and kurtosis coefficients were 0.09 and -0.64 respectively with the variation of 39.52 %. Single panicle sterile seed was highest in T2684 (96) and lowest in TN1 (12) with overall mean of 40.44. Positive skewness (0.65) and heavy tailed kurtosis (1.65) was observed for this character with a variation of 35.41%. The genotype TKM3 recorded highest mean of 260 for the trait single panicle fertile seed and Cult3000 was observed with lowest value 40. The overall mean of germplasm accessions was 138.42. The skewness and kurtosis coefficients for this character were 0.36 and 0.45 respectively with the variation of 29.34 per cent. Mean length of flag leaf was 27.91 cm with the minimum and maximum value of 14.15 cm in the genotype CO39 and 54.60 cm in Cult14106, respectively. It also showed higher coefficient of variation of 27.91 per cent with positive skewness (0.83) and kurtosis (0.40). The trait

Table 1. Variation in quantitative traits of rice genotypes

Variables	Mean	Min	Genotype	Max	Genotype	SD	CV (%)	Skewness	Excess Kurtosis
CD	4.38	2.66	Nootripathu	7.85	Cult14106	1.04	23.73	0.84	0.67
HD	100.69	68.00	CO39	185.00	Chethuvali, ASD9	25.03	24.86	1.09	0.61
PH	111.68	62.80	CO39	192.80	T1904	26.06	23.34	0.65	-0.23
FLL	30.44	14.15	CO39	54.60	Cult14106	8.50	27.91	0.83	0.40
FLW	1.10	0.65	TKM5	2.00	WGL14	0.21	18.96	1.01	1.99
PL	22.90	14.50	CO39	32.20	T1035	3.40	14.86	0.25	-0.10
PT	13.94	5.10	Uplri	23.80	T1915	3.52	25.27	0.47	0.02
SPFS	138.42	40.00	Cult3000	260.00	TKM3	40.62	29.34	0.36	0.15
SPSS	40.44	12.00	TN1	96.00	T2684	14.32	35.41	0.65	1.63
SPTS	178.86	89.50	Dular	313.00	TKM3	45.64	25.51	0.28	-0.28
SW	19.85	11.50	Whitesannam	27.60	Karanellu	3.60	18.16	-0.41	-0.68
SPY	16.95	3.69	Cult14106	31.71	HKR47	6.70	39.52	0.09	-0.64

CD: Culm diameter; HD: Heading date; PH: Plant height; FLL: Flag leaf length; FLW: Flag leaf width; PL: Panicle length; PT: productive tillers; SPFS: Single panicle fertile seed; SPSS: Single panicle sterile seed; SPTS: Single panicle total seed; SW: 1000 seed weight; SPY: Single plant yield

single panicle total seed was observed highest in TKM3 (313) and lowest in Dular (89.50) with overall mean of 178.9. Positive skewness (0.28) and light tailed kurtosis (-0.28) distribution was observed for the single panicle total seed with the variation of 25.51%. The larger variation was observed for number of productive tillers with coefficient of variation of 25.27 per cent. The genotype T1915 recorded maximum number of productive tillers per plant (23.8) and the genotype Uplri had registered less number of productive tillers (5). The overall mean was 13.94 productive tillers per plant. The skewness and kurtosis coefficients were 0.47 and 0.02 respectively which shows right skewed and platykurtic distribution. The genotype Chethuvali ASD9 had taken the longest duration for heading (185 days) and CO39 had shortest (68 days). The overall mean for heading for the germplasm accessions was 101days. The skewness and kurtosis coefficients were 1.09 and 0.61 respectively with the variation of 24.86 per cent. Overall mean for culm thickness of 152 germplasm lines were 4.38 cm with the minimum and maximum value of 2.66 cm in the genotype Nootripathu, CO39 and 7.85cm in Cult14106 respectively. It also showed higher coefficient of variation of 23.73 per cent with positive skewness (0.84) and kurtosis (0.67). The genotype T1904 was the tallest with mean height of 192.8cm and CO39 was shortest (62.8cm) with mean plant height of 111.68 cm was observed among the germplasm accession. A total

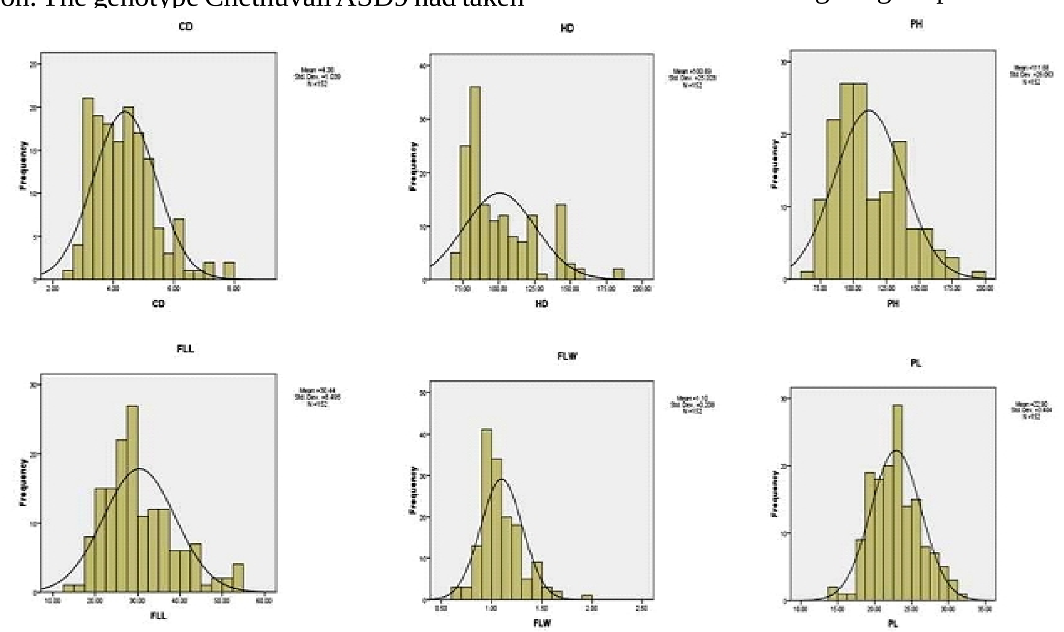

Fig. 1a. Frequency distribution of different quantitative traits

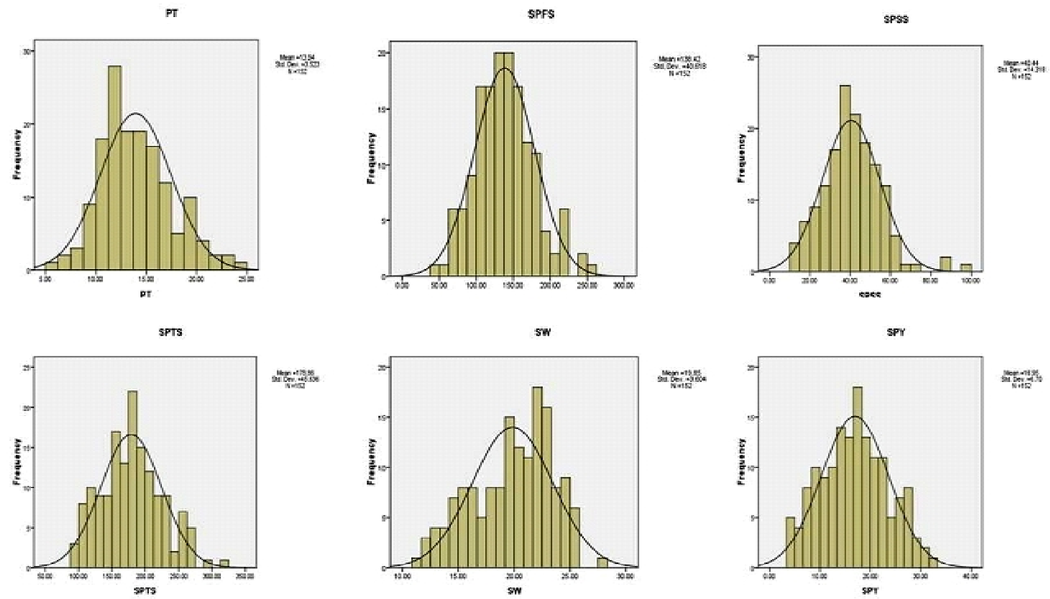

Fig. 1b. Frequency distribution of different quantitative traits

variation of 23.34 per cent with positive skewness (0.65) and negative kurtosis (-0.23) was observed. The trait flag leaf width was recorded the overall mean of 1.1 cm; the genotype WGL14 was broader (2cm) and TKM5 was narrow (0.65). It showed a variation of 18.96% with positive skewness (1.01) and kurtosis (1.99). The genotype Karanellu was heavy (27.6g) and whitesannam was lighter (11.5g) for the trait 1000 seed weight. Overall mean of 1000 seed weight was 19.85g with variation of 18.16 per cent. Left handed skewness (-0.41) and low tailed kurtosis (-0.68) distribution was observed. The genotype T1035 possessed long panicle (32.2cm) and CO39 was with smallest (14.5 cm) with overall mean of 22.9 cm. A total variation of 14.86 per cent with positive skewness (0.25) and negative kurtosis (-0.10) was observed.

Four significant principal components were identified and accounted for a cumulative variation of 74.90%. The first principal component accounted for 34.97 %, second for 19.56%, third for 10.55% and fourth for 9.81% of total variation (Table 2). The scores of quantitative traits were taken into account and subjected to PCA using PAST 3. Eigenvectors and principal components based on non-rotated loadings were estimated for all the traits (Table 2; Fig 2 & 3). First principal component (PC1) was correlated with culm thickness (0.43), panicle length (0.4), days to heading (0.39), flag leaf length (0.38), plant height (0.35) and width of flag leaf (0.34). Second principal component (PC2) was associated single panicle, total seed (0.58) and single panicle fertile seed (0.57). Third principal component (PC3) was related with productive tillers (0.64) and single plant yield (0.55). Fourth principal

Table 2. Eigen value and percent of total variation and component matrix for the principal component axes

PC	PC 1	PC 2	PC 3	PC 4
Eigen value	4.20	2.35	1.27	1.18
% variance	34.97	19.56	10.55	9.81
Cumulative %	34.97	54.53	65.08	74.90
	Component Matrix			
	PC 1	PC 2	PC 3	PC 4
CD	0.425	-0.058	0.039	-0.081
HD	0.394	-0.021	0.075	-0.248
PH	0.348	-0.232	0.128	0.157
FLL	0.383	-0.186	0.122	0.050
FLW	0.341	0.073	0.139	0.051
PL	0.403	-0.077	0.083	0.141
PT	-0.161	0.149	0.640	-0.235
SPFS	0.090	0.573	-0.070	0.267
SPSS	0.239	0.226	-0.414	-0.294
SPTS	0.155	0.581	-0.193	0.145
SW	-0.046	-0.212	-0.084	0.776
SPY	0.028	0.334	0.552	0.213

CD: Culm diameter; HD: Heading date; PH: Plant height; FLL: Flag leaf length; FLW: Flag leaf width; PL: Panicle length; PT: productive tillers; SPFS: Single panicle fertile seed; SPSS: Single panicle sterile seed; SPTS: Single panicle total seed; SW: 1000 seed weight; SPY: Single plant yield

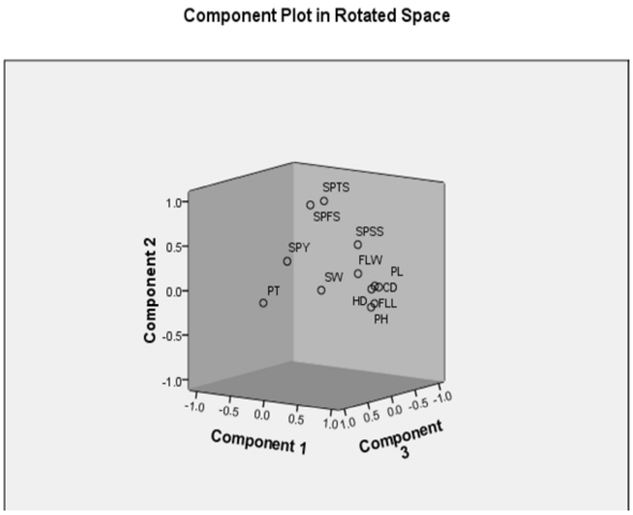

Fig. 2. Component plots for the twelve quantitative traits in Rice accessions

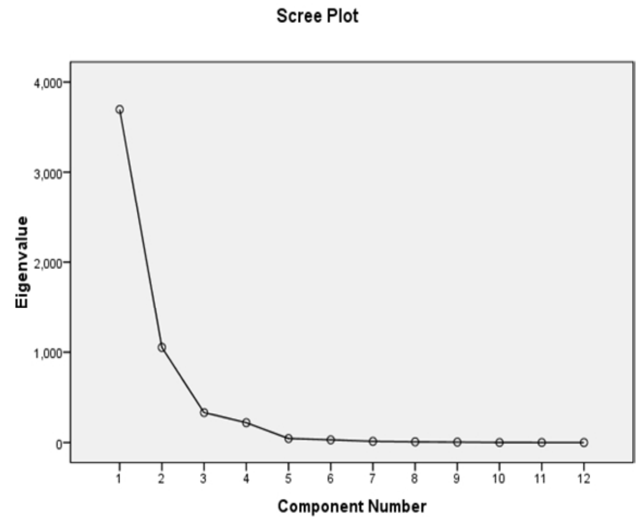

Fig. 3. Scree plot showing the eigen value variation for twelve quantitative traits in rice

component (PC4) was associated with 1000 seed weight (0.78). Nachimuthu *et al*. (2014) also obtained 80% variability contributed by five principal components in 192 germplams using 12 agro morphological traits.

A scatter plot was drawn using PC1 and PC2 factor scores and clear pattern of grouping of genotypes was observed in the factor plane (Fig 4). Convex of the hull was occupied by the genotypes namely TKM3, ADT 47, CO 39, Nootripathu, Veeradangan, Cult3000 and TJ035 as these genotypes were placed in the highest point on the hull. These genotypes were exhibited the highest level of variation. They can be used as diverse parents in crossing programme for exploitation of heterosis. A vector line drawn from the origin to the place where traits have been mapped. Based on the length of the vector, the traits namely culm thickness, panicle length, days to heading, flag leaf length, plant height, width of flag leaf were possessing lengthy vector (these traits would be used as salient descriptor for characterization), single panicle total seed and single panicle fertile seed were medium in length, productive tiller, 1000 seed weight and single plant yield were short.

