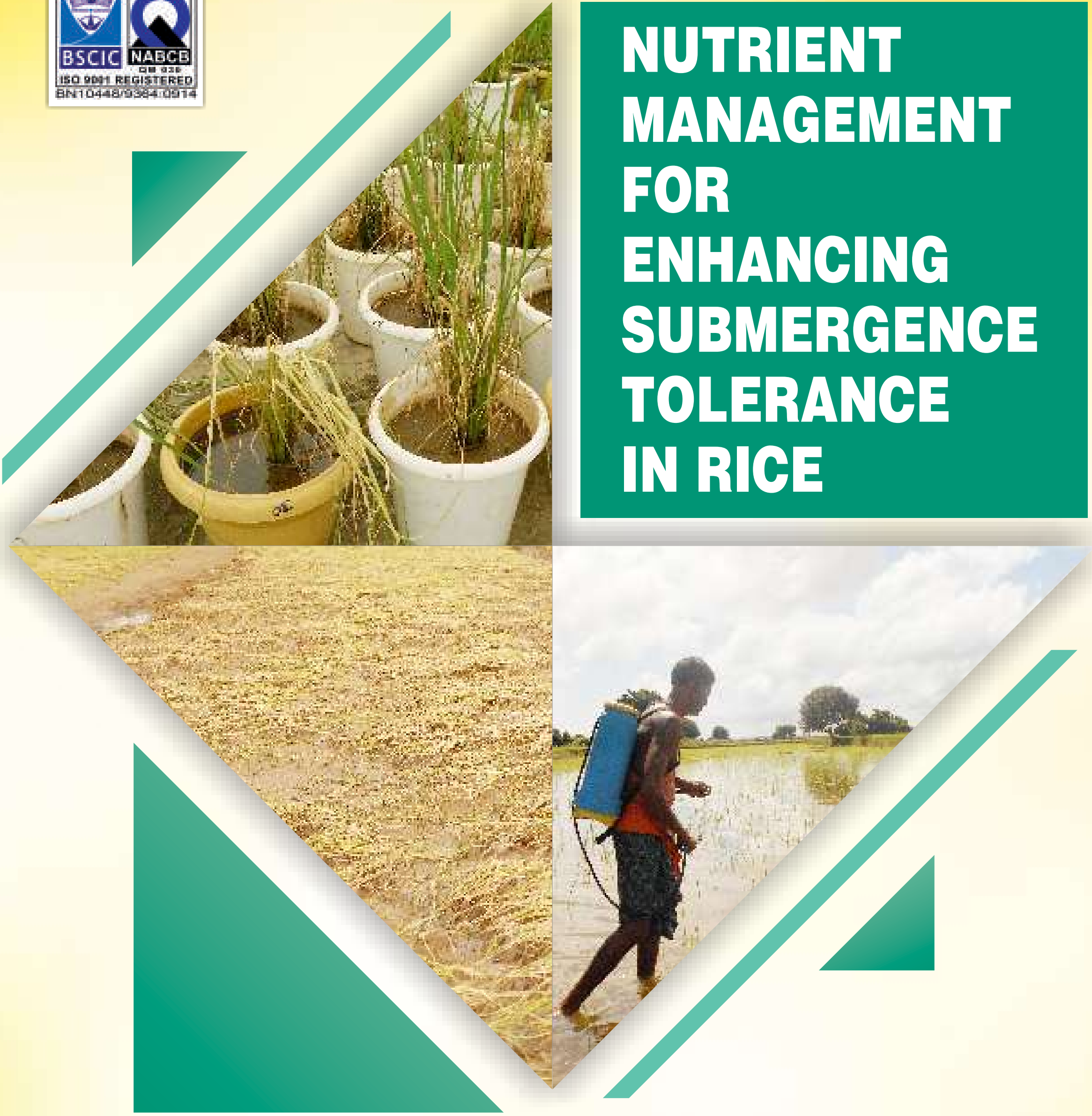
NRRI Research Bulletin No. 13

NUTRIENT MANAGEMENT FOR ENHANCING SUBMERGENCE TOLERANCE IN RICE



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CORRECT CITATION

Gautam Priyanka, Lal B, Nayak AK, Tripathi R, Shahid M, Baig MJ, Maharana S, Raja R, Mohanty S, Chatterjee D, Guru PK, Panda BB, and Kumar U. 2017. Nutrient Management for Enhancing Submergence Tolerance in Rice. NRRI Research Bulletin No.13 ICAR-National Rice Research Institute, Cuttack, Odisha 753006, India. pp 102.