Pearson correlation coefficient was worked out for 12 different morphological traits, among the inter correlation, 40 were showed significance (Table 3). The trait culm thickness positively and significantly correlated with heading date, plant height, flag leaf length, flag leaf width, panicle length and single panicle

Table 3. Correlation among twelve agromorphological traits

	CD	HD	PH	FLL	FLW	PL	PT	SPFS	SPSS	SPTS	SW	SPY
CD	1.00											
HD	0.77**	1.00										
PH	0.56**	0.49**	1.00									
FLL	0.65**	0.55**	0.68**	1.00								
FLW	0.57**	0.50**	0.34**	0.45**	1.00							
PL	0.64**	0.57**	0.62**	0.68**	0.52**	1.00						
PT	-0.25**	-0.13	-0.24**	-0.15	-0.22**	-0.25**	1.00					
SPFS	0.06	0.07	-0.12	-0.03	0.17*	0.07	0.05	1.00				
SPSS	0.36**	0.36**	0.16*	0.19*	0.23**	0.29**	-0.25**	0.20*	1.00			
SPTS	0.18	0.17*	-0.06	0.03	0.23**	0.16	-0.03	0.95**	0.49**	1.00		
SW	-0.08	-0.20*	0.11	0.03	-0.10	0.04	-0.19*	-0.09	-0.26**	-0.16*	1.00	
SPY	0.01	-0.02	0.01	-0.11	0.22**	0.08	0.30**	0.34**	-0.01	0.30**	-0.06	1.00

*Significance at 5%, ** Significance at 1%

CD: Culm diameter; HD: Heading date; PH: Plant height; FLL: Flag leaf length; FLW: Flag leaf width; PL: Panicle length; PT: productive tillers; SPFS: Single panicle fertile seed; SPSS: Single panicle sterile seed; SPTS: Single panicle total seed; SW: 1000 seed weight; SPY: Single plant yield

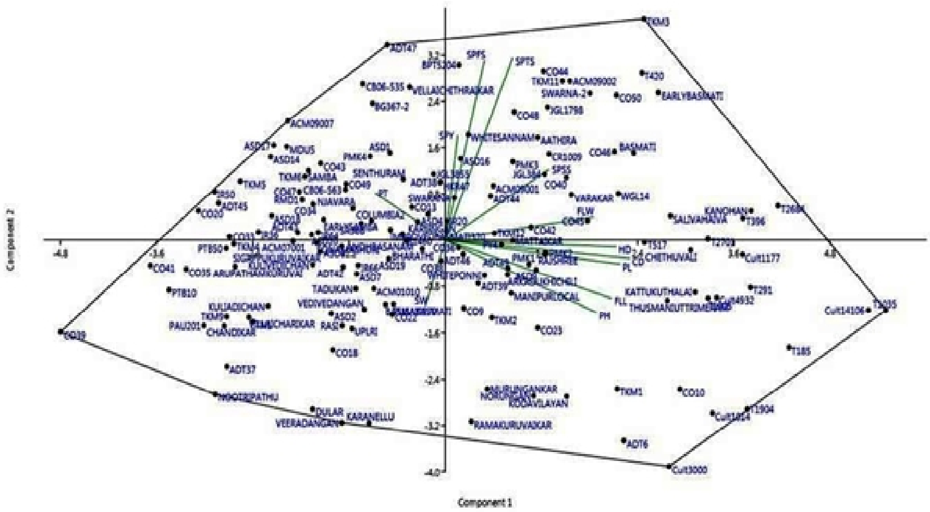

Fig. 4. Distribution of genotypes across the two components

sterile seed. However, it had significant negative correlation with productive tillers. The trait heading date significantly positively correlated with plant height, flag leaf length, flag leaf width, panicle length and single panicle sterile seed. But negatively correlated with 1000 seed weight. Plant height was positively and significantly correlated with flag leaf length, flag leaf width, panicle length and single panicle sterile seed. It was negatively correlated with productive tillers. Flag leaf length was significantly and positively correlated with flag leaf width, panicle length and single panicle sterile seed. Flag leaf width was negatively correlated with productive tillers, but in contrast it was positively associated with panicle length, single panicle fertile seed, single panicle sterile seed, single panicle total seed and single plant yield. Panicle length was positively correlated with single panicle sterile seed and negatively associated with productive tillers. Productive tiller was positively and significantly associated with single plant yield, it was negatively correlated with single panicle sterile seed. Single panicle fertile seed was positively and significantly associated with single panicle sterile seed, single panicle total seed and single plant yield. Single panicle sterile seed was positively and significantly associated with single panicle total seed, but negatively correlated with 100 seed weight. Single panicle total seed was positively and significantly associated with single plant yield and negatively correlated with 1000 seed weight.

The factors corresponding to four PCs were subjected to cluster anlaysis based on Euclidean

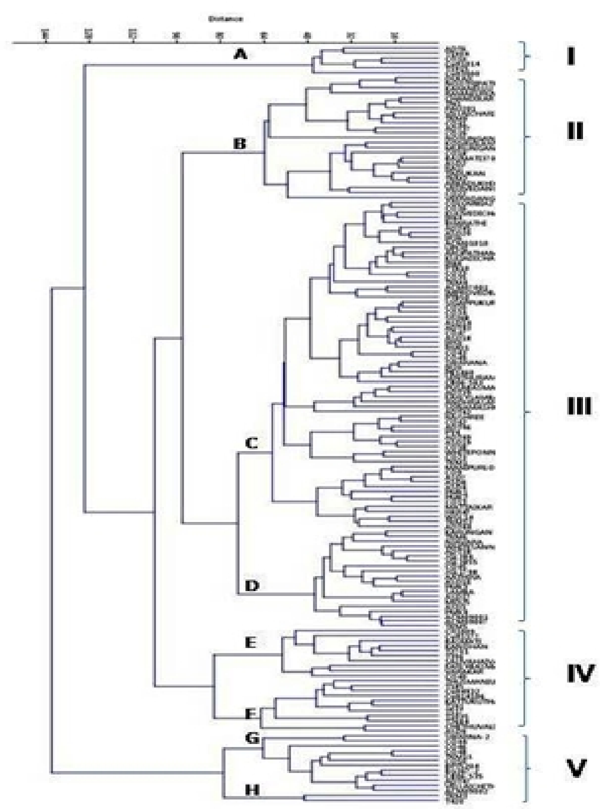

Fig. 5. Dendrogram based on morphological traits in Rice accessions

Table 4. Grouping of genotypes based on agro morphological traits

Group	Sub group	Genotypes	Salient characters
1	1	ADT6, T1904, CO10, Culture 1014, Culture 3000 and T1915	Higher plant height, flag leaf length and Panicle length Lower in single panicle. fertile seed, single panicle total seed and single plant yield
2	1	Dular, Nootripathu, Karanellu, Rama Kuruvaikar, Chandikar, TN1, Villaicharikar, TKM9, ADT37, CO39, CO41, Murungan Kar, Norungan, Kodavilayan, Basmati370, TKM2, ASD2, CO18, CO22, Rasi, Vedivedangan and Veeradangan	Higher productive tillers and 1000 seed weight. Lower culm thickness, heading date, flag leaf width, panicle length and single plant sterile seed
3	1	Columiba 2, CO36, Kulivedichan, IR64, Bharathi, ADT45, ASD20,IR36, ACM01010, Uplri, Arupathamkuruvai,Kuliadichan, IR66, PTB10, CO33, CO35, TKM4, ACM07001, Improved Basmati370 and PTB50	Lower plant height and flag leaf length
	2	SR36B, CO34, Sigappukuruvai Kar, CO20, ASD14, ASD18, IR50, RMD1, CO43, CO49, CO47, PB1460, Swarana, IR20, Senthuram and CB06-563	
	3	Pusa Basmati, ADT39, Early Samba, Andhra Sanam, Sonamashuri and ADT42	
	4	TKM1, CO23, White Ponni, CO38, ASD19, ADT49, PY4, ADT46, Rajshree and CO42	
	5	ASD4, ASD8, ASD7, Manipur local, CO9, PMK1, PMK2, Mattaikar, CO13, HKR47, TKM12, WGL14 and ADT44	
	6	Karungan, Njavara, White Sannam, TKM6, JGL1798, ADT38, JGL384, JGL3855, CO45, PTB51, MDU5, Samba, ASD16, PMK3, ASD1, ASD17, PMK4, ACM09001, ACM09007 and TKM5	
4	1	T2701, Kanohan, Basmati, CR1009, Culture 1177, Salivahaiva, Early Basmati, T396, Varakar and CO40	Higher culm thickness, heading date, flag leaf width and single panicle sterile seed
	2	Thusmani uttrimeram, T185, Culture 4932, Culture 14106, Kattu Kuthalai, T291, T517, T1035, T2684, Chethuvali and ASD9	Lower productive tiller
5	1	Swargathara, Vellaichithrai Kar, BG367-2, ACM09002, ADT43, ADT47, BPT5204, CO44, CO46, CO48, CO50, TKM11 and CB06-535	Higher single panicle total seed, single panicle fertile seed and single plant yield
	2	TKM3 and T420	

distances and grouped by unweighted paired group method using arithmetic average (UPGMA) using DARwin 5. The dendrogram depicted five distinct clusters (Table 4; Fig. 5). Group 1 consisted of 6 genotypes with characteristics of higher plant height, flag leaf length and panicle length. Group 2 had 22 genotypes with higher productive tillers and 1000 seed weight. A set of 88 genotypes were present in group 3. Group 4 had 21 genotypes with higher culm thickness, heading date, flag leaf width and single panicle sterile seed. Group 5 consisted of 15 genotypes with higher single panicle total seed, single panicle fertile seed and single plant yield.

The indigenous rice varieties cultivated by farmers in traditional tracts may contain huge agronomical genes that can serve as a source of germplasm to use in varietal improvement programmes. Landraces around centre of diversity and domestication are key natural resources important for maintaining the future food security in the current scenario of climate change (Pusadee *et al*. 2009). Conserving the existing various farmers varieties and landraces are greater priorities. Once the characterization has been done, it is very easy to maintain these landraces without any change in their genetic structure and also useful for plant varietal production in the era of intellectual property rights (IPR).

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SHORT COMMUNICATION

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Effect of varying sowing dates and nitrogen levels on growth and physiology of scented rice

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ABSTRACT

A field experiment was carried out to determine the influence of sowing dates and nitrogen (N) levels on different growth and physiological parameters of scented rice cv. Pusa Sugandh-3. Significant variation in terms of phenological stages and growing degree days (GDDs) accumulation on rice crop was recorded due to different sowing dates and nitrogen levels. The earlier sown crop took more number of days and GDDs to reach various phenological stages as compared to late sown crop. Among the nitrogen levels, 80 and 60 kg N ha $^{-1}$ took significantly more number of days and GDDs to reach different phenological stages. The 15th standard meteorological week (SMW) and 16th SMW sown crop recorded significantly higher number of primary and secondary tillers while tertiary tillers were significantly higher for 18th SMW crop. Pertaining to dry matter partitioning among different levels of nitrogen, significantly more photosynthates were translocated to leaves as well as stem from 45 DAT to harvest at the nitrogen level of 80 kg ha $^{-1}$ and least in control. Also, more photosynthates were translocated to panicle for 80 kg N ha $^{-1}$ and least for the control. At harvest significantly higher nitrogen uptake was recorded for the 15 th SMW (106.86 kg N ha $^{-1}$) and 16th SMW (103.64 kg N ha $^{-1}$) sowing. Nitrogen uptake was significantly higher for 80 and 60 kg N ha $^{-1}$.

Key words: *Dry matter partitioning, nitrogen uptake, phenology, Pusa Sugandh-3, sowing date, tiller count*

Rice (*Oryza sativa* L.), the single most important crop, is the primary food source for more than one third of the global population (Hasamuzzaman *et al*. 2009) and grown in 11 per cent of the world's cultivated area (Islam *et al*. 2009). The crop has wide physiological adaptation and is grown successfully in tropics, subtropics and temperate regions up to 2000 m above mean sea level (Okon *et al*. 1998). It has the capacity to grow under continuous flooded condition as well as mild to moderate water deficit stress (Kumar *et al*. 2016, 2017). It is endowed with amazing genetic diversity including more than one hundred thousand landraces and improved cultivars maintained in the germplasm collections spread world over. A unique sub-group that has distinguished itself as a result of natural and human selection, which found wider acceptance all over the world as a speciality rice is called "scented rice".

For utilization of solar radiation effectively and storage of ensuing photosynthate (assimilate), plants need a transport system to transfer assimilate from areas of synthesis to areas of consumption. Assimilate produced by green tissue is transferred all over the plant for growth, development, storage and cell maintenance. This division of assimilate among these processes, termed as partitioning, not only affects productivity but also survival of plant (Gardener *et al*. 2010). The duration of plant growth has two conspicuously different features, *i.e.*, phasic and morphological development. Phasic development comprises alteration in stages of growth and is almost invariably coupled with major changes in biomass partitioning patterns. Morphological development refers to the commencement and ending of development of various plant organs within the plant life cycle (Ritchie *et al*. 1998). The productiveness of rice depends not only on accrual of dry matter but also on its efficient partitioning to leaf, stem and grain as this is key to yield stability (Kumar *et al*. 2006). Numerical estimation of the impact of meteorological parameters on crop growth and development can help to realize the stabilization of crop production to great extent. The dry matter production and its partitioning into different plant parts provides a lucid insight of plant efficiency (Palit *et al*. 1976; Jand *et al*. 1994).

The sowing date of the rice crop is important for three major reasons. Firstly, it ensures that vegetative growth occurs during a period of satisfactory temperature and high levels of solar radiation. Secondly, the optimum sowing date for each cultivar ensures that the cold sensitive stage occurs when the minimum night temperatures are historically the warmest. Thirdly, sowing on time guarantees that grain filling occurs when milder autumn temperatures are more likely, hence good grain quality is achieved (Farrell *et al*. 2003). Owing to the short rice crop growing period under Kashmir conditions, synchronising of critical stages of the crop with optimal temperature regime is of utmost importance. Under field conditions the impact of temperature on phenology and yield of crop plants can be examined via accumulated heat unit system since plants require a definite temperature before they attain certain phenological stage (Rajput *et al*. 1987; Bishnoi *et al*.1995; Brar *et al*. 2011).

Application of nitrogen at right dose, right time and right source (Mohanty *et al*. 2017) is an important aspect of overall nitrogen management in aromatic rice for its efficient utilization, higher productivity and better quality. Excess application of nitrogen fertilizer can cause delay in crop maturity as well as high incidence of insect pest attack and lodging (Sidhu *et al*. 2004). Owing to translocation, nitrogen absorption by rice throughout vegetative growth stages promotes growth during reproduction and grain-filling stages (Norman *et al*. 1992; Bufogle *et al*. 1997; Wani *et al*. 2016). Information on the seasonal N uptake patterns and partitioning within the crop is valuable in assessing the amount, timing and method of N fertilization to prevent the occurrence of N deficiencies or over fertilization (Saito 1991; Islam *et al*. 1996; Mohanty *et al*. 2017). Keeping in view the aforementioned facts, the study, effect of varying sowing dates and nitrogen levels on growth and physiology of scented rice was undertaken.

The study was conducted at the Agronomy Farm of Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir during summer season 2013. The study area is situated at 34° 05' N latitude and 74° 89' East longitude having an altitude of 1587 m above mean sea level. The mean temperatures ranged from 13.11 ^{0}C and 26.90 ^{0}C, respectively. Twelve treatment combinations (D_1N_0, D_1N_1, D_1N_2, D_1N_3, D_2N_0, D_2N_1, D_2N_2, D_2N_3, D_3N_0, D_3N_1, D_3N_2, D_3N_3) of 3 sowing dates, *viz*., 15th, 16th and 18th standard meteorological week (SMW) at an interval of 10 days and 4 nitrogen levels ('0', '40', '60' and '80' kg N ha^{-1}), respectively were tested and randomized in split plot design with three replications. The soil of the experimental field was silty clay loam in texture with neutral pH, normal electrical conductivity (0.23dSm^{-1}), medium available nitrogen (407.68 kg ha^{-1}), low available potassium (178.08 kg ha^{-1}), high available phosphorus (26.57 kg ha^{-1}) and organic carbon (0.95%).

The nursery was raised in low polythene tunnels during a period of low temperature (<10°C). Forty days old seedlings were transplanted at a spacing of 15 cm × 15 cm. A uniform dose of phosphorous (P), potassium (K) and zinc (Zn) at the rate of 60 kg P_2O_5, 40 kg K_2O and 15 kg $ZnSO_4$ ha^{-1} was applied in all plots. The nitrogen was applied as per treatments in three splits. Entire quantity of P, K and Zn and 50% N was applied before transplanting and the remaining nitrogen was applied in two equal splits at mid tillering, *i.e.*, 30 days after tillering (DAT) and panicle initiation (60 DAT) stages as per treatments. Anthesis was determined when 50 % of panicles were visible in the centre of the plot. The crop reached physiological maturity when 95 % of spikelets had turned yellow. Number of days taken by the crop from each plot to reach mid tillering, panicle initiation, anthesis, milking, dough and maturity stage from the days after transplanting were keenly observed and recorded. Panicle initiation was determined by dissecting five main stems of plants from penultimate rows of each plot every other day after mid tillering to check for primordial growth. When 90 per cent of the main stems showed primordial growth, it was considered as the panicle initiation stage. Growing degree days (GDD) was calculated for different phenophases according to the equation developed by Summerfield *et al*. (1992). The plants used for dry weight from each plot were separated into leaves, culm (stem) and panicle to study the amount of translocates partitioned to different parts after taking the dry weight. The dry weight of each plant part (leaf, stem and panicle) from its respective treatment was measured in kg ha^{-1} and then converted to q ha^{-1}. Plant samples were collected at maximum tillering, panicle initiation, and harvesting stages of the crop at all the three sowing dates (Kumar *et al*. 2017). Nitrogen content in plant samples was estimated by modified Kjeldahl method (Jackson 1973). Nitrogen uptake was determined by multiplying dry matter accumulation at that particular stage by respective percentage of nitrogen content in plant samples and recorded in kg ha^{-1}.

Data were analysed using split plot design for analysis of variance (ANOVA). Significant ($P \leq 0.05$) differences between treatments were determined using critical difference. The software program used for the analysis was IRRISTAT data analysis package (IRRI 2000).

Days taken to different phenological stages

The phenological stages of rice crop *viz*., mid tillering, panicle initiation, flowering, milking, dough and maturity varied significantly due to effect of various treatments (Table 1). As far as sowing dates are concerned, earlier sown crop took more number of days to reach various phenological stages as compared to late sown crop. The 15th SMW sowing date took significantly highest number of days to reach mid tillering (39.97), panicle initiation (64.81), flowering (95.06), milking (105.04), dough (126.06) and harvest (135.06), whereas the 18th SMW sowing date took significantly least number of days to reach different phenological stages (Table 1). It might have been due to the higher temperature experienced by the 18th SMW sowing date during the vegetative stage which shortened its basic vegetative phase while the prolonging of the vegetative phase of the 15th SMW sowing might be due to lower temperature during initial growth stages. Sowing date primarily influences the length of vegetative period of rice with early sown rice requiring a greater number of days to accumulate the same number of degree days units compared with later sown rice was also reported by Norman *et al*. (1999). Lee *et al*. (2001) reported that days from sowing to flowering were shortened as sowing dates were delayed from 25th April to 5th June in the field and phytotron experiments. Linscombe *et al*. (2004) found that days from seedling emergence to 50 per cent panicle emergence decreased as planting was delayed. Dixit *et al*. (2004) ascertained that panicle initiation stage started late in early sown crop (5th and 10th June) and 50 per cent flowering was earlier in late crop (25th June). Chopra *et al*. (2006) reported that days to 50 and 100 per cent flowering were significantly affected due to delay in transplanting.

Among the nitrogen levels, 80 & 60 kg N ha^{-1} took significantly more number of days to reach different phenological stages while significantly lower number of days were taken by 40 and 0 kg N ha^{-1} to reach different phenological stages, indicating that higher doses of nitrogen increase the crop growth period. Delayed flowering with higher nitrogen dose may be due to more vegetative growth, as reflected by increased plant height (data not shown), which delayed maturity. Abou-Khalifa *et al*. (2007) found that maximum tillering, panicle initiation, heading date, crop growth rates, leaf area index, and grain yield increased with increased levels of nitrogen up to 165 kg N ha^{-1}. Mahajan *et al*. (2010) reported that the high level of N fertilizer (60 kg N ha^{-1}) delayed flowering by 2-3 days in 'Pusa 1121' and 'Punjab Basmati 2'; while in unfertilized plots, flowering was early by 2 days, irrespective of the cultivar used. However, the interaction was non significant.

Growing degree days (GDD) accumulated by crop to reach different phenological stages

With change in sowing dates and nitrogen levels, GDD accumulation also varied for the crop. Sowing dates behaved differently to the number of growing degree days required to reach various phenological stages (Table 2). No significant difference was noticed among all sowing dates as regards GDD accumulation to reach mid tillering and panicle initiation. However, treatments sown earlier *i.e*., 15th and 16th SMW required significantly higher number of GDDs to reach to different phonological stages *viz*., flowering, milking, dough and harvest stages whereas late sown

Table 1. Effect of sowing dates and nitrogen levels on phenology of rice

Treatment	Mid tillering	Panicle initiation	Flowering	Milking	Dough	Harvesting
Sowing dates						
15th Standard Meteorological week	39.97	64.81	95.06	105.04	126.06	135.06
16th Standard Meteorological week	35.99	60.06	92.05	102.31	124.06	132.06
18th Standard Meteorological Week	35.08	59.03	90.06	101.22	118.85	127.23
SEm±	0.23	0.35	0.59	0.35	1.09	1.29
CD ($p \leq 0.05$)	0.77	0.95	1.86	1.03	3.42	3.96
Nitrogen levels (kg ha^{-1})						
Control (N_0)	36.95	58.74	90.17	100.13	120.02	128.81
40 kg N ha^{-1} (N_{40})	36.97	59.78	91.23	101.36	121.15	130.12
60 kg N ha^{-1} (N_{60})	37.06	62.82	94.16	104.18	125.02	132.92
80 kg N ha^{-1} (N_{80})	37.06	63.85	95.20	105.23	126.15	133.95
SEm±	0.19	0.27	0.29	0.33	0.35	0.53
CD ($p \leq 0.05$)	NS	0.87	1.03	1.09	1.12	1.55
Sowing dates ×Nitrogen levels			NS			

treatments ie during 18th SMW required significantly the lower number of GDDs to reach different phenological stages. Since the duration to reach different phenological stages was more in case of 15th SMW sowing and least for the 18th SMW sowing date, hence 15th SMW sowing date required more GDDs to complete its growing cycle and least was required for the 18th SMW sowing date (Table 2). Chopra and Chopra (2004) reported that growing degree days from transplanting to maturity (total phenophases) got reduced almost linearly with delay in transplanting. Similar results were also reported Reddy *et al*. (2004). Accordingly, early sown crop accumulated more GDDs to reach different phenological stages compared to late sown crop. Brar *et al*. (2011) reported that 10 to 20 days delay in transplanting led to 13 and 24 days reduction in total growing cycle of the crop under June 25 and July 5 transplanted crops as compared to June 15 transplanted crop, respectively. Consequently, this led to reduction in accumulated GDDs to the tune of 86 and 20 heat units to attain the maturity under June 25 and July 5 transplanted crop as compared to June 15 transplanted crop, respectively.

Regarding the nitrogen levels, significantly more number of GDDs were required for higher nitrogen levels of 80 and 60 kg N ha^{-1} while significantly lower number of GDDs were accumulated by treatments receiving lower nitrogen levels *i.e.*, 40 and 0 kg N ha^{-1}, to complete the various phenological stages. This may be attributed to the fact that treatments (60 and 80 kg N ha^{-1}) received higher application of nitrogen took more days to complete different phenological stages and hence more growing degree days. Similar results were reported by Mahla *et al*. (2011). However, the interaction was non significant.

Dry matter partitioning (q ha^{-1})

The dry matter partitioning to leaf and stem were recorded at 15, 30, 45 and 60 DAT while from 75 DAT

Table 2. Effect of sowing dates and nitrogen levels on GDD's taken by rice to reach different phenological stages

Treatment	Mid tillering	Panicle initiation	Flowering	Milking	Dough	Harvesting
Sowing dates						
15th Standard Meteorological week	484.59	861.84	1292.05	1400.55	1598.96	1757.98
16th Standard Meteorological week	482.72	847.11	1271.52	1408.45	1558.47	1751.49
18th Standard Meteorological Week	480.38	834.08	1230.05	1389.50	1507.48	1660.49
SEm±	1.92	4.31	8.68	4.76	11.07	14.19
CD ($p \leq 0.05$)	NS	NS	26.81	14.55	33.21	43.14
Nitrogen levels (kg ha^{-1})						
Control (N_0)	482.31	823.18	1236.55	1393.16	1530.50	1701.16
40 kg N ha^{-1} (N_{40})	482.33	831.28	1248.78	1383.37	1540.67	1712.36
60 kg N ha^{-1} (N_{60})	482.80	864.06	1281.61	1405.58	1569.22	1734.25
80 kg N ha^{-1} (N_{80})	482.80	874.18	1292.88	1415.88	1579.50	1745.50
SEm±	1.3	1.18	6.23	3.67	9.35	12.11
CD ($p \leq 0.05$)	NS	3.66	18.84	11.19	28.43	36.71
Sowing dates ×Nitrogen levels			NS			

i.e., during reproductive phase, partitioning to leaf, stem and panicle was recorded (Table 3). Most of the dry matter (photosynthates) was partitioned to stem up to 60 DAT but the magnitude of difference of dry matter partitioned to stem and leaf decreased during later stages up to 90 DAT. Gardner *et al*. (2010) reported that during vegetative growth roots, stem and leaves are competitive sinks for assimilate. However, from 75 DAT to harvest a constant increase in dry matter partitioning towards panicle took place and was much higher as compared to the dry matter partitioned to leaf and almost comparable to the dry matter partitioned to stem. The manner in which the dry matter is distributed among different parts of the plants, determines the magnitude of economic yield (Kumar *et al*. 2017). This might be due to the fact that leaves which produce photosynthates get stored in stem of the plant due to which stem constitutes more portion of the plant weight, and the subsequent increase in leaf weight is due to the increase in the leaf number. Moreover, the increase in the weight of panicle from 75 DAT to harvest is attributed to the fact that apart from accumulation of photosynthates in panicle (sink) resulting from flag leaf photosynthesis, mobile carbohydrates, proteins and mineral nutrients from different sources are also moved to panicle during the grain filling stage. Jain (2016) reported that the amount of assimilates transported to harvest organ is much more in comparison to other organs of the plant.

Regarding the dry matter partitioning to stem among all the sowing dates, no significant difference was recorded up to 90 DAT but significantly higher dry matter partitioning was recorded for the 15th SMW and 16th SMW sowing dates as compared to the 18th SMW. While the dry matter partitioning to leaf was not found statistically different among all the sowing dates at all crop growth stages. Moreover, no significant difference was noticed in dry matter partitioning to panicle among all the sowing dates. Brar *et al*. (2011) ascertained that total dry matter accumulation and its partitioning was influenced significantly under three (15 and 25 June and 5 July) days of transplanting at various stages of the crop.

Pertaining to levels of nitrogen application, the partitioning of dry matter to stem was not significantly different up to 30 DAT, while highest dry matter partitioning to stem was found to occur from 45 DAT to harvest in the nitrogen level of 80 kg N ha^{-1} and least in control (Table 3). This might be due to the fact that more photosynthates were formed in the leaf of the plants receiving higher fertilization levels (N_1 and N_2) which were translocated to stem resulting in significantly higher dry matter partitioning of stem. The dry matter partitioning to leaf was not significantly different up to 30 DAT and at 105 and 120 DAT, but the dry matter partitioning to leaf from tillering to flowering stage *i.e*., at 45, 60, 75 and 90 DAT was significantly different among various nitrogen levels. The highest dry matter partitioning to leaf was found in the nitrogen level of 80 kg N ha^{-1} and least in control. However, 60 kg N ha^{-1} was at par with 80 kg N ha^{-1} at all growth stages. The differences in the dry matter partitioning to leaf at 45,

Table 3. Effect of sowing dates and nitrogen levels on dry matter partitioning (q ha^{-1}) of rice

Treatment	**Days after transplanting**							
	15		**30**		**45**		**60**	
	Stem	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**
Sowing dates								
15th Standard Meteorological week	0.82	0.21	4.88	1.50	21.75	9.24	38.79	21.74
16th Standard Meteorological week	0.82	0.22	5.36	1.51	21.97	9.27	38.71	21.77
18th Standard Meteorological Week	0.83	0.22	5.20	1.50	21.94	9.33	38.01	21.37
SEm±	0.01	0.03	0.08	0.05	0.63	0.41	0.64	0.69
CD ($p \leq 0.05$)	NS	NS	NS	N.S	NS	NS	NS	NS
Nitrogen levels (kg ha^{-1})								
Control (N_0)	0.81	0.22	4.63	1.45	18.92	8.09	34.59	19.46
40 kg N ha^{-1} (N_{40})	0.82	0.22	5.25	1.49	20.96	8.84	37.21	20.82
60 kg N ha^{-1} (N_{60})	0.83	0.22	5.32	1.52	23.03	9.71	39.93	22.46
80 kg N ha^{-1} (N_{80})	0.83	0.22	5.43	1.55	24.64	10.48	42.26	23.77
SEm±	0.01	0.02	0.17	0.06	0.65	0.51	0.69	0.80
CD ($p \leq 0.05$)	NS	NS	NS	NS	1.95	1.52	2.08	2.38
Sowing dates ×Nitrogen levels				NS				

60, 75 and 90 DAT might be due to the presence of higher leaf number per plant in the plots receiving higher doses of nitrogen, which consequently contributes to higher leaf dry weight. The dry matter partitioning to panicle was not found significantly different at 75 DAT but from 90 DAT to harvest, the higher dry matter partitioning to panicle was found in the nitrogen level of 80 kg N ha^{-1} and was significantly different from other nitrogen levels 40 and 0 kg ha^{-1}. The higher dry matter partitioning to panicle from 90 DAT to harvest might be due to the greater reserves of photosynthates present in the stem of the plants receiving higher nitrogen levels which were translocated to the panicle resulted in significant difference in the dry weight of panicle from the plants received lower nitrogen applications. Qinglin *et al*. (2000) reported that higher nitrogen application significantly enhanced dry matter partitioning at the vegetative stage. Moreover, leaf partitioning of absorbed nitrogen compared to dry matter was higher and varied little during early vegetative growth, but varied greatly from panicle initiation onwards probably due to competition for nitrogen among leaves, stem and the developing panicle. Azarpour *et al*. (2014) reported that the dry matter partitioning to various parts of the plant is influenced by the amount of nitrogen. However, the interaction was non significant.

Nitrogen content and uptake

The data on nitrogen content recorded at various phenological stages of the rice crop revealed that nitrogen content did not show any significant variation by altering the sowing dates and nitrogen levels (Table 4). Regarding nitrogen uptake there was no significant difference among the sowing dates at maximum tillering and panicle initiation stage (Table 5). However, at harvest significantly higher nitrogen uptake was recorded for the 15th SMW (106.86 kg N ha^{-1}) and 16th SMW (103.64 kg N ha^{-1}) sowing. The lowest nitrogen uptake was recorded for the 18th SMW sowing date (87.76 kg N ha^{-1}). Pandey *et al*. (2008) reported that nitrogen uptake in hybrid rice planted on July 5 or 20 was significantly higher compared to 5 or 20 August planting dates. Among levels of nitrogen significant difference for nitrogen uptake was recorded. The application of 80 kg N ha^{-1} recorded significantly highest nitrogen uptake at all the stages of crop growth while the level of 60 kg N ha^{-1} recorded significantly higher nitrogen uptake than 40 kg N ha^{-1}. The lowest nitrogen uptake was recorded in the control. The nitrogen uptake was significantly different among the nitrogen levels and increased with increase in nitrogen levels (Table 5). Increased nitrogen uptake with application of nitrogen levels from 0 to 80 kg N ha^{-1} might be due to increased root growth that absorbs more N from the soil at higher N level resulting in higher nitrogen concentration in dry matter. Also this might be attributed to the fact that high nitrogen uptake of the crop is favoured by additional supply of nitrogen during maximum growth phase. Tayefe *et al*. (2011) observed that total N uptake varied significantly with the increment of the amount of nitrogen applied. Rao et al. (2013) reported that at all growth stages nitrogen uptake was maximum at 240 kg ha^{-1} which was significantly superior over low level (120 kg N ha^{-1}). Moreover, nitrogen uptake increased with increase in the levels of nitrogen up to 240 kg ha^{-1}. The beneficial effect of increasing nitrogen levels on the nitrogen uptake was also reported by Ebaid and Ghanem (2000). The results were also in accordance with those of Srivastava *et al*. (2006), Zaidi *et al*. (2007), Prudente *et al*. (2008). Pandey *et al*. (2008) reported that nitrogen uptake increased significantly with increasing levels of nitrogen from 50-150 kg N ha^{-1}. However, the interaction was non significant.