Published By :

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Director
ICAR - National Rice Research Institute
Cuttack-753006, Odisha, India

Layout Design :
Sunil Kumar Sinha

October, 2017

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Laser typeset at the ICAR-National Rice Research Institute, Cuttack-753006 (Odisha) India, and printed in India by the Print-Tech Offset Pvt. Ltd., Bhubaneswar-751024, Odisha Published by the Director, for the ICAR-National Rice Research Institute, Cuttack-753006 (Odisha) India.

FOREWORD

Rice is grown in a wide range of ecologies ranging from irrigated uplands, rainfed lowlands, deepwater, and tidal wetlands. About 30% of India's total rice area i.e., around 13 million ha is rainfed lowland, which contributes 20% to the national rice production. Rainfed lowlands prone to flash floods (submergence) constitute highly fragile ecosystems with a productivity of only 0.5–1.2 t ha^{-1}. The seasonal flash floods are extremely unpredictable and may occur at any growth stage of the rice crop. However, these flood-prone ecosystems have potential for higher production because of the predominance of good soils and freshwater resources to meet the increasing demands for rice supply. Research on submergence tolerance in rice has mainly focused on germplasm tolerance. Tolerant rice varieties that can survive transient submergence have become available through the incorporation of the submergence tolerant SUB1A gene. Limited options are available from the viewpoint of crop management practices to alleviate the damage in areas prone to flash flooding by enhancing plant survival during submergence and recovery afterwards.

The bulletin entitled **"Nutrient Management for Enhancing Submergence Tolerance in Rice"** is an attempt to provide a holistic information of submergence tolerance in relation to time and method of application of different nutrients and their interaction, effect of water turbidity, seedling age and establishment methods and farmers field demonstration.

I appreciate the efforts of the authors in bringing out this bulletin and hope that farmers, researchers, students, planners and extension agents will find this publication very useful.

Place: Cuttack, Odisha
October, 2017

(H. PATHAK)
Director, NRRI

PREFACE

Rice is a unique crop due to its adaptability to different flooding conditions. The adaptability of a plant to water logging or submergence is enhanced by the development of either metabolic or anatomical characteristics. Damage to rice plants in wetlands due to flooding has been increasing along with the expansion of rainfed lowland rice cultivation. Therefore, damage to rice plants by submergence differs depending on the distinctive water environment, as determined by water depth, duration of submergence, temperature, turbidity, rate of nitrogen fertilizer application and light intensity. Eastern India alone has approximately 10 million ha of rice lands affected by flash floods and complete submergence with poor drainage. Although rice is the only cereal crop that can withstand submergence at all, most rice varieties will die if fully submerged for more than seven days because of leaf or stem elongation, loss of dry mass, and also lodging after the flood water recedes. The reaction of the rice plant to submergence differs greatly, depending on genotype and environmental conditions before, during, and after submergence. Tolerant rice varieties that can survive transient submergence have become available through the incorporation of the submergence tolerant *SUB1A* gene. Using a marker-assisted backcrossing (MABC) approach, a small genomic region containing the *SUB1* locus was initially introgressed into six modern high yielding varieties (Swarna, Samba Mahsuri, BR11, IR64, CR1009 and TDK1).

Numerous reports on attempts to improve rice productivity in flood-prone areas through germplasm enhancement are available. However, there is negligible work done regarding the nutrient management strategies under submergence that can enhance plant survival during submergence and recovery afterward ultimately productivity in rainfed lowlands.

This bulletin gives a complete package of practices on nutrient management strategies for higher plant survival and productivity of rice prone to flooding, along with effect of water turbidity and seedling age. We hope that this bulletin will act as a useful tool for rice researchers, extension workers, students, and farmers, and would help to boost up the rice production with some alteration in time and methods of nutrient management.