Primary, secondary and tertiary tillers m^{-2} at the time of harvest

The data on primary, secondary and tertiary tillers m^{-2} recorded at the time of harvest is presented in Table 6. The highest number of primary tillers was observed in early sown crop *i.e.*, 15th SMW sowing date and lowest in late sown crop *i.e.*, 18th SMW which might be due to longer vegetative period of earlier sown date (18th SMW), which favoured primary tiller development. However, the 16th SMW sowing date was found at par with both 15th SMW as well as 18th SMW sowing dates. Pertaining to number of secondary tillers m^{-2}, significant differences were recorded among the sowing dates wherein 15th SMW and 16th SMW sowing dates recorded significantly higher number of secondary tillers m^{-2} and were at par with each other while lowest number of secondary tillers m^{-2} were recorded for the 18th SMW sowing date. On the contrary, 18th SMW sowing date recorded significantly higher number of tertiary tillers m^{-2} than other sowing dates. This may

Table 4. Effect of sowing dates and nitrogen levels on dry matter partitioning (q ha $^{-1}$) of rice

Treatment	Days after transplanting											
	75			90			105			At harvest		
	Stem	Leaf	Panicle	Stem	Leaf	Panicle	Stem	Leaf	Panicle	Stem	Leaf	Panicle
Sowing dates												
15th Standard Meteorological week	45.59	33.97	9.83	39.48	29.94	26.96	43.10	16.46	37.76	44.56	12.59	39.71
16th Standard Meteorological week	45.54	33.93	9.91	39.18	29.62	26.76	42.24	16.32	37.44	44.18	12.48	39.38
18th Standard Meteorological Week	44.15	32.73	9.52	37.17	28.11	25.38	40.04	15.44	35.42	41.78	11.80	37.24
SEm±	0.86	0.51	0.29	0.84	0.39	0.35	0.23	0.49	0.97	0.38	0.21	1.04
CD ($p \leq 0.05$)	NS	NS	NS	NS	NS	NS	0.91	NS	NS	1.48	NS	NS
Nitrogen levels (kg ha^{-1})												
Control (N_0)	41.05	30.58	8.96	35.12	26.55	23.99	37.87	14.63	33.56	39.60	11.19	35.29
40 kg N ha^{-1} (N_{40})	44.26	32.97	9.54	37.55	28.39	25.64	40.50	15.64	35.89	42.35	11.97	37.75
60 kg N ha^{-1} (N_{60})	46.76	34.84	10.08	40.28	30.45	27.51	44.14	16.75	38.43	45.35	12.81	40.42
80 kg N ha^{-1} (N_{80})	48.33	35.78	10.42	41.50	31.49	28.34	44.68	17.26	39.60	46.72	13.20	41.64
SEm±	0.78	0.80	0.67	0.90	0.86	0.84	0.87	0.80	0.79	0.88	0.80	0.76
CD ($p \leq 0.05$)	2.32	2.39	NS	2.69	2.56	2.51	2.59	NS	2.35	2.63	NS	2.27
Sowing dates ×Nitrogen levels						NS						

Table 5. Effect of sowing dates and nitrogen levels on nitrogen content and uptake (kg ha $^{-1}$) by rice

Treatment	N content (%)			N uptake (kg ha^{-1})		
	Maximum-tillering	Panicle initiation	Harvesting	Maximum-tillering	Panicle initiation	Harvesting
Sowing dates						
15th Standard Meteorological week	2.10	2.14	1.19	124.63	137.69	106.86
16th Standard Meteorological week	2.10	2.13	1.18	124.74	136.53	103.64
18th Standard Meteorological Week	2.11	2.13	1.18	125.12	134.08	87.76
SEm±	0.06	0.08	0.06	3.36	3.86	3.42
CD ($p \leq 0.05$)	NS	NS	NS	NS	NS	13.35
Nitrogen levels (kg ha^{-1})						
Control (N_0)	2.06	2.08	1.14	102.75	109.81	80.34
40 kg N ha^{-1} (N_{40})	2.11	2.14	1.20	118.19	130.86	93.90
60 kg N ha^{-1} (N_{60})	2.11	2.15	1.20	133.15	145.53	107.39
80 kg N ha^{-1} (N_{80})	2.12	2.15	1.21	145.24	158.20	116.04
SEm±	0.05	0.06	0.06	2.78	2.85	2.62
CD ($p \leq 0.05$)	NS	NS	NS	8.26	8.48	7.81
Sowing dates ×Nitrogen levels			NS			

be due to the fact that prevalence of lower temperature during the later stages might have favoured the tertiary tiller production. Oda and Honda (1996) also reported that the number of tertiary tillers increased with decreasing temperature.

Perusal of data with respect to different nitrogen levels indicated that primary, secondary and tertiary tillers m^{-2} recorded at the time of harvest were significantly different. The highest number of primary and secondary tillers (138.23 m^{-2}) was recorded for nitrogen level of 80 kg N ha^{-1}. However, the application of 60 kg N ha^{-1} was at par for the number of primary and secondary tillers m^{-2} with the 80 kg N ha^{-1} treatment. The lowest number of primary and secondary tillers was recorded for the control (128.51 m^{-2}). The

Table 6. Primary, secondary and tertiary tillers m^{-2} (at harvest) as influence by sowing dates and nitrogen levels

Treatment	Primary tillers (m^{-2})	Secondary tillers (m^{-2})	Tertiary tillers (m^{-2})
Sowing dates			
15th Standard Meteorological week	138.99	180.58	12.55
16th Standard Meteorological week	135.00	171.99	14.12
18th Standard Meteorological Week	127.00	154.09	24.37
SEm±	3.46	3.59	0.57
CD ($p \leq 0.05$)	10.48	14.11	2.23
Nitrogen levels (kg ha^{-1})			
Control (N_0)	128.51	147.73	14.30
40 kg N ha^{-1} (N_{40})	133.96	162.19	16.78
60 kg N ha^{-1} (N_{60})	134.62	175.84	17.93
80 kg N ha^{-1} (N_{80})	138.23	188.45	19.03
SEm±	2.88	3.75	0.72
CD ($p \leq 0.05$)	8.70	11.15	2.14
Sowing dates ×Nitrogen levels		NS	

highest number of tertiary tillers m^{-2} was also recorded for the higher doses of nitrogen used and lowest for the control treatment. The reason that could be attributed is the role of nitrogen in the growth and production of tillers. The beneficial effect of nitrogen on tillering and vegetative growth was reported by Reddy (1988). The higher tertiary tiller number recorded for the higher nitrogen levels might be due to the residual soil nitrogen present in these plots which favoured the tertiary tiller production at later stages of crop. Similar findings were also reported by Pramanik and Bera (2013). However, the interaction between sowing dates and nitrogen was non significant.

The study revealed that the days taken by the crop to reach various phenological stages and accumulation of GDDs were more in case of 15th SMW sowing and closely followed by 16th SMW while 18th SMW sowing recorded lowest values for the respective parameters. As far as nitrogen uptake is concerned, higher nitrogen uptake was recorded in the 15th and 16th SMW sowing and lowest for the 18th SMW sowing. The nitrogen content was not found significantly different among the sowing dates. Days taken to reach various phenological stages were more in case of higher nitrogen levels of 80 & 60 kg N ha^{-1} and lower in case of 40 kg N ha^{-1} and control. Nitrogen uptake was found highest in the nitrogen level of 80 kg N ha^{-1} and was closely followed by nitrogen level of 60 kg N ha^{-1}, while the lowest values were recorded for control. However, nitrogen content was not significantly different in all the nitrogen levels tested.

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SHORT COMMUNICATION

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Uptake of major nutrients by rice (*Oryza sativa* L.) as influenced by different levels of potassium and green manure at harvest stage

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ABSTRACT

A field experiment was conducted during kharif, 2015 at Agricultural college farm, Mahanandi to study the concentration and uptake of major nutrients by rice as influenced by green manure and different levels of potasium at harvest stage. The results revealed that yield, concentration and uptake of major nutrients by rice were increased with increasing levels of potassium up to 120 kg K_2O ha^{-1}. However, there were no statistical differences between three levels of K 40, 80 and 120 kg K_2O ha^{-1} in increasing yield, concentration and uptake of major nutrients. Application of green manure in situ (G.M) in combination with K recorded higher values of above mentioned parameters than when applied alone. The higher concentration and uptake of major nutrients were obtained with GM+120 kg K_20 ha^{-1} which was on par with GM+80 kg K_20 ha^{-1} and GM+40 kg K_20 ha^{-1}.

Key words: *Rice, green manure, yield, uptake of major nutrients*

Rice is an important food crop in the world. It is the staple food in South-East Asia and at present more than half of the world population depends on this crop. It is also one of the most important cereals in India and occupies second position in cultivation after wheat. Rice is one of the major field crops in Kurnool district and the crop is cultivated in an area of 91,568 ha (Department of Agriculture 2014). Incorporation of dhaincha at flowering stage before transplanting of rice was followed by most of the farmers in major rice growing areas of Kurnool district. The available potassium content was increased by the incorporation of dhaincha (Singh *et al*. 2009 and Singh *et al*. 2006). Hence judicious application of potassic fertilizer is required for better crop production were reported by Prasad (2014) and Swamanna (2015). Though much work has been reported on green manure in combination with N and P in rice crop but no investigation have been carried out in green manure along with K fertilizer of rice crop. Hence, present investigation will be carried out to know the yield, dry matter production, concentration, uptake of major nutrients and economics of rice as influenced by the different levels of potassium and green manure.

A field experiment was conducted at Agricultural College Farm, Mahanandi in Kurnool district of Andhra Pradesh during Kharif, 2015. The soils of experimental field was sandy loam with soil pH 7.97, EC 0.33 dSm^{-1}, organic carbon 0.55%, low in available N (239 kg ha^{-1}), high in P_2O_5 (82 kg ha^{-1}) and K_2O (1075 kg ha^{-1}) respectively. The eight treatments consisted of 0, 40, 80 and 120 kg K_2O ha^{-1} alone and in combinations with green manure, which were laid out in randomized block design and replicated thrice. Nitrogen in the form of urea was applied in three equal splits as basal, at tillering and at panicle initiation stages. Phosphorus in the form of single super phosphate was applied basally. Potassium in the form of muriate of potash was applied in two equal splits as basal and at panicle initiation stage as per the treatments. Green manure (dhaincha @ 5t ha^{-1}) was grown in the treatments T_5, T_6, T_7 and T_8 ploughed *in situ* at flowering one week before transplanting. The content of N, P and K in green manure was 3.5 %, 0.3 % and 1 % respectively. Plants samples were collected by destructive sampling at tillering and panicle initiation stage in m^{-2} area in each plot. At harvest stage the grains from each net plot was cleaned and sun dried until constant weight was recorded and expressed in kg ha^{-1} and the straw in each plot was allowed to dry in the field until a constant weight obatained and the field weight was recorded and expressed in kg ha^{-1}. The collected plants were dried in oven at 65^0C till constant weights recorded and expressed in kg ha^{-1}. After recording the dry weights, the straw and grain samples were grounded in a willey mill and were analyzed for the concentrations of major nutrients as per the procedures out lined by Tandon (1993). The uptakes of major nutrients were computed with using the formula:

$$\text{Uptake of nutrients (kg ha}^{-1}\text{)} = \frac{\text{Nutrient concentration (\%) x wt. of dry matter (kgha}^{-1}\text{)}}{100}$$

Yield

All the treatments recorded significantly higher grain and straw yield than control except T_2 (40 kg K_2O ha^{-1}) (Table 1). Grain and straw yield increased with increasing levels of K up to 120 kg K_2O ha^{-1}. However, there was no statistical difference between the three levels of K (40, 80 and 120 kg K_2O ha^{-1}) in increasing grain and straw yield. The increased grain and straw yield by the application of K fertilizer was due to the continuous supply of K during crop growth period which might be due to increased number of total tillers, dry mater accumulation, effective tillers, number and weight of filled grains. These findings are in close conformity with those of Meena *et al*. (2003).

Application of green manure in combination with K recorded higher grain and straw yield than when applied alone. The highest grain and straw yield were obtained with T_8 (GM + 120 kg K_2O ha^{-1}), but which were on par with T_7 (GM + 80 kg K_2O ha^{-1}) and T6 (GM + 40 kg K_2O ha^{-1}). Green manure in combinations with K fertilizers increased the grain yield due to long stature of plants, higher number of tillers m^{-2}, higher dry matter production. Green manure in combinations with K fertilizers increased the straw yield due to the highest plant height and dry matter production were associated with these treatment. This might be due to immediate release of nutrients through inorganic sources and later by mineralization of nutrients through green manure leading to steady supply of nutrients. Similar findings were reported by Sharma *et al*. (2001) .

Nitrogen concentration and uptake

Application of K fertilizers gradually increased the concentration and uptake of nitrogen in both grain and straw up to 120 kg K_2O ha^{-1} (Table 1). However, 80 and 120 kg K_2O ha^{-1} both are equally effective in increasing in concentration and uptake in grain and straw of rice crop. The higher values of concentration and uptake of nitrogen with K addition could be attributed to enhanced vigour of crop growth with

Table 1. Effect of different levels of potassium and green manure on Yields, N, P, K Concentration and Uptake of paddy at harvest stage

Treatment	Grain yield (kg ha-1)	Straw yield (kg ha-1)	Concentration (% N)		Uptake of N (kg ha-1)		Concentration (% P)		Uptake of P (kg ha-1)		Concentration (% K)		Uptake of K (kg ha-1)	
			Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
T_1 : (Control)	5008	6173	1.28	0.55	64.28	33.85	0.24	0.14	12.01	8.84	0.32	1.31	15.93	80.87
T_2 : 40 kg K_2O ha^{-1}	5281	6716	1.47	0.74	77.75	49.85	0.31	0.17	16.56	11.41	0.33	1.36	17.26	91.34
T_3 : 80 kg K_2O ha^{-1}	5433	7664	1.50	0.92	81.51	70.55	0.34	0.18	17.75	14.05	0.36	1.36	19.56	104.44
T_4 : 120 kg K_2O ha^{-1}	5517	7830	1.61	0.97	88.95	76.69	0.35	0.19	19.30	15.11	0.38	1.40	20.76	112.68
T_5 : GM (dhaincha) in situ only	5473	8979	1.65	1.02	90.14	93.51	0.37	0.24	20.43	21.65	0.39	1.49	21.51	133.41
T_6 : GM+ 40 kg K_2O ha^{-1}	5551	9617	1.73	1.06	96.17	102.19	0.39	0.25	21.81	24.04	0.40	1.56	22.39	149.05
T_7 : GM+ 80 kg K_2O ha^{-1}	5671	10403	1.99	1.09	110.62	113.54	0.40	0.29	23.42	30.45	0.48	1.57	27.03	163.37
T_8 : GM+ 120 kg K_2O ha^{-1}	5748	10931	2.03	1.11	116.87	120.02	0.43	0.31	24.72	33.68	0.49	1.60	28.17	176.83
SE(m)±	95	465	0.05	0.06	3.09	3.88	0.01	0.01	0.49	1.35	0.01	0.01	0.52	6.88
CD(p=0.05)	292	1424	0.14	0.18	9.46	11.87	0.03	0.02	1.50	4.15	0.02	0.04	1.58	21.08
CV %	3.0	7	4.73	10.66	5.89	8.14	8.99	4.76	4.34	11.79	3.17	1.64	4.14	9.42

increased nitrogen utilization and translocation in to the plant resulting in the enhancement of yield similar findings were also reported by Sharma *et al*. (2003) with potassium application in cauliflower and onion.

Green manure either alone or in combination with K fertilizer showed higher values of nitrogen concentration and uptake both in grain and straw than when K fertilizer alone. The highest concentration and uptake of nitrogen in grain and straw was observed with T_8 (G.M +120 kg K_2O ha^{-1}) but it was on par with T_7 (G.M +80 kg K_2O ha^{-1}). This was attributed to the added advantage that green manure application increased soil physical, chemical and biological properties resulting in creation of favorable conditions suitable for better root growth and proliferation lead to higher absorption of water and nutrients. Due to the steady and continuous availability of nitrogen in the rhizosphere coupled with enhanced dry matter production resulted in higher uptake of nitrogen. These results were in conformity with findings of Singh *et al*. (2002).

Phosphorus concentration and uptake

The concentration and uptake of phosphorus also showed the same trend as that of concentration and uptake of nitrogen in grain and straw (Table 1). The phosphorus concentration increased at 150 % RDK (120 kg K_2O ha^{-1}) over control was 45.83 percent and 35.71 percent in grain and straw respectively. Similar increase in P uptake with increasing levels of K application was reported by Surekha *et al*. (2003).

Similar to N, the P concentration and uptake showed higher values with green manure either alone or in combinations with K fertilizer than K fertilizer alone. The highest concentration and uptake of phosphorus in both grain and straw was observed under T_8 (G.M+120 kg K_2O ha^{-1}), but it was at par with T_7 (G.M + 80 kg K_2O ha^{-1}).The concentration and uptake of P increased due to the application of green manure along with inorganic fertilizer were also reported by Singh *et al*. (2006).

Potassium concentration and uptake

Application of potassium gradually increased the concentration of rice crop at all the stages of crop growth up to 120 kg K_2O ha^{-1} (Table 1). Similar increase in concentration of K due to the levels of K fertilizer application in rice crop was also reported by Surekha *et al*. (2003) and Swamanna (2015).

Green manure either alone or in combination with K fertilizer showed higher values of concentration than K fertilizer alone. The highest potassium concentration was observed under T_8 (G.M +120 kg K_2O ha^{-1}) which was at par with T_7 (G.M +80 kg K_2O ha^{-1}) at all the stages of crop growth.

Uptake of K increased with increase in rates of application of K and also due to green manure incorporation. Among all treatments the highest K uptake was observed with T_8 (G.M +120 kg K_2O ha^{-1}) which was on par with T_7 (G.M +80 kg K_2O ha^{-1}) at all the stages of crop growth.

The increased uptake of N, P and K due to the application of green manure along with K fertilizer might be due to the role of organic matter in supplying nutrients as well as improvement in the physical properties and water holding capacity of soil, which in turn brings the nutrients in to soluble and available form. The positive effects of organic manures on nutrient availability and their extraction due to increased activity of roots seemed to have improved the nutrient status of the plant roots. The variation in N, P and K uptake among different treatments might be due to their inherent capability to supply nutrients during the crop growth period, which in turn influenced the dry matter production and hence nutrients uptake by plants. These results were in accordance with findings of Kavitha and Rao (2010).

The results concluded that the highest yield, concentration, uptake of major nutrients increased with increasing levels of K up to 120 kg K_2O ha^{-1} but significant difference was observed at 80 and 120 kg K_2O ha^{-1}. Application of green manure in combination with K fertilizers recorded higher yield, concentration and uptake of major nutrients were obtained with incorporation of green manure as dhaincha (GM) + 120 kg K_2O ha^{-1} but which was on par with GM+80 kg K_2O ha^{-1} and GM + 40 kg K_2O ha^{-1}. Hence, the incorporation of green manure (dhaincha) at flowering stage before transplanting along with 40 kg K_2O ha^{-1} may be recommended for rice crop. However, the results will have to be confirmed by conducting extensive field trials in farmers fields on long term basis.

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SHORT COMMUNICATION

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Planting techniques on productivity of organically grown scented rice (*Oryza sativa* L.) in Assam

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ABSTRACT

A field experiment on scented (joha) rice var. 'Badshahbhog' was carried out in the organic block located at Instructional-cum Research Farm of Assam Agricultural University, Jorhat during kharif seasons of 2013 and 2014 to evaluate the effects of 3 planting patterns and 5 staggered planting with different types of seedling under rainfed condition. Among different planting patterns grain yield was recorded highest for rectangular planting (1.40 t/ha) followed by skip row planting (1.37 t/ha). Among different planting dates, transplanting on 10th September with 70 days old (35+35 days) double planted seedlings (DPS) produced the highest grain yield of 1.61t/ha with H.I of 37.25 per cent On the other hand, the straw yield was recorded lowest on 10 September planting with (35+35 days)double planted seedlings. The highest straw yield was recorded on 1 August planting using 30 days nursery seedling (DNS) and decreased with the advancement of planting dates. The increase in grain yield with 10th September planting over 1st,10th, 20th and 30th August planting was 7.83,17.39, 22.36 and 27.95 %. Total uptake of N, P and K by grain + straw recorded the highest when planting was done on 1st August using 30 DNS. The highest value in terms of net returns and benefit-cost ratio were recorded on 1 st August planting followed by 10th September planting.

Key words: *Planting techniques, productivity, scented rice*

The scented (*joha*) rice of Assam, India is known for its unique aroma, superfine kernel, good cooking quality and excellent palatability. The agriculture in Assam is still under the natural makeup without much degradation of ecosystem as crop pressure on soil is still less (142% cropping intensity) and use of agrochemicals are also below toxic levels. Presently, organic agriculture has come out to be the viable alternative for quality food production, eco sustainability, soil and human health issues along with other social and cultural issues. It is now established globally that organic farming can improve the quality of scented rice (Das *et al*. 2010). In Assam, more than 80% rice area is situated under high risk, ecologically handicapped rainfed ecosystem and due to vagaries of monsoon, particularly late receipt of monsoon showers during *kharif* season and early floods, farmers find it difficult to decide whether they would transplant with aged seedlings or grow new seedlings. Selection of rice variety for late transplanting with aged seedling is important for higher production (Choudhary *et al*. 1997, Kurmi *et al*. 1993; Nayak and Choudhary, 1997). The practice of double transplanting of rice avoids ill effects of over-aged seedlings in the nursery and it is also useful in seedling scarcity situations and can cover 8-10 times more area as compared to normal planting with nursery raised seedlings. In double transplanting practice, uprooting of seedlings is done carefully after 30-35 days from the first nursery and transplanting is done in second nursery at closer spacing (10cm x10cm) with 2 seedlings per hill after puddling. The hills from second nursery are uprooted again after 30-35 days. Tillers are separated out from the hills and planting is done in the main field as per recommended practice.

Some reports suggested that skip row planting produces as much rice yield as conventional planting during the wet season. Also, border method may enable the farmers to economize fertilizer and seed by 25per cent by leaving every fourth row unsown and unfertilized (AICARP 1986). Rice is highly sensitive to diverse ecological situation, as such seedling age and specific row arrangement at transplanting play a crucial role in realizing the potential yield. No precise work on these aspects have been done so far in Assam. Hence, the present study was undertakento find out proper planting pattern in relation to planting date and performance of staggered planting on growth and yield of scented rice by manipulating planting pattern anddates of planting.

The field experiment was carried out in the organic block of Assam Agricultural University, Jorhat during *kharif* season of 2013 and 2014 under rainfed condition. The soil collected from 0-15 cm depth was sandy loam with pH 5.9, organic carbon 0.53%, available nitrogen 269.96 kg/ha, available phosphorus 23 kg/ha and available potassium 175.05 kg/ha. Total rainfall received during the crop growth period was 1171.43 and 1285.58 mm distributed in 68 and 72 rainy days during 2013 and 2014, respectively.

The seedlings of *joha* rice var. Badshahbhog were planted staggering under five dates of planting (1, 10, 20, 30 August and 10 September) with increasing age of seedlings, starting from 30 days of nursery seedling (DNS) up to 60 DNS at an interval of 10 days and subsequently to 70 days (35+35 days) using double planted seedling (DPS) on 10 September in three planting patterns *viz.*, rectangular (20×15 cm), square (20×20cm) and skip row (3:1, 20×15cm). The experiment was laid out in split-plot design, keeping planting patterns in main plot and staggered plantings with type of seedlingin sub-plot, with 3 replications. Plant height of randomly selected tillers from each treatment was recorded from soil level to the tip of flag leaf with the help of a meter scale. At maturity plant samples from each plot were harvested manually and separated into straw and panicles. The dry weight of straw was determined after oven drying at 70°C to constant weight. All agronomic parameters, yield and yield attributing characters were recorded following standard procedures (Kumar *et al*. 2016, 2017).

Effect of planting pattern

All the growth and yield attributing characters of scented (*joha*) rice were influenced significantly due to staggered planting, but remained unaffected with planting patterns (Table 1). All the yield attributes such as length of panicle, weight of panicle, filled grains per panicle and 1000 grains weight were not influenced significantly due to different planting patterns. However, the length of panicle, filled grains per panicle and 1000 grains weight were recorded higher in skipped row planting (3:1; 20×15 cm). On the other hand, weight of panicle was obtained higher in rectangular planting with

Table 1. Growth and yield attributing characters of scented rice as influenced by planting pattern and staggered planting with type of seedling (mean data of 2 years)

Treatment	Plant height (cm)	Flag leaf area(cm^2)		No. of tillers/ hill	Length of panicle (cm)	Weight of panicle(g)	No.of filled grains/ panicle	Per cent unfilled grains/ panicle	1000 grains weight (g)
		Flowering	Harvest						
Planting pattern									
Rectangular(20x15cm)	120.04	14.93	10.15	6.82	24.58	1.49	119.95	10.41	12.17
Square (20x20cm)	119.95	16.97	10.98	7.19	25.68	1.47	123.79	10.00	12.28
Skip row (3:1;20x15cm)	121.91	16.27	11.38	6.86	5.34	1.43	124.60	11.48	12.34
SEm ±	1.331	0.304	0.239	0.249	0.146	0.036	3.857	0.284	0.151
CD (P= 0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS
Staggered planting with type of seedling									
1 August (30 DNS)	124.25	17.01	11.44	7.64	24.76	1.42	117.24	9.36	12.08
10 August (40 DNS)	124.43	15.81	10.22	7.39	24.84	1.37	120.97	9.42	12.17
20 August (50 DNS)	119.66	15.67	10.57	7.03	24.53	1.30	117.49	11.07	12.13
30 August (60 DNS)	113.62	14.79	10.24	6.68	23.09	1.08	91.78	13.70	12.22
10 September (70 days DPS)	121.22	16.98	11.72	6.03	27.12	2.15	166.42	9.61	12.71
SEm ±	1.085	0.546	0.366	0.213	0.235	0.054	2.959	0.211	0.150
CD (P= 0.05)	3.17	1.59	1.07	0.62	0.68	0.16	8.64	0.62	0.4

*DNS: Days nursery seedling, DPS: Double planted seedling, NS: Non-Significant a spacing of 20×15 cm. The higher yield attributes in closer spacing of 20×15 cm either in rectangular or skip row planting might be due to the fact that closer spacing produced less number of tillers per hill which may developed better and more stronger plants resulting better development of yield attributes. Among the three planting patterns, rectangular (20×15 cm) planting produced the highest grain and straw yields (Table 2) over the other planting patterns . The higher grain and straw yield in closer planting pattern of 20×15cm (rectangular) might be due to higher number of total tillers per unit area, more ear bearing shoots per m^2 and more number of functioning leaves per hill as well as better development of yield attributes. This might be also due to more number of hills per hectare (3.33 lakh) in rectangular planting as compared to other two planting patterns where 2.5 lakh hills/ha were accommodated. Similar findings have also been obtained by Balasubhramanian and Palaniappan (1991), Gupta and Sharma (1991), Padmaja and Reddy (1998), Patra and Nayak (2001) and Powar and Deshpande (2001). The residual available N, P and K contents and their uptake were not influenced significantly due to different planting patterns (Table 3).

Effect of staggered planting with type of seedling

Effect of different planting dates with type of seedling on growth and yield attributing characters of rice such as length of panicle, weight per panicle, number of filled grains per panicle and 1000-grain weight were significantly higher when 70 days old (35+35 days) double planted seedlings (DPS) were planted on 10 September than the 30, 40, 50 and 60 days nursery raised seedlings planted on 1,10, 20 and 30 August, respectively. All the yield attributing characters decreased with delay in transplanting dates and use of aged nursery seedlings for planting from 1st August to 30th August. Similar findings were also reported by Babu (1988).

The grain yield and harvest index were significantly higher in double planted seedling (DPS) transplanted on 10th September and lowest was on 30th August planting with 60 days nursery seedling. Increase in grain yield on 10 September planting with 70 days DPS over 1st,10th, 20th and 30th August planting with nursery raised seedling was 7.83,17.39, 22.36 and 27.95%. Advantage of double transplanting of late transplanted rice was also reported by Rautaray (2006) and Ashem *et al*. (2010). Higher grain yield with 70 days old DPS planted on 10th September might be due to significant increase in length of panicle, weight of panicle, number of filled grains per panicle, 1000-grain weight and decrease in per cent unfilled grains per panicle. Another reason might be due to thicker culm, better shoot and root growth thereby more food reserves in double planted seedlings in comparison to conventional seedlings. Again, double planted seedlings led to quick establishment and less mortality of seedlings in main field even in late planting. Higher flag leaf area recorded on 10th September and 1st August plantings might have some contribution towards the photosynthate production which helped in higher grain yield. The results

Table 2. Yield and economics of scented rice as influenced by planting pattern and staggered planting with type of seedling (mean data of 2 years)

Treatment	Grain yield (t/ha)	Straw yield (t/ha)	H.I (%)	Net return (Rs/ha)	Benefit-cost ratio
Planting pattern					
Rectangular(20x15cm)	1.40	3.55	28.57	47,281.00	1.79
Square (20x20cm)	1.33	3.37	28.30	44,281.00	1.72
Skip row (3:1;20x15cm)	1.37	3.35	28.18	45,781.00	1.78
SEm ±	0.017	0.072	0.493	-	-
CD (P= 0.05)	NS	NS	NS	-	-
Staggered planting with type of seedling					
1 August (30 DNS)	1.48	4.29	25.52	54,871.70	2.13
10 August (40 DNS)	1.33	4.02	24.98	47,521.70	1.84
20 August (50 DNS)	1.25	3.34	27.35	40,921.70	1.59
30 August (60 DNS)	1.16	2.75	30.38	34,371.70	1.33
10 September (70 days DPS)	1.61	2.70	37.25	51,001.70	1.90
SEm ±	0.060	0.117	0.673	-	-
CD (P= 0.05)	0.10	0.34	1.96	-	-

DNS: Days nursery seedling, DPS: Double planted seedling, NS: Non-Significant

Table 3. Organic carbon (%), residual available nutrient status in soil and nutrient uptake by scented rice as influenced by planting pattern and staggered planting with type of seedling (mean data of 2 years)

Treatment	Organic carbon (%)	Available nutrient (kg/ha)			Total uptake of nutrient(kg/ha)		
Planting pattern		N	P	K	N	P	K
Rectangular(20x15cm)	0.80	274.41	18.11	175.83	52.76	7.91	42.01
Square (20x20cm)	0.79	273.08	18.79	176.05	51.79	7.88	40.55
Skip row (3:1;20x15cm)	0.78	272.62	18.25	176.27	51.07	7.96	39.77
SEm ±	0.011	0.848	0.450	3.397	0.949	0.441	1.066
CD (P= 0.05)	NS	NS	NS	NS	NS	NS	NS
Staggered planting with type of seedling							51.24
1 August (30 DNS)	0.78	273.07	17.83	175.76	61.79	10.52	46.27
10 August (40 DNS)	0.78	273.06	18.61	176.03	56.77	8.67	38.41
20 August (50 DNS)	0.79	274.01	18.41	175.29	49.70	8.13	31.50
30 August (60 DNS)	0.80	272.35	18.34	176.53	41.47	5.95	36.34
10 September (70 days DPS)	0.79	274.35	18.70	176.14	49.64	6.31	0.868
SEm ±	0.007	1.420	0.381	0.953	1.132	0.402	2.53
CD (P= 0.05)	NS	NS	NS	NS	NS	NS	NS

DNS: Days nursery seedling, DPS: Double planted seedling, NS: Non-Significant

corroborate the findings of Bashar *et al*. (1995). Lower grain yield in late planting on 30 August might have exposed the crop to relatively more adverse environmental condition in terms of water stagnation at the tillering phase, low temperature at the reproductive phase and infestation of insect-pests (case worm) which might have pulled down the yield as compared to earlier plantings. However, straw yield decreased with delaying the planting dates from 1st August to 10th September which might be due to more taller plants and higher number of tillers per hill in early planting than the successive plantings. Early planting on 1st August favours better rooting density and better uptake of N, P and K and thereby increase in growth and yield attributing characters and ultimately reflected on straw yield.

Economics

The net return and benefit-cost ratio were recorded higher in rectangular planting than that of square and skipped row planting which was mainly due to higher grain and straw yields of rice. Among various dates of planting, 30 days nursery seedling (DNS) planted on 1st August recorded the highest value in terms of net return and benefit-cost ratio which was followed by 10th September with 70 days (35+35 days) DPS. This was due to the fact that 10 September and 1 August planting registered relatively higher value in respect of grain and straw yield, respectively. These findings are in align with that of Singh *et al*. (1997).

Overall, it can be concluded that rectangular planting spaced at 20 ×15 cm or skip row (3:1; 20 ×15 cm) planting with 30 days nursery seedling on 1st August or 70 days (35+35 days) double planted seedling on 10th September found to be the best for obtaining higher production as well as maximum economic returns.

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SHORT COMMUNICATION

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Studies on the efficacy of synthetic jasmonates and salicylates on parasitism of brown planthopper, *Nilaparvata lugens* (Stal.) by *Anagrus* sp.