Authors

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1. Introduction

Rice is the staple food for more than half of the world population and is grown over a wide range of climatic and edaphic conditions. Its geographical distribution is largely determined by temperature and water availability. Rice is now grown on over 144 million hectares worldwide, from 50°N in northern China to 35°S in Australia and Argentina. It is also grown from 3 m below sea level in Kerala, India, to as high as 3000 m in Nepal and Bhutan. Rice is also grown on a variety of soils and under variable water regimes and hydrologic conditions, ranging from aerobic soils in uplands, to flooded soils in irrigated and rainfed lowlands, and to long-duration inundated conditions in flood-prone areas. Rainfed lowland and deepwater rice altogether accounts for approximately 33% of global rice farmlands worldwide. The large gap in the productivity of rainfed ecosystems compared with irrigated ecosystem is largely caused by abiotic stresses such as drought, floods, and poor soils, coupled with the lack of well adapted high-yielding varieties. About two thirds of the shallow and intermediate rainfed lowland rice lands are in India, Thailand, and Bangladesh. Out of 42 biotic and abiotic stresses surveyed in rainfed lowlands of Asia, submergence stress is considered the third most important limitation to rice production, frequently affecting over 15 million ha area (Singh *et al*, 2009). Lowland rice is typically cultivated in paddies of 5 to 25 cm of standing water, which are highly vulnerable to monsoon flash floods of 50 cm or more that can rapidly and completely submerge plants. Prolonged submergence is a major production constraint and affects 22 million ha of rainfed lowland rice in South and Southeast Asia, of which over 6 million ha area is in India (Sarkar *et al*, 2006). Environmental factors associated with flooding are variable in different locations, even over short distances (Setter *et al*, 1995). The visual damage caused by submergence is generally not apparent immediately, rather it manifests only after the water recedes during recovery from complete submergence. The adverse effects of flooding constitute a complex phenomenon that varies with genotype, carbohydrate status of the plant before and after submergence, developmental stage of the plant when flooding occurs, duration and severity of flooding, and degree of turbidity of floodwater (Ramakrishnayya *et al*, 1999; Das *et al*, 2005). Submergence imposes a complex abiotic stress in flood-prone ecosystem, because it substantially reduces crop stand especially if it occurs during early vegetative stage and prolongs for more than a week (Mohanty *et al,* 2000). The flooding of root systems and partial to complete submergence of aerial organs can dramatically reduce crop productivity.

1.1 Germination and early seedling stage

Flash-floods normally affect rice during vegetative stage, however, in areas where direct seeding is practiced, heavy rains immediately after seeding cause severe damage to germinating seeds, resulting in poor crop stand. This submergence during germination (also called anaerobic germination, AG) can occur in both rainfed and irrigated lowlands. Damage to direct seeded rice in irrigated areas can happen when lands are not well levelled, or when floodwater is used for weed suppression. In all cases, poor crop establishment results in severe yield losses. Rice varieties tolerant of flooding during germination have been identified and characterized (Angaji *et al*, 2010, Ismail *et al*, 2009). Some of the characteristics associated with tolerance include the ability to maintain carbohydrate catabolism, coleoptile elongation, anaerobic respiration and maintenance of cellular extensibility of the growing embryo in flooded soils (Ismail *et al*, 2009). As the seedling elongates to more aerated zones, it develops aerenchyma tissue or lacunae through which oxygen is provided to submerged plant parts including roots. Aeration of submerged parts also allows progressive detoxification of oxygen radicals in seeds and other toxins that develop in anoxic soils, preventing further injury. Tolerant rice varieties like Khao Hlan On, Ma Zhan Red and Khaiyan were identified and QTLs conferring tolerance to germination in anaerobic soils were mapped. These tolerant varieties maintain higher α-amylase activity and higher soluble sugars, and deplete stored starch faster (Ismail *et al*, 2009). *RAmy3D* was found to be upregulated in tolerant varieties during germination in flooded soil. This gene is involved in degrading complex oligosaccharides and its regulation is independent of *RAmy1* and *RAmy2* gene families that are active under normoxia, but is activated under hypoxia (Ismail *et al*, 2013). *RAmy3D* together with other active amylases help maintain the supply of fermentable carbohydrates to provide energy for the growing embryo. Tolerant genotypes also show higher upregulation of the alcoholic fermentation pathway and its enzymes. The major enzymes are pyruvate decarboxylase (PDC), aldehyde dehydrogenase (ALDH) and alcohol dehydrogenase (ADH), mostly involved in the quick detoxification of products such as acetaldehyde and ethanol. Differential up regulation of ALDH in tolerant genotypes is probably related to ethanol conversion to acetate to avoid the accumulation of acetaldehyde during re-aeration (Meguro *et al*, 2006). Moreover, the synthesis of acetate would also involve the recycling of carbon and its subsequent use in other pathways to maintain metabolism and energy generation to maintain growth under anaerobic conditions.

1.2 Submergence during vegetative stage

Complete submergence annually affects about 16 million ha of rice in South and Southeast Asia, and about one third of the total rice growing area in Africa. The effect of damage caused by transient submergence is dependent on the characteristics of flood waters, including temperature, turbidity, concentration of dissolved gases, and extent of light penetration (Das *et al*, 2009). The main traits associated with tolerance of submergence are maintenance of high stem carbohydrates, optimum rates of alcoholic fermentation, aerenchyma formation, energy supply through underwater photosynthesis, and root aeration (Sarkar *et al*, 2006; Colmer *et al*, 2014; Winkel *et al*, 2013). Major genetic improvements have taken place in recent years, after cloning and characterization of the *Sub1A* gene from FR13A, an Indian variety (Xu *et al*, 2006; Bailey-Serres *et al*, 2010). This gene has subsequently been introgressed into several popular varieties with a reported yield increase of 1 to 3.5 t ha^{-1} over non-tolerant varieties following submergence for 4-18 days (Mackill *et al*, 2012, Ismail et al, 2013). *Sub1* confers a state of quiescence, thus avoiding excessive stem elongation, decreasing carbohydrate depletion and halting chlorophyll degradation (Colmer *et al*, 2014). The whole mechanism seems to be regulated uniquely by the *Sub1A* gene, an ethylene responsive factor (ERF), which is expressed only under submergence, causing reduction in ethylene synthesis and sensitivity, suppressing gibberellic, acid synthesis, and halting elongation. Moreover, only the *Sub1A-1* allele present in few indica landraces can confer tolerance, whereas varieties containing the *Sub1A-2* allele or lacking the *Sub1A* gene altogether, are sensitive as in most indicas, all japonicas and all rice wild relatives tested so far (Fukao *et al*, 2006; Xu *et al*, 2006; Bailey-Serres *et al*, 2010).

Mechanisms that rice adopts after desubmergence are also important, but not clear whether these are also regulated by the *Sub1A-1* allele. Post-submergence injury involves water deficits, where leaves of sensitive genotypes wilt immediately after submergence (Setter *et al*, 2010). Besides, high concentrations of reactive oxygen species (ROS) and toxic oxidative products are usually generated when leaves are aerated just after submergence, which could result in substantial damage to leaves causing them to wither and die. Ability to detoxify these products or halt their synthesis is therefore, essential for survival and recovery, and tolerant genotypes were found to upregulate their scavenging mechanisms during, and immediately after submergence (reviewed in Colmer *et al*, 2013). Complete submergence is worsening, with floods lasting over 20 days in some cases, and increasing frequency in recent years. More efforts are needed to characterize additional sources of tolerance to identify novel genes that can add to the tolerance provided by the *Sub1A* gene, to develop resilient varieties.

In areas, where flash floods cause momentary inundation during the seedling and crop establishment stage, submergence tolerance in rice needs to be improved either by growing rice varieties tolerant to flood that can survive submergence with least elongation or by management options. Along with varietal improvement, agronomic management practices like seedling age, nutrient management will also be helpful for plants to adapt to these areas. Submergence tolerance can be enhanced by genetic and environment modification or by G x E interaction.

1.3 Genetic improvement

Four different types of tolerance responses seem to satisfy the basic requirements of this ecosystem:

(i) Cultivars with high tolerance for submergence with minimum underwater shoot elongation, for flash-flood-prone areas where complete submergence predominantly occurs for 10–14 days.