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ABSTRACT

A field experiment to evaluate the effect of synthetic jasmonates and salicylates on parasitisation of rice brown planthopper (BPH)by egg parasitoid, Anagrus sp. *was conducted during October - December, 2015. Jasmonic acid (JA), Methyl jasmonate (MeJ), Salicylic acid (SA) and Methyl salicylate (MeS) at three different concentrations (1, 10 and 100 mM) were tested for their effect on rice BPH population and their ability to attract egg parasitiod. Significantly, higher % parasitism and thereby less BPH population was noticed on plants treated with Jasmonates and salicylates than buffer and control. Even though all the evaluated treatments attracted parasitoids, Jasmonic acid @10 mM recorded the maximum mean parasitism of 34.69 % against control with 10.69 % only. These potential synthetic herbivore induced plant volatiles (HIPVs) can be explored in rice fields for attracting Anagrus* sp.

Key words: *Brown planthopper, jasmonates, parasitoid, salicylates, synthetic HIPV*

Plants can influence the natural enemies of herbivores by emitting behaviour-modifying volatile organic compounds (VOCs). Plants damaged by herbivores often produce a blend of volatiles (Pareand Tumlinson 1999), commonly referred to as herbivore-induced plant volatiles (HIPVs) (Mumm and Dicke 2010). Plants are known to release more than 30,000 different Volatile Organic Carbons (VOCs) including alkanes, alkenes, alcohols, ketones, aldehydes, ethers, esters and carboxylic acids (Niinemets *et al*. 2004). VOCs emitted as a consequence of herbivore attack may have a role in plant indirect defences, attracting natural enemies of herbivores and helping them to find the attacked plants (Induced Synomones) (Dicke and Sabelis 1988; Turlings *et al*. 1990; Agrawal *et al*. 1999; Walling 2000). The herbivore attack induces a variety of plant hormones, including jasmonic acid, salicylic acid and ethylene, which subsequently regulate defensive responses, including the release of VOCs in rice plants (Lou *et al*. 2005, 2006; Lu *et al*. 2006; Zhou *et al*. 2009).

The volatiles emitted from rice plants in response to *N. lugens* attack attracts the parasitoid, *Anagrus nilaparvatae* (Lou and Cheng 1996; Lou *et al*. 2002). Exogenous application of JA can mimic the defensive reaction of rice (Lou *et al*. 2005; Zhou *et al*. 2009) and can enhance the parasitism of *N. lugens* eggs by *A. nilaparvatae* in the glasshouse and the field (Lou *et al*. 2005). The parasitism of *N. lugens* eggs by *A. nilaparvatae* on plants that were surrounded by JA-treated plants is more than two fold higher than on control plants in the greenhouse and field (Lou *et al*. 2005). The egg deposition by herbivores can also induce a volatile response in plants and consequently attract egg parasitoids (Meiners and Hilker 1997 2000; Hilker and Meiners 2002; Colazza *et al*. 2004). This study explored the efficacy of synthetic jasmonates and salicylates on attraction of egg parasitoids of BPH under field conditions.

Field experiment to evaluate the efficacy of synthetic jasmonates and salicylates on parasitism of BPH eggs byegg parasitoid was conducted in rice variety NLR 3449 at Kondayampatti village, Madurai, Tamil Nadu ($9^0$56' N, $78^0$19' E) during Oct-Dec, 2015. Jasmonic acid ($\geq$ 97.0 %), methyl jasmonate ($\geq$ 94.5 %), methyl salicylate ($\geq$ 99.0 %) and Salicylic acid ($\geq$ 99.0 %) were tested at three different dosages (1, 10 and 100mM). The treatments were compared with buffer and control and were replicated thrice. Size of each plot was 5m x 4 m. Five plants per treatment were selected and labelled. Each selected plant was individually damaged with a needle on rice leaves with 200 pricks and then the damage site was treated by applying 40 µl of each JA , MeJA, MeSA and SA (Sigma Aldrich) at different dosages (50mM sodium phosphate buffer titrated with 1 M citric acid until pH 8, including 0.01% Tween). In buffer treatment sodium phosphate buffer was treated @ 40 µl of 50mM solution whereas the control plants were kept non-manipulated without application. In each treatment number of BPH eggs(parasitizedand un parasitized) per plant were dissected and recorded before application as well as on 1, 3, 5 and 7 days after the application (DAA) of synthetic jasmonates and salicylates. Parasitism was determined by eggs with yellowish red colour and then per cent parasitism was calculated.

Experiment data was analysed using two-way analyses of variance (ANOVA) followed by Duncan's multiple range test when significant differences were detected. Statistical analyses were executed using software AGRES.

The mean BPH population recorded after the application of synthetic jasmonates and salicylates ranged from 69.14 to 106.80 nos./5 hills over the study period. Jasmonic acid @ 10mM (69.14 nos./5 hills) recorded the lowest mean BPH population followed by methyl salicylate @10mM and Salicylic acid @100mM with 80.30 and 81.23 nos./5 hills, respectively. The BPH population in jasmonic acid @10mM was approximately 1.32 times lesser as compared to the pre-count BPH population and 1.69 times lesser compared to the control (Fig. 1). At 5 DAA of the Jasmonates and salicylates the BPH population was minimum of 79.95 nos./5 hills followed by 3 DAA with 81.38 nos./5 hills. Jasmonic acid @10mM at 5 DAA and 3 DAA recorded a minimum population of

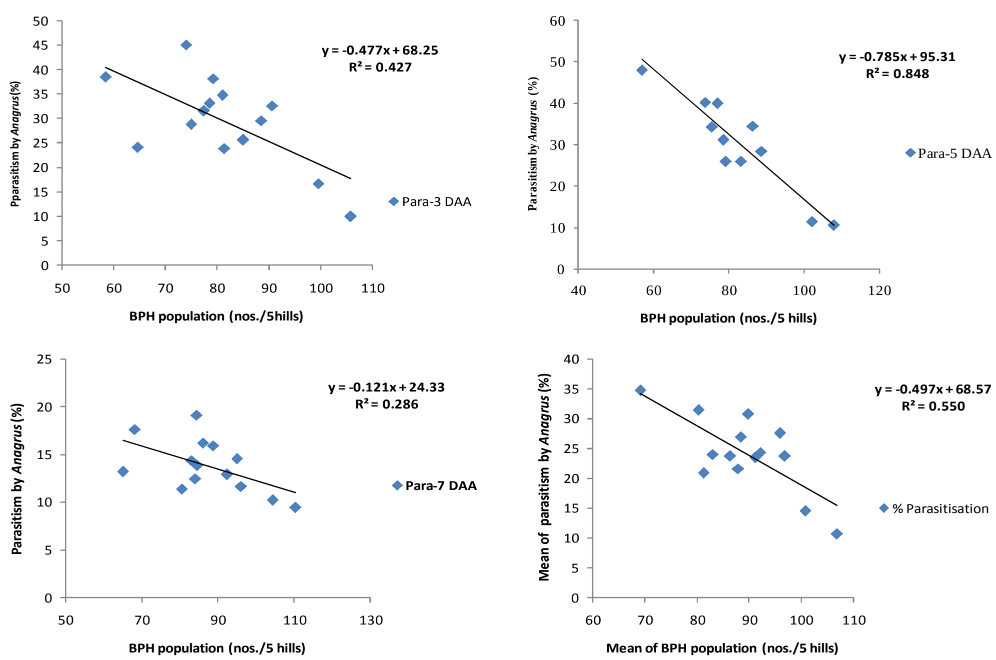

Fig. 1. Correlation between BPH population and per cent parasitism on 1, 3, 5 and 7 days after application of jasmonates and salicylates.

56.88 nos./5 hills and 58.45 nos./5 hills, respectively and were significantly different from each other as well as from all other treatments (P = 0.05). At 7 DAA, low BPH population was seen in Jasmonic acid @10mM (65.00 nos./5 hills) followed by methyl salicylate @100mM (68.11 nos./5 hills) and methyl salicylate @1mM (80.43 nos./5 hills). James *et al*. (2008) revealed that deployment of herbivore-induced plant volatiles in controlled release dispensers in hops and grapes orchards increased the populations of beneficial insects as well as improved the conservation of biological control.

The highest mean % parasitism was recorded in methyl salicylate @10 mM of 31.40 and was followed by methyl jasmonate @10 mM (30.71) and Methyl jasmonate @100 mM (27.55). The lowest mean % parasitism was seen in control and buffer @50 mM of 10.69 and 14.52, respectively. At 5 DAA, the maximum % parasitism of 49.35 was observed in methyl jasmonate @10 mM and was followed by jasmonic acid @10 mM with a % parasitism of 47.98. The next best treatments were methyl salicylate @10 mM at 3 DAA and 5 DAA and jasmonic acid @100 mM at 5 DAA representing a % parasitism of 45.03, 40.19 and 39.96, respectively and the later two treatments were also on par with each other (Fig. 2). The results are in agreement with the findings of Lou *et al*. (2004 and 2005) who reported that both BPH and its parasitoids preferred to settle on JA-treated rice plants immediately after release and because of the action of these parasitoids the population of BPH decreased after 24h of application.

There was an abrupt decline in % parasitism from 49.35 (5 DAA) to 16.19 (7 DAA) in methyl jasmonate @10 mM and Salicylic acid @100 mM recorded the maximum per cent parasitism of 19.10 at 7 DAA. It was followed by methyl jasmonate @10mM and methyl jasmonate @100 mM representing a per cent parasitism of 16.19 and 14.52, respectively.The per cent parasitism in Jasmonic acid @10 mM at 5 DAA was approximately 5.20 times higher compared to before application and 4.54 times higher compared to the control. At 5 DAA of jasmonates and salicylates the per cent parasitism was maximum (30.94) followed by 3 DAA with 29.43 per cent. Lou *et al*. (2004 and 2005) reported that the parasitoid *A. nilaparvate* was more attracted to the volatiles emitted from jasmonic acid treated rice plants than to volatiles from control plants. This was evident from greenhouse and field experiments in which parasitism of *N. lugens* eggs by *A. nilaparvatae* on plants that were surrounded by JA-treated plants was two fold higher than on control plants. Correlation between BPH population and per parasitism by egg parasitoidon 3 DAA of JA and SA (r= 0.42*), 5 DAA (r= 0.84*), 7 DAA(r= 0.28*) and Mean (r= 0.55*) showed significant and positive correlation with BPH population (Fig. 1).

It could be concluded that rice plants treated with Jasmonic acid @10 mM at 5 DAA harboured maximum number of *Anagyrus* sp. and minimum number of BPH as compared to all other treatments. The % parasitism in Jasmonic acid @10 mM at 5 DAA was approximately 5.20 times higher as compared to the % parasitism before application and 4.54 times higher compared to the control. The BPH population showed a 1.69 fold decrease when compared to control. Even though, our preliminary results have shown that egg parasitiod responded to the tested jasmonates and salicylates, further experiments to verify the functions of these synthetics over multiple seasons are needed.

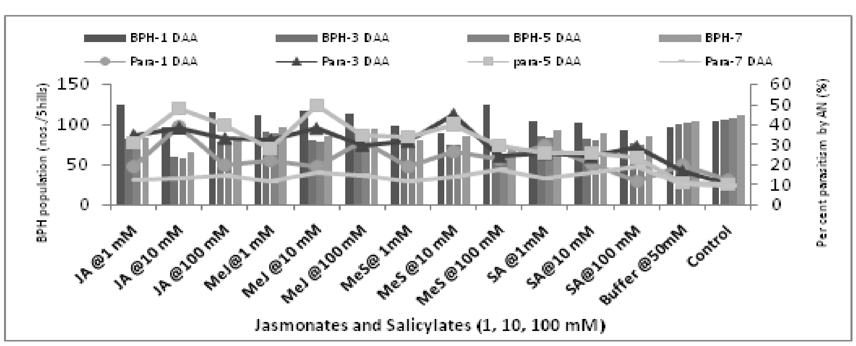

Fig. 2. Influence of jasmonates and salicylates on BPH population and per cent parasitism on 1, 3, 5 and 7 DAA.

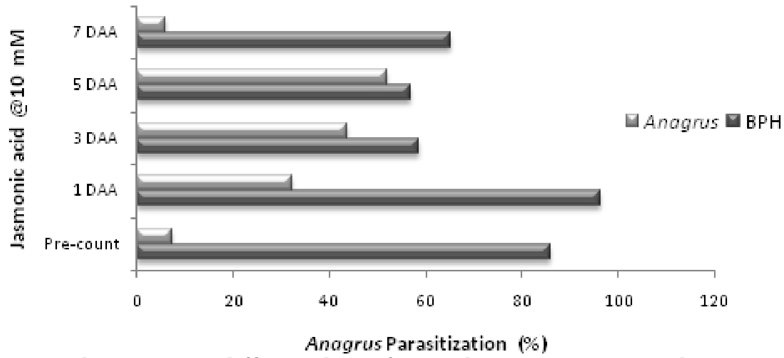

Fig. 3. Effect of Jasmonic acid @10mM at different days after application on BPH and Anagrus population

Current research in this area focuses on the possibility of exploiting the practical application of these in crop protection and hence this study provides baseline information.

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SHORT COMMUNICATION

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Field evaluation of pre-mixture insecticide sulfoxaflor and chlorpyriphos for the control of rice leaffolder, *Cnaphalocrocis medinalis* (Guenee) and its effect on coccinellids.

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ABSTRACT

Field trials were conducted during 2012 and 2013 at Agricultural College and Research Institute, Madurai and in a farmer's holding at Kallandhiri village, Melur Block of Madurai District respectively to evaluate the efficacy of a pre mixture insecticide sulfoxaflor 3.75 + chlorpyriphos 37.5 SE against rice leaf folder and natural enemy (Coccinellids) revealed that the test compound at 516 and 619 g a.i./ha was highly effective in reducing leaf folder incidence (71.2 to 80.2 per cent reduction over control during the period of study) and in case of coccinellids, negligible population reduction were noticed at 516 and 619 g a.i/ha (4.3 to 4.7/hill) when compared to untreated check (5.7 to 6.3/hill). The check insecticides like sulfoxaflor, buprofezin, chlorantraniliprole and chlorpyriphos were observed to be less effective than the test compounds.

Key words: *Efficacy, field evaluation, pre mixture insecticide, rice leaf folder, coccinellids*

The rice leaffolder, *Cnaphalocrocis medinalis* (Guenee) is considered a major pest of rice. This insect retains the potential to cause substantial damage during the vegetative growth. It leads to leaf damage of 60 to70 % inflicting significant yield losses up to 80 % (Prabal and Saikiya 2000). In the past a number of conventional insecticides have been tested however, satisfactory control has not yet been encountered. Therefore, combination products are gaining importance. Keeping in view, an attempt was made to evaluate the efficacy of new pre-mixture insecticide, sulfoxaflor 3.75 + chlorpyriphos 37.5 % SE against rice leaffolder and this premixture insecticide, sulfoxaflor 3.75 + chlorpyriphos 37.5 SE belongs to a new class of combination of sulfoximines and phosphorothioate insecticides. Studies have demonstrated that sulfoxaflor 24 SC has a unique interaction with the nicotinic acetylcholine receptor (nAchR) (Watson *et al.* 2011).

Twenty five day old rice seedlings of a variety Seeraga Samba were transplanted in season I and II of 2012 and 2013, respectively with all recommended agronomic practices in a plot size of (5X5) m^2 with a spacing of (20X10) cm with three replications arranged in a randomized block design. The test molecule sulfoxaflor 3.75 + chlorpyriphos 37.5 SE was applied at 413, 516 and 619 g a.i./ha along with four check insecticides *viz.*, sulfoxaflor 24 SC (75 g a.i/ha), chlorantraniliprole 20 SE (30 g a.i/ha), buprofezin 25 EC (200 g a.i/ha), chlorpyriphos 20 EC (375 g a.i/ha) and untreated check. The treatments were imposed at 45 days after transplanting and repeated three times at 15 days interval using high volume knapsack sprayer with the spray volume of 500 l/ha to a level of run off. Observation on leaffolder incidence (per cent leaf damage) and population of nymphs and adults of coccinellids were recorded at 1 day before first spray and at 7, 10 and 15 days after treatment (DAT) of each spray from 10 randomly selected hills/plot/replication during both the years. Plot wise grain yield was computed and expressed as q/ha after necessary conversion. All the data were subjected to statistical analysis as per RBD procedure. The data from various field experiments were scrutinized by RBD analysis of variance (ANOVA) after getting transformed into x+0.5, logarithmic and arcsine percentage values where appropriate (Gomez and Gomez 1984). Critical difference values were calculated at five per cent probability level and treatment mean values were compared using Duncan's Multiple Range Test (DMRT) (Duncan 1951).