(ii) Cultivars with rapid underwater shoot elongation for deep and very deepwater areas,

(iii) Good submergence tolerance with rapid regeneration ability for repeated intermittent flood conditions, and

(iv) Cultivars with tolerance for stagnant floods that can occur independently or subsequent to flash floods and can result in partial submergence for longer duration.

Efforts to identify landraces with sufficient tolerance of these abiotic stresses were initiated by IRRI and a few national programs in Asia during the 1960s and 1970s. As a result of these efforts, genotypes with considerable tolerance of major abiotic stresses were identified. To date, the most significant finding in flood-tolerance rice research is the major QTL *Sub1* derived from FR13A, a tolerant donor from Odisha, India providing tolerance of 2–3 weeks complete submergence, was previously fine-mapped on the top of chromosome 9 (Xu and Mackill, 1996; Xu *et al*, 2000). The QTL region was subsequently cloned and revealed as a cluster of three ERF genes, namely *Sub1A*, *Sub1B*, and *Sub1C* (Xu *et al*, 2006), and it was demonstrated that *Sub1A* was the key player underlying the tolerance (Xu *et al*, 2006; Septiningsih *et al*, 2009). Under flooding scenarios, complete submergence stress triggers high production of ethylene causing the intolerant varieties to elongate in attempt to reach the water surface for oxygen. In the tolerant varieties, the accumulation of ethylene regulates the expression of *Sub1A* gene, which leads to suppression of plant elongation. Upon submergence, the intolerant varieties cannot survive due to depletion of energy reserves, while the tolerant ones have enough carbohydrate supplies to recover and grow new leaves (Fukao and Bailey-Serres, 2008). In the first phase, *Sub1* was introduced into six mega varieties: Swarna (MTU 7029), Samba Mahsuri (BPT 5204) and CR1009 (Savitri) from India, IR64 from the Philippines (IRRI), Thadokkham 1 (TDK1) from Laos, and BR11 from Bangladesh (Neeraja *et al*, 2007; Septiningsih *et al*, 2009; Iftekharuddaula *et al*, 2011) using marker-assisted backcrossing (MABC). As a result of repeated backcrossing to the respective recipient parent, the improved submergence-tolerant varieties carry the FR13A *Sub1* locus but are otherwise identical to the original variety. These new varieties are called *"Sub1"* or "Scuba" rice. The *Sub1A* gene of the polygenic rice (*Oryza sativa*) Submergence1 (*Sub1*) locus was shown to confer submergence tolerance through a 'quiescence' strategy in which cell elongation and carbohydrate metabolism is repressed (Xu *et al*, 2006; Fukao *et al*, 2006). *Sub1A* encodes an ethylene-responsive element (ERF) domain–containing transcription factor. The lack of *Sub1A* or the presence of a slightly modified allele is associated with reduced submergence tolerance. Varieties having *Sub1* gene can survive during complete submergence for around 15 d because they resist elongation during submergence, conserving energy for survival and quick recovery after de-submergence. These cultivars also experience slower sugar and starch depletion and higher levels of alcoholic fermentation as a way of providing energy for maintenance processes in the absence of adequate oxygen underwater (Sarkar *et al*, 2006). *Sub1* gene containing modern varieties are identical to the original varieties in nearly all traits (Sarkar *et al*,. 2009; Mackill *et al*, 2012). Consequently, they have been extensively adopted by farmers within few years of their release (Mackill *et al*, 2012; Ismail *et al*, 2013; Singh *et al*, 2013).

Rice is unique in being capable of growing well in waterlogged and submerged soils because of its well-developed aerenchyma system that facilitates aeration of the roots and the rhizosphere, thus alleviating most of the stresses experienced under low oxygen (Setter *et al*, 1997; Jackson and Ram, 2003). But it is extremely intolerant to anaerobic conditions during germination and early growth of the embryo (Yamauchi *et al*, 1993; Ismail *et al*, 2009; Angaji *et al*, 2010). Rice seeds can germinate and, to some degree extend their coleoptiles under hypoxic and even anoxic conditions, but fail to develop roots and leaves (Taylor, 1942; Ella and Setter,1999), because of its limited ability to mobilize and use energy reserves when oxygen is limiting (Ismail *et al*, 2009). Cell division is active during the first 48 h of submergence, and that is the period when oxygen is mostly required (Atwell *et al*, 1982). Since cellular expansion consumes less energy than cell division, the latter is the main process governing elongation of the coleoptiles under anoxia. Several major quantitative trait loci (QTLs) for tolerance of anaerobic germination (AG) were identified from different landraces (Angaji *et a*l, 2010; Septiningsih *et al*, 2013), and one of them, qAG-9-2 (AG1) was derived from Khao Hlan On, a landrace from Myanmar (Angaji *et al*, 2010). Near isogenic lines (NILs) containing AG1 in the background of IR64 were developed, two of them were IR93312-30-101-20-3-66-6 (IR64-AG131) and IR93312-30-101-20-13-64-21 (IR64-AG132). Subsequent studies confirmed that there was no yield penalty due to the introgression of this QTL in several genetic backgrounds, including IR64 (Toledo *et al*, 2015). Furthermore, the genes underlying *AG1, OsTPP7,* was identified through map-based cloning. These genes increases the sink strength in proliferating heterotrophic tissues by maintaining signaling for low sugar availability, thus enhancing starch mobilization to drive growth kinetics of the germinating embryo and elongating coleoptile, which consequently enhance AG tolerance (Kretzschmar *et al*, 2015). Genotypes that are more tolerant of flooding during germination seem to have better capabilities for breaking starch into simple sugars, as demonstrated by faster depletion of starch in their germinating seeds compared with intolerant genotypes (Ismail *et al*, 2009). When flooding occurs just after direct seeding, tolerant genotypes germinate faster and their coleoptiles grow at a relatively faster rate to emerge from flooded soils. These genotypes are also capable of forming roots and leaves in shallow water depths (Ismail *et al*, 2009; Angaji *et al*, 2010).

Varieties suited to these situations will also provide opportunities for better land productivity by being more responsive to inputs and adjustments in cropping systems, helping farmers to cope with current problems and future challenges caused by climate change. By providing options for use of nutrients and other inputs to enhance yields further, these varieties have provided new opportunities for farmers in submergence-prone areas to secure higher annual productivity (Ismail, 2013).

2. Nutrient management options for enhancing submergence tolerance

The availability of tolerant varieties provides more opportunities for developing and validating proper management options effective in flood-prone areas, which could further boost and stabilize the productivity of these varieties (Ella and Ismail, 2006). However, nutrient recommendations have not been specially developed for flood-prone areas and farmers often avoid using inputs as a risk aversion strategy. Optimal nutrition before submergence and post-submergence is necessary to equip plants with cellular and metabolic requirements essential for survival of flooding, and also for fast recovery after floodwater recedes (Ella and Ismail, 2006; Gautam *et al*, 2014; Lal *et al*, 2014). It is unclear if some nutrient elements can actually increase submergence tolerance if applied at rates above balanced applications. Therefore, improving plant health through nutrient management may lead to better crop establishment. Post-submergence nutrient management particularly N can also contribute substantially towards increasing productivity in flood-prone areas. But the farmers' practice of applying only nitrogenous fertilizers in the nursery to get taller and greener seedlings may not have positive consequences in flood-prone areas because high N content in seedlings before flooding adversely affects survival after flooding and results in poorer recovery (Ella and Ismail, 2006). There exists vast possibility for increasing rice production and harnessing the productivity potentials of submergence-affected areas with the use of submergence-tolerant varieties, particularly when combined with the best nutrient management practices specific for these areas.

2.1 Nutrient management during and after submergence

Most of the Asian farmers do not apply the N and P fertilizers with a belief that the rice crop may not give response to fertilization under flood water stress situations and also due to high risk involved in successful production of crop and runoff losses during floods. They normally apply 40–50 kg N, 15–25 kg P_2O_5, and 10–15 kg K_2O ha^{-1} against the recommended rates of 60–80 kg N and 40 kg P_2O_5 ha^{-1} (Pathak, 1991). Most of these farmers do not apply fertilizers before transplanting or direct seeding because of extensive inundation during heavy rains that can break their field bunds and result in fertilizer losses. At the recession of terminal floods, several farmers broadcast only nitrogenous fertilizers at a very low rate against the recommended split dose of fertilizer (half at basal before planting and remaining half at panicle initiation). Fertilizer application of 30–40 kg N ha^{-1} is optimum for deepwater areas, but farmers seldom apply any fertilizer. Deepwater rice areas normally receive sufficient organic matter and nutrients during floods and are supposed to sustain good crop growth. However, N application at the time of flowering is essential since a sizeable amount of nutrients are lost due to runoff, volatilization, and deep percolation during floods. Since submergence in flood-prone areas affects crop establishment more seriously than other crop performance indicators, the solution lies in nursery management and “hardening” of the seedlings prior to transplanting for better crop establishment.

2.2 Nutrient management in the nursery

Submergence greatly affects N and P availability and assimilation, which can influence submergence responses and which have been implicated in differences in tolerance between cultivars. Submergence rapidly depletes the protein reserves of the plants through hydrolysis to amino acids and other soluble N-containing compounds (Yamada, 1959). Palada and Vergara (1972) found the normal increase in percentage N content that occurs between 10 and 20 d after germination (from 3.1 to 4.3%) to be hindered by submergence or even reversed if the water was turbid. However, attempts to raise N levels by feeding ammonium sulfate was not beneficial and even prejudicial to survival (79% survival without vs 15% survival with ammonium sulfate application), an effect associated with 61% and 34% decrease in pre-submergence starch and total sugar concentrations, respectively. Furthermore, submergence-tolerant cultivars are not noticeably richer in N after prolonged submergence than intolerant. Yet, when nitrate concentration was analyzed before submergence, the shoots of tolerant lines such as FR13A were found to be much richer in nitrate than that of sensitive types.

The nutritional status of seedlings before transplanting is also of immense importance, especially when plants are submerged during the early growth stages. Farmers' convention of applying only nitrogenous fertilizers in the nursery to get taller and greener seedlings may not have any positive consequence for flood-prone areas, since the high N content in seedlings at the time of transplanting adversely affects survival after submergence and results in poor recovery growth. However, when high N is accompanied by high P content, it seems to improve seedling vigor and tolerance to withstand ensuing submergence stress and with effectively better recovery afterwards (Jackson and Ram, 2003; Ella and Ismail, 2006; Gautam *et al*, 2014b; Lal *et al*, 2014). Singh *et al* (2004) also pointed out the harmful effects of high N application in the nursery when applied one week before transplanting. However, application of N 10–15 d prior to planting reduced plant mortality by 50% when the soil is N-deficient. Ella and Ismail (2006) and Gautam *et al* (2014b) observed poorer plant survival when high N was applied late (just before transplanting) relative to early N application, irrespective of the tolerance level of the genotype. In contrast, application of P, either alone or together with N, improved the survival of plants in P-deficient soil after 12 d of complete submergence. High leaf N before submergence showed a negative correlation with photosynthetic gas exchange during the recovery phase after desubmergence. Leaf chlorophyll content increased with application of N either alone or in combination with P, and the response was better in P-deficient soil. Late N application showed lower relative chlorophyll content during the first 3 d of recovery, with maximum recovery being noted in control plants (Ella and Ismail, 2006).

Increasing levels of N application before submergence were not beneficial but instead were pre-judicial to survival (Yamada, 1959; Jackson and Ram, 2003; Gautam *et al*, 2014b; Lal *et al*, 2014), and resulted in depletion of chlorophyll and non structural carbohydrates (NSC) (Gautam *et al*, 2014b; Lal *et al*, 2014). Soluble carbohydrates after submergence are more important for survival than at the initial level, Gautam *et al* (2014a) reported that, IR64 *Sub 1* could survived better under submergence because it possessed 12.5% more NSC after submergence as compared to IR64. Leaves are known to senescence more rapidly under the conditions of nitrogen deficiency. The senescence is accompanied by enhanced generation of reactive oxygen specis (ROS), whereas the activity of antioxidant enzymes could increase or decrease.

In a study, Gautam *et al* (2014b) reported that foliar spray of post-submergence N resulted in higher plant survival, and maintained higher level of chl a & b content and NSC with encouraging effects in plants submerged under turbid water might be due to the fact that urea spray removed the adhering silt from the leaf surface and enable the plants to photosynthesize and survive. Foliar spray of post-submergence N with basal P seems to be beneficial, as application of P contributes to submergence tolerance, reduces the ethylene accumulation which ultimately helps in conservation and maintenance of carbohydrates while N spray helps in regeneration and survival, ultimately increasing submergence tolerance.