During season I (2012), the per cent leaf damage due to leaffolder varied from 28.0 to 30.0 per cent before imposing treatments (Table 1). After first spray at 7 DAT, there was significant reduction in the per cent leaf damage. The lowest leaf damage was recorded on plots sprayed with sulfoxaflor 3.75 + chlorpyriphos 37.5 SE 619 g a.i./ha and 516 g a.i./ha (11.5 and 13.7%) respectively. While check insecticides registered 17.3 to 23.6 per cent leaf damage, among the check insecticide sulfoxaflor 24 SC 75 g a.i/ha (17.3 %) followed by chlorantraniliprole 20 SC 30 g a.i/ha (17.5%) as against 35.6 per cent leaf damage in untreated control. There was further reduction in the leaf damage due to sulfoxaflor 3.75 + chlorpyriphos 37.5 SE 619 g a.i./ha (7.7 and 4.7%) and 516 g a.i./ha (9.4 and 6.3%) at 10 and 15 DAT, respectively. More or less same trend of per cent leaf damage was observed after the second and third sprays. The new pre-mixture insecticides caused 80.2 to 62.9 per cent reduction in leaffolder incidence as compared to check insecticides.

Population of nymphs and adults of coccinellids like *Harmonia* sp., *Coccinella transversalis* (Fabricius) and *Chilomenus sexmaculata* (Fabricius) were recorded and given in Table 3. There was negligible reduction on coccinellids population due to sulfoxaflor 3.75 + chlorpyriphos 37.5 SE 619 g a.i./ha which ranged from 3.6 to 3.7/hill; 3.7 to 4.4/hill and 4.1 to 4.8/hill from 7 to 15 DAT after first, second and third sprays, respectively. This was followed by sulfoxaflor 3.75 + chlorpyriphos 37.5 SE 516 g a.i./ha which resulted in coccinellids population of 3.6 to 3.9 per hill, 4.2 to 4.5 per hill and 4.1 to 5.0 per hill from 7 to 15 DAT after first, second and third sprays respectively when compared to other treatments.

During season II (2013), the leaf damage due to leaffolder ranged from 22.1 to 23.4 per cent before imposing first spray (Table 2). There was significant reduction on leaf damage at 7,10 and 15 DAT after first spray due to sulfoxaflor 3.75 + chlorpyriphos 37.5 SE at 619 g a.i./ha (10.1, 7.8 and 6.7 % respectively)

Table 1. Effect of sulfoxaflor 3.75 + chlorpyriphos 37.5 SE against *Cnaphalocrocis medinalis* on rice - I season (2012)

Treatments (a.i./ha)	Per cent leaf damage on DAT										Mean	Per cent reduction over control
	Pre count	1st spray			2nd spray			3rd spray				
		7	10	15	7	10	15	7	10	15		
Sulfoxaflor 3.75 + chlorpyriphos 37.5 SE 413 g	28.2	18.4 d	15.3 e	11.3 e	12.3 e	9.5 e	8.6 e	8.4 d	6.3 c	4.5 c	10.5 e	62.9
Sulfoxaflor 3.75 + chlorpyriphos 37.5 SE 516 g	28.0	13.7 b	9.4 b	6.3 b	8.5 b	5.4 b	4.4 b	7.3 b	5.5 b	3.4 b	7.1 b	74.9
Sulfoxaflor 3.75 + chlorpyriphos 37.5 SE 619 g	28.6	11.5 a	7.7 a	4.7 a	7.3 a	4.5 a	3.4 a	5.7 a	3.6 a	2.1 a	5.6 a	80.2
Sulfoxaflor 24 SC 75 g	29.9	17.3 c	11.4 c	8.5 c	10.1 c	8.1 c	7.1 c	8.0 c	6.1 c	5.1 d	9.1 c	67.8
Chlorpyriphos-20 EC 375 g	28.4	23.6 f	18.5 g	15.1 g	14.7 f	11.9 g	10.8 g	7.6 b	5.7 b	3.6 b	12.4 f	56.2
Chlorantraniliprole-20 SC 30 g	29.7	17.5 c	14.4 d	10.8 d	10.8 d	8.7 d	7.8 b	9.3 e	7.5 d	5.3 d	10.2 d	64.0
Buprofezin 25 SC 200 g	28.1	21.7 e	16.3 f	14.8 f	12.8 e	10.9 f	10.0 f	10.1 f	8.5 e	7.3 e	12.5 f	55.8
Untreated control	30.0	35.6 g	39.8 h	46.1 h	22.7 g	24.8 h	30.4 h	16.7 g	18.7 f	24.8 f	28.3 g	-
CD (0.05)	-	0.38	0.39	0.37	0.49	0.60	0.52	0.40	0.47	0.39	0.12	-
SEd	-	0.18	0.18	0.17	0.22	0.28	0.24	0.19	0.22	0.18	0.06	-

Data are mean values of three replications, DAT - Days After Treatment
Values were transformed by arc sine transformation and the original values are given
Means with columns lacking common bold upper case superscript are significantly different (P<0.05)

Table 2. Effect of sulfoxaflor 3.75 + chlorpyriphos 37.5 SE against *Cnaphalocrocis medinalis* on rice- II season (2013)

Treatments (a.i./ha)	Per cent leaf damage on DAT										Mean	Per cent reduction over control
	Pre count	1st spray			2nd spray			3rd spray				
		7	10	15	7	10	15	7	10	15		
Sulfoxaflor 3.75 + chlorpyriphos 37.5 SE 413 g	22.1	14.8 ^{c}	11.3 ^{d}	9.4 ^{c}	9.4 ^{c}	7.8 ^{c}	5.5 ^{c}	7.4 ^{b}	5.7 ^{c}	3.5 ^{c}	8.3 ^{c}	64.4
Sulfoxaflor 3.75 + chlorpyriphos 37.5 SE 516 g	22.4	12.5 ^{b}	8.6 ^{b}	6.9 ^{b}	8.2 ^{b}	6.1 ^{b}	4.1 ^{a}	6.4 ^{a}	4.4 ^{b}	2.9 ^{b}	6.7 ^{b}	71.2
Sulfoxaflor 3.75 + chlorpyriphos 37.5 SE 619 g	22.0	10.1 ^{a}	7.8 ^{a}	6.7 ^{a}	7.3 ^{a}	5.4 ^{a}	3.3 ^{a}	6.3 ^{a}	3.2 ^{a}	2.3 ^{a}	5.8 ^{a}	75.1
Sulfoxaflor 24 SC 75 g	23.1	14.6 ^{d}	10.8 ^{c}	8.2 ^{e}	11.3 ^{e}	9.7 ^{d}	8.5 ^{b}	9.3 ^{cd}	7.4 ^{d}	5.2 ^{e}	9.4 ^{e}	59.6
Chlorpyriphos- 20 EC 375 g	22.6	17.3 ^{f}	14.5 ^{f}	3.6 ^{e}	11.4 ^{e}	9.3 ^{d}	8.2 ^{e}	10.0 ^{d}	6.1 ^{d}	4.3 ^{d}	10.6 ^{f}	54.5
Chlorantraniliprole- 20 SC 30 g	23.4	15.6 ^{e}	12.4 ^{e}	8.3 ^{d}	10.4 ^{d}	7.3 ^{c}	6.4 ^{b}	7.9 ^{d}	5.1 ^{c}	3.9 ^{cd}	8.6 ^{d}	63.1
Buprofezin 25 SC 200 g	22.9	20.3 ^{g}	14.7 ^{f}	11.5 ^{e}	11.8 ^{e}	9.6 ^{d}	8.8 ^{d}	9.1 ^{c}	7.4 ^{d}	6.3 ^{f}	11.0 ^{g}	52.8
Untreated control	23.0	27.2 ^{h}	32.6 ^{g}	38.4 ^{f}	17.6 ^{f}	20.4 ^{e}	25.6 ^{f}	13.3 ^{e}	15.5 ^{e}	18.7 ^{g}	23.3 ^{h}	-
CD (0.05)	-	0.51	0.49	0.69	045	0.58	0.43	0.74	0.71	0.63	0.22	-
SEd	-	0.23	0.22	0.32	0.21	0.27	0.20	0.34	0.33	0.29	0.10	-

Data are mean values of three replications

Values were transformed by arc sine transformation and the original values are given

Means with columns lacking common bold upper case superscript are significantly different (P<0.05)

Table 3. Effect of sulfoxaflor 3.75 + chlorpyriphos 37.5 SE against coccinellids on rice- I season (2012)

Treatments (a.i./ha)	Number of grubs and adults/hill on DAT										Mean population of grubs and adults/ hill	Grain Yield q/ha
	Pre count	1st spray			2nd spray			3rd spray				
		7	10	15	7	10	15	7	10	15		
Sulfoxaflor 3.75 + chlorpyriphos 37.5 SE 413 g	4.2	3.9 ^{b}	4.0 ^{b}	3.8 ^{b}	4.5 ^{b}	4.4 ^{bcd}	5.0 ^{ab}	4.9 ^{b}	5.3 ^{b}	5.5 ^{b}	4.4 ^{b}	32.9 ^{c}
Sulfoxaflor 3.75 + chlorpyriphos 37.5 SE 516 g	4.0	3.9 ^{b}	3.6 ^{b}	3.7 ^{b}	4.5 ^{b}	4.2 ^{bc}	4.4 ^{b}	4.1 ^{c}	4.8 ^{c}	5.0 ^{bc}	4.3 ^{c}	34.6 ^{b}
Sulfoxaflor 3.75 + chlorpyriphos 37.5 SE 619 g	3.8	3.6 ^{b}	3.9 ^{b}	3.7 ^{b}	3.7 ^{c}	4.3 ^{bc}	4.4 ^{b}	4.2 ^{bc}	4.1 ^{d}	4.8 ^{c}	4.0 $_{d}$	36.7 ^{a}
Sulfoxaflor 24 SC 75 g	3.6	3.7 ^{b}	3.8 $_{b}$	3.9 ^{b}	4.5 ^{b}	4.5 ^{b}	4.5 ^{b}	4.3 ^{c}	5.0 ^{bc}	5.2 ^{bc}	4.4 ^{bc}	34.2 ^{b}
Chlorpyriphos 20 EC 375 g	4.3	2.1 ^{d}	2.2 ^{d}	2.3 ^{d}	3.0 ^{d}	3.2 ^{e}	3.0 ^{c}	3.0 ^{e}	3.0 ^{f}	3.2 ^{e}	2.7 ^{f}	30.4 ^{d}
Chlorantraniliprole 20 SC 30 g	3.5	2.9 ^{c}	3.0 ^{c}	3.0 ^{c}	3.4 ^{c}	3.5 ^{cde}	3.3 ^{c}	3.8 ^{d}	4.1 ^{d}	4.0 ^{d}	3.4 ^{e}	32.5 ^{c}
Buprofezin 25 SC 200 g	4.7	2.8 ^{c}	2.8 ^{c}	2.9 ^{c}	3.5 ^{c}	3.3 ^{be}	3.4 ^{c}	3.5 ^{d}	3.7 ^{e}	3.9 ^{d}	3.4 ^{e}	32.8 ^{c}
Untreated control	4.4	4.7 ^{a}	4.8 ^{a}	5.1 ^{a}	5.8 ^{a}	5.8 ^{a}	5.7 ^{a}	6.3 ^{a}	6.4 ^{a}	6.2 ^{a}	5.7 ^{a}	26.1 ^{e}
CD (0.05)	-	0.14	0.15	0.14	0.11	0.21	0.16	0.13	0.08	0.10	0.04	0.01
SEd	-	0.07	0.07	0.07	0.05	0.1	0.08	0.06	0.04	0.05	0.02	0.004

Data are mean values of three replications

Values were transformed by arc sine transformation and the original values are given

Means with columns lacking common bold upper case superscript are significantly different (P<0.05)

and sulfoxaflor 3.75 + chlorpyriphos 37.5 SE at 516 g a.i./ha (12.5, 8.6 and 6.9 % respectively) when compared to other treatments. The per cent reduction in leaf folder incidence was higher in sulfoxaflor 3.75 + chlorpyriphos 37.5 SE 619 g a.i./ha (75.1 %) followed by sulfoxaflor 3.75 + chlorpyriphos 37.5 SE 516 g a.i./ ha (71.2 %).

Population of grubs and adults of coccinellids on insecticide treated and control plots are given in the Table 4. Initial population of coccinellids ranged from 3.2 to 4.8 per hill before imposing first spray. Mean coccinellids population were higher in sulfoxaflor 3.75 + chlorpyriphos 37.5 SE 413, 516 and 619 g a.i./ha (ranged from 4.0 to 4.4/hill) followed by sulfoxaflor 24 SC 75 g a.i./ha (4.4/hill). Lowest coccinellids population was recorded in chlorpyriphos 20 EC 375 g a.i./ha (2.7/

Table 4. Effect of sulfoxaflor 3.75 + chlorpyriphos 37.5 SE against coccinellids on rice - II season (2013)

Treatments (a.i./ha)	Pre count	Number of grubs and adults/hill on DAT									Mean population of grubs and adults/hill	Grain Yield (q/ha)
		1st spray			2nd spray			3rd spray				
		7	10	15	7	10	15	7	10	15		
Sulfoxaflor 3.75 + chlorpyriphos 37.5 413 g	4.8	4.6 [ab]	4.2 [b]	4.1 [b]	4.8 [b]	4.8 [b]	4.8 [b]	5.6 [b]	5.4 [bc]	5.8 [b]	4.9 [b]	29.7 [e]
Sulfoxaflor 3.75 + chlorpyriphos 37.5 516 g	4.3	4.4 [ab]	4.1 [bc]	4.0 [b]	4.6 [b]	4.6 [b]	4.6 [b]	5.2 [bc]	5.4 [bc]	5.5[bc]	4.7 [b]	32.2 [b]
Sulfoxaflor 3.75 + chlorpyriphos 37.5 619 g	3.8	4.0 [bc]	3.5 [cd]	3.9 [b]	4.2 [bc]	4.4 [b]	4.4 [bc]	5.0 [cd]	4.8 [c]	5.0 [c]	4.3 [c]	34.0 [c]
Sulfoxaflor 24 SC 75 g	4.4	4.1 [bc]	4.3 [b]	4.2 [b]	4.6[b]	4.8 [b]	4.4 [bc]	5.5 [bc]	5.8 [ab]	5.4 [bc]	4.8 [b]	31.3 [c]
Chlorpyriphos 20 EC 375 g	4.0	2.0 [e]	1.9 [e]	1.6 [d]	2.0 [e]	2.0 [d]	2.1 [e]	2.4 [f]	2.2 [f]	2.6 [e]	2.0 [f]	27.6 [f]
Chlorantraniliprole 20 SC 30 g	3.9	3.6 [c]	3.5 [cd]	3.4 [c]	3.8 [d]	3.7 [c]	4.0 [cd]	4.4 [d]	4.4 [d]	4.4 [d]	3.9 [d]	30.5 [d]
Buprofezin 25 SC 200 g	3.8	3.0 [d]	3.0 [d]	3.0 [c]	3.5 [d]	3.4 [c]	3.5 [d]	3.9 [e]	3.7 [e]	4.1 [d]	3.4[e]	29.8 [e]
Untreated control	3.2	4.8 [a]	5.3 [a]	5.6 [a]	6.0 [a]	6.3 [a]	6.8 [a]	6.6 [a]	6.6 [a]	7.2 [a]	6.3 [a]	23.3 [g]
CD (0.05)	-	0.14	0.17	0.11	0.18	0.15	0.13	0.12	0.17	0.12	0.06	0.01
SEd	-	0.07	0.08	0.05	0.08	0.07	0.06	006	0.08	0.06	0.03	0.004

Data are mean values of three replications

Values were transformed by square root transformation and the original values are given

Means with columns lacking common bold upper case superscript are significantly different ($P<0.05$)

hill). During both the years, sulfoxaflor 3.75 + chlorpyriphos 37.5 SE 619 g a.i./ha registered highest grain yield of 36.7 and 34 q/ha which was significantly different than rest of its other dosage and the check insecticides (Table 3 & 4). The premixture insecticides at its two higher dosages were observed to be more potential than the check insecticides.