The yield advantage, achieved by raising crops from seedlings obtained from well nourished seedbeds, was 16–20% in one farmer's field at Faizabad, India where submergence stress was not very severe. In the other farmer's field, the benefit of nursery nutrient management was about 160%, when multiple floods occurred at three different growth stages (data not shown). This indicates that seedling health in the nursery is extremely important as it affects crop establishment and survival, especially when submergence occurs at the early stages of crop growth and also under recurrent floods. The beneficial effects of seedling health prior to transplanting seem to be more evident following severe submergence conditions and are directly related to seedling survival and rapid recovery growth after the recession of floods.

Proper nursery management can, therefore, substantially help enhance and stabilize productivity in flood-prone areas, provided that balanced nutrients are used and excessive early seedling growth is avoided (Ella and Ismail 2006; Gautam et al, 2014b; Lal et al, 2014).

2.3 Nutrient management in the main field

Nutrient management in the main rice fields before transplanting and also after the floods recede is also important for improving rice productivity in flood-prone ecosystems. Plant growth and yield depend not only on carbohydrate production through photosynthesis but also on mineral absorption by the roots and its assimilation. Rice crops in flooded soil absorb N both from the floodwater and the soil. Absorption of N fertilizer broadcast onto floodwater in the rice field is very rapid if fertilizer application is timed carefully to match the plant's demand. However, the N that is not absorbed rapidly is lost through gaseous emission, percolation, or runoff. Consequently, use of N fertilizer tends to be very inefficient even in irrigated ecosystems, where average recovery across Asia is less than 30%. The rapid uptake of N from the floodwater is due to surface roots in the water and in the adjacent topsoil. These roots differ morphologically and physiologically from those in the anoxic soil bulk. The entire N in flooded soil is absorbed in the form of NH_4^+, which is the main available form of N. However, N is also absorbed as NO_3^- and amino acids in flooded rice fields, and plant growth and yield are generally improved when plants absorb N as a mixture of NO_3^- and NH_4^+ compared with either of them used separately (Kirk and Kronzucker, 2005).

In flood-prone areas of eastern India and Bangladesh, nutrient management activities are mainly done after the recession of the floods because of the inherent risks of crop loss if submergence is severe. Farmers mostly apply N after the floods for rapid recovery growth of surviving rice plants. Another option followed by the researchers to facilitate the slow release of N is to apply it through urea supergranules (USG) or coated urea or as foliar spray of post-submergence N for better survival and recovery of rice seedlings (Gautam *et al*, 2014c). Reddy and Sharma (1992) suggested that if the water level remains at an intermediate depth (~50 cm) in the field, 40 kg N ha^{-1} as USG or neem cake-coated or coal tar-coated urea may be applied, together with 20–40 kg P_2O_5 in the seed furrow at the time of sowing. However, if the crop is transplanted, fertilizer may be applied as basal at the time of final puddling or coated USG can be broadcast a few days later after transplanting. Singh *et al* (1992) reported that the placement of USG in the soil at a depth of 8–10 cm in about 15 cm of standing water resulted in higher yield than the basal application of either prilled urea or neem cake-coated urea during puddling. The efficacy of slow-release nitrogenous fertilizers is yet to be validated at a large scale in farmers' fields and at target locations, together with the consideration of its economic benefits. Submergence-induced membrane damage is one of the most serious threats to plant survival and plants need a large amount of energy for repair and maintenance processes under anaerobic stress. Supply of sufficient P might thus have positive impacts on submergence tolerance of rice plants, presumably through the maintenance of a high level of energy. Application of P at 80 kg ha^{-1} as diammonium phosphate along with 60 kg N ha^{-1} at sowing enhanced initial seedling vigour and shoot carbohydrate concentration before submergence. Plant survival after 7–10 d of complete submergence and regeneration growth during recovery were better in P-treated plants of several lowland rice varieties. However, P application 24 h prior to submergence did not show any beneficial