These results are in accordance with the findings of Rath and Dash (2009) who reported that Ducord 17 EC (chlorpyriphos 16 % + alpha cypermethrin 1%) at 1250 and 1000 ml/ha produced pronounced effect on leaffolder causing least leaf infestation. Seetharamu *et al*. (2005) reported that chlorpyriphos effectively reduced the larval count and reported 8.17 % leaf damage. DRR (2011) reported that chlorpyriphos was relatively better against rice pest complex and in increasing grain yield. Monilal Chatterjee and Amalendu Ghosh (2012) reported that sulfoxaflor showed excellent fit with high levels of insecticidal potency and showed lesser hazards to natural enemies. Anonymous (2012) indicated that sulfoxaflor 24 SC @ 375 g per ha recorded the maximum yield of 4.96 t per ha.

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First Circular

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on

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Frontiers of Rice Research for Improving Productivity, Profitability and Climate Resilience

Rice (*Oryza sativa L.*) may be originated at least 130 million years ago and dispersed as a wild grass, the super continent that eventually broke up and drifted apart to become Asia, Africa, Australia, and Antarctica. Rice has always been one of the most important food crops in the world. It is estimated that 40% of the world's population take rice as their major source of food; 1.6 billion people in Asia take rice as their mainstay food. Rice is produced in a wide range of locations and under a variety of climatic conditions, from the wettest areas in the world to the driest deserts which is produced in 111 countries in the world and grown on 144 million farms worldwide -more than for any other crop. The developing countries, especially the Asian countries - the regions with high population density and the most rapid population growth produce and consume the most rice. Rice production is an important source of livelihood for around 140 million rice-farming households and for millions of poor people working on rice farms as hired labour.

Global demand for food is rising because of population growth, enhanced income and changing food pattern. The population of world is expected to stabilize around more than 9 billion people by year 2050. The population growth will mainly restricted to rice-eating countries for which global food production needs to be increased by over 70% by 2050 to meet the overwhelming demand. Further, water scarcity and increasing competition for arable land put added pressure on agricultural production. In addition, climate change may affect the food production system and reduce reliance through altered weather patterns and increased pressure from pests and diseases particularly in rice. Therefore, the challenge of providing the farmers with tools and resources to enhance rice production with saving of natural resources in shrinking arable lands is an uphill task. Furthermore, this has to be achieved in a climate of increasing variability through climate resilient agricultural technology in which rice cultivation has to reduce its impact and participate to its mitigation. However the problem can be solved through development of improved, environment-friendly and precise agricultural practices and high potential resilient varieties for different agro-ecosystems.

Recent breakthroughs in structural, functional and evolutionary rice genome biology have narrowed the gap between genetic variation and the phenotype performance and allowed the deciphering of the function of important genes underlying agronomically relevant traits pushing current scientific knowledge to address the need of sustainably increasing crop yields and global food security. Biotechnological tools have been deployed wherever necessary to enhance breeding efficiency and to save time. Transgenic rice and hybrid rice technology has been evolved as an essential tool for engineering new plant type, abiotic and biotic stress tolerance varieties. Modern scientific approaches and new technologies are making it possible to increase rice productivity in a sustainable manner, add nutritive value to rice, reduce losses from drought and flood, reduce the environmental footprint of rice production and make the rice production system "climate-smart." Similarly, new opportunities are now available for enhancing rice value chains, reducing post-harvest losses, adding value through secondary processing and ensuring higher quality and safety of rice and rice products. Regional networks for the sharing of rice technology and market information are being established to raise productivity and stabilize the market supply through improved trading arrangements to achieve the national objective of doubling farmers' income.

SCIENTIFIC THEMES

I. Genetic advancement for yield, quality and stress tolerance

- Plant genetic resource and its utilization
- Climate resilient and stress tolerant rice
- Breeding for resource use efficiency
- New plant type and hybrids
- Quality improvement and biofortification
- Novel approaches for rice improvement

II. Molecular interventions for trait improvement

- Functional genomics for trait improvement
- Bioprospecting of genes and allele mining
- Transgenic rice
- Bioinformatics

III. Efficient resources utilization and system analysis

- Resource conservation technologies and Conservation agriculture
- Climate smart production technology
- Nutrient and water use efficiency
- Innovative farming and cropping systems and its management
- Utilization of microbial resources
- Crop simulation and modelling

IV. Biotic and abiotic stress physiology and management

- Pest dynamics under changing climatic scenario
- Host-Plant Resistance
- Next Generation Pesticides
- Integrated pest, weed and disease management
- Physiology and biochemistry under stresses
- Problem soil management
- Climate change adaptation and mitigation

V. Farm mechanization and agro-processing

- Farm machinery development and refinement
- Drudgery reduction and energy efficiency
- Storage, processing and by-product utilization

VI. Socioeconomics and agro-technology transfer

- Socio-economic assessment and gender issues
- Yield gap analysis and technology transfer
- IT based knowledge transfer
- Rice value chain models and marketing issues
- Agribusiness and entrepreneurship development
- Farm profitability and income enhancement

SYMPOSIA PRESENTATIONS

Lead Paper Presentations

These would offer an insight into the various themes of the symposia. A Technical Committee will identify 2 - 3 speakers in each theme. Each lecture will be of 15-20 minutes followed by discussion. Lead speakers of the symposium will be requested to provide 3-4 pages extended summaries of their presentation for inclusion in the symposium proceedings.

Contributory Paper Presentations

This would provide an opportunity for oral presentation of research papers received / submitted for poster presentation. Limited number of papers will be selected by the committee for presentation based on importance of subject matter and its relevance to the subthemes of the symposium and speakers will be informed well in advance for making the presentation. The presentation will be of 7-8 minutes.

Poster Presentations

There will be separate 'Poster Sessions' covering all the themes to encourage wider interaction and information sharing. The extended summary contributed for presentation will be screened as per the theme. Each poster session will have Chairman and Co-Chairman. They will prepare and present summary of the concerned Poster Session for formulation of meaningful recommendations.

Exhibition and Advertisement

An exhibition will be organized at the venue. The details of the space available and charges etc. will be made available in the second circular and on the Society Website. Advertisement can be included in the conference publications.

Registration Fee

Foreign Delegates

Scientists / Researchers	:	US $250 (SAARC countries); US $350 (Other countries)
Students / Research Scholars	:	US $150 (SAARC countries); US $250 (Other countries)
Accompanying member	:	US $100

Indian Delegates

Member	: ₹. 6,000 (after due date ₹. 7,000)
Non-Member	: ₹. 7,000 (after due date ₹. 8,000)
Students	: ₹. 4,000 (after due date ₹. 5000)
Industry & Private Organization	: ₹. 10,000 (after due date ₹. 12000)
Accompanying Member	: ₹. 4,000

Rupee is the national currency of India. All major currencies can be exchanged at the International Airports, 5-star hotels and Banks. Major currencies are accepted at the hotels and important shopping centers. International credit cards are widely accepted. The registration fee in the form of demand draft in favour of 'ARRW' payable at Cuttack may be sent to "The Secretary, Association of Rice Research Workers, Cuttack (Odisha)- 753 006". Alternatively, fees can be transferred online to the account of the Association (Account No. 10329387060, State Bank of India, Nayabazar, Cuttack, Code-2094, IFS Code-SBIN0002094, MICR No.- 753002016) and receipt may be emailed to the organizing secretary.

Key Dates to Remember

Submission of Participation Form	: September 30, 2017
Submission of Extended Summary	: October 30, 2017
Acceptance of Extended Summary	: November 30, 2017
Registration fee (without late fee)	: December 15, 2017
Accommodation Request	: December 15, 2017
Symposium	: February 6 – 9, 2018

Symposium Overview

The symposium will be of four days duration. The venue is ICAR - National Rice Research Institute, Cuttack. English will be the official language of the Symposium. The interested participants may fill up the 'Participation Form' and send back by September 30, 2017 exercising their options, wherever applicable. Registration fee covers all ancillary expenses including lunch, dinner and refreshment during the session. The delegates will, however, bear the expenses of their boarding, lodging and travel. A wide range of hotel accommodation is available varying from 5-star hotels to medium range hotels. The likely tariffs will be given in second circular. Hotel arrangements will be made for participants on request.

Participants will submit extended summary of their research paper before the last date. These extended summaries will be scrutinized for poster presentations and authors will be communicated for preparation of manuscripts accordingly. All modern facilities of public address system and projection system are available. Guidelines for preparation of extended summary are given separately in the circular. All updates are available at the website 'www.arrworyza.com'.

About City

The silver city 'Cuttack; is the former capital and one of the oldest and second largest city in the Eastern Indian state of Odisha. The name of the city is an anglicized from of Kataka that literally means The Fort. It is located about 28 km to the north east of Bhubaneswar, the capital city of Odisha. Cuttack is famous for its unique silver filigree work woven textiles. The city is well connected with all other important cities of India by the network of 'Indian Railway' as well as by air via 'Biju Patnaik Airport' Bhubaneswar.

The architecture wonders and heritage sites in Odisha offer breathtaking views and a wholesome experience to the tourists visiting the State. The 'Jagannath Temple', located in the coastal town of Puri, 90 km away from Cuttack, is a famous 'Hindu Temple' dedicated to Lord Jagannath. Sun Temple, Konark, located at a distance of nearly 80 km from Cuttack, is famous all over the world is the world heritage monument declared by the UNESCO.

The open air zoo 'Nandankanan National Park' is located at a distance of 20 km from Cuttack. It is famous for the rare white tigers, reptiles and snakes. 'Lingaraj Temple', dedicated to Lord Shiva is located in the Temple city Bhubaneswar.

Dhauli Hill Shanti Stupa', one of the most visited Bhddhist pilgrim destinations in India, is located at a distance of 8 km from Bhubaneswar. The edicts at Dhauli are a living testimony to Emperor Ashoka's adoption of the doctrine of non-violence. 'Khandagiri and Udayagiri' caves of Bhubaneswar are famous shrines of Jains.

The Chilika lake, the largest coastal lagoon in India and the second largest lagoon in the world is a brackish water lagoon, spread over the Puri, Khurda and Ganjam districts of Odisha state on the east coast of India, at the mouth of the Daya River, flowing into the Bay of Bengal, covering an area of over 1,100 km^2.






Guidelines for Preparation of Extended Summary

- Extended Summary should not exceed 1000 words excluding illustration (one) and references. The title should be short, specific and phrased to identify the content of the article. Heading should be in capital letter.
- Extended Summary should be typed in MS-Word, Times New Roman with normal fonts. Font's size for different parts of extended summary are Title - 14 points bold, Authors - 12 point, Institute name -11 points italic, Content - 11 points. It should include the author(s) name(s), email id and place of work including the name of the university / organization, State, PIN code and the country. In case, the present address of any of the author is different, it should be given with complete address as footnote.
- The extended summary should begin with brief introduction and objectives, followed by methodology, results, conclusion and references (maximum two) and illustration (one). Heading of Methodology, Results, Conclusion and References should be given in capital letters. No heading for introduction is required. The guidelines are also available at Society Website: www.arrworyza.com
- The extended summaries of all the contributory papers will be published and provided to the delegates as soft copy during the symposium. These must reach the Organizing Secretary in the prescribed format latest by October 30, 2017. Extended summary prepared without following the style and format and guidelines will not be accepted for publication. A soft copy of the extended summary prepared in Microsoft word doc may be emailed to Organizing Secretary Email: secretaryarrw@gmail.com

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PARTICIPATION FORM

3rd ARRW INTERNATIONAL SYMPOSIUM

Frontiers of Rice Research for Improving Productivity, Profitability and Climate Resilience

February 6-9, 2018, Cuttack-753 006 (Odisha), India

(Please mail or fax this form so as to reach by September 30, 2017)

Name : Prof. /Dr. / Mr. /Ms. ....

Country : ....

Address : ....

....

....

Tel. No. : ....

Fax No. : ....

E-mail Id : ....

ARRW Member : ☐ Yes ☐ No

Title of the paper : ....

....

....

Sub-theme of Interest : ....

I am interested in : (Tick mark the appropriate option)

- Attending the Congress: ....
- Presenting a paper : Oral ☐ Poster ☐
- Post-Congress tour: ....

Signature

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BRIEF INSTRUCTIONS TO AUTHORS

Oryza publishes original research articles in English on all aspects of rice, rice-based cropping systems and rice based farming system in the form of full-length papers and short communications. Authors should be member of the Association of Rice Research Workers.

1. ***Submission of manuscript.*** The manuscript developed in MS-WORD (Font Times New Roman, Size-12pt.) along with tables, figures, digital photo etc. should be sent to the Editor-in-Chief, ORYZA by e-mail : editororyza@gmail.com. Original graph as excel file and photos in JPEG format and address (including e-mail ID) of three reviewers should be sent along with the manuscript. The manuscript should be submitted through Indian Agricultural Research Journals website (weblink: http://epubs.icar.org.in/ejournal/index.php/index/user). Authors may also submit their manuscript through E-mail(editororyza@gmail.com)

2. ***Preparation of manuscript.*** Papers should be written in simple and clear language, strictly following the latest ORYZA style. Avoid footnotes in the text and, if a footnote has to be used, number it with an arabic numeral. The complete scientific name (genus, species and author) of all the experimental organisms should be given at the first mention both in the Abstract and Materials and Methods. International System of Units in abbreviated form should be used for all the measurements. Spell out the acronyms in the first instance.

Manuscript, should be typed in double-spacing. Tables must not exceed 12 columns in potrait and 18 in landscape. Leave liberal margins on both the sides. Arrange the manuscript in the order of title, short titel, author(s), address and e-mail ID of each author, abstract (approx. 200 words), key words, introduction, materials and methods, results, discussion, acknowledgements (if any) and references. ₹ 2000/- per page will be charged to the author intending to print coloured materials (graphics/photographs etc.).

2.1. ***Title.*** A short title of the paper should appear on the top of the article with a maximum of 40 characters, followed by the long title in bold letters. The short title appears on alternate printed pages of each article.

2.2. ***Author(s).*** Author(s) name(s) should be typed in bold letters, first initials and then surname. Corresponding author' s name should be specified by an asterisk mark and his **e_mail address must be indicated.**

2.3. ***Address.*** The address of corresponding author should be typed in italics indicating the place where the work was carried out. If the present address is different, it should be given as footnote in the first page.

2.4. ***Abstract***. Maximum 250 words conveying the objectives and the most important results.

2.5. ***Key words.*** Maximum of 5-6 key words in italics should be provided for subject indexing.

2.6. ***Introduction***. It should be concise and include the scope of the work in relation to the state of art in the same field along with specific objectives.

2.7. ***Materials and Methods.*** A full technical description of the methods followed for the experiment(s) should be given, providing enough information. Detailed methodology should be given when the methods are new while for standard methods, only references may be cited.

2.8. ***Results and Discussion.*** In this section, only significant results of the experiment(s) should be reported. Along with tables and figures. Data should be properly analysed. The discussion should deal with interpretation of results and relate the author' s findings with the past work on the same subject. Result and discussion may also be submitted as separate section.

2.9. ***Conclusion.*** This should indicate important findings of the experiment. This should state clearly the main conclusions and include an explanation of their relevance or importance to the field.

3.0. ***References.*** Refer this copy as sample for references. References in the text should be quoted by the author' s name and year in parentheses. Whenever there are more than two authors, references should be quoted by the name of the first author, followed by et al*.,* year Distinction for the same author and same year be done as e.g. 1969a, 1969b. Unpublished data, personal communication and articles in preparation are not acceptable as references but may be referred to parenthetically in the text.

3.1. ***Tables.*** Number the tables consecutively in arabic numerals. Tables should have comprehensible legends. Any short legends given for treatments or otherwise should be given expanded form below the table. Conditions specific to a particular experiment should be stated. Zero results must be represented by 0 and not determined by n.d. The dash sign is ambiguous. For values < 1, insert a zero before the decimal point.

3.2. ***Illustrations.*** All graphs, diagrams and half-tones should be referred to as Figure and should be numbered consecutively in arabic numerals. The figures should either match with the column width (8.5 cm) or the printing area (17.8 x 22 cm). The legends should be brief and self-explanatory. Define in the footnote or legend any non-standard abbreviations or symbols used in a table or figure.

3.3. ***Short communication.*** New results of special interest will be published as Short Communication. This need not provide separate headings for each section but the details should be separated by paragraphs. Short communication should have an Abstract.

4. ***Reviews.*** Review articles whether full or mini are only invited. Very special review articles are also considered.

In general, the reviewed paper is sent to the author for incorporating necessary changes and the revised paper is reviewed again. The Editors finally decide the acceptance of the manuscript.

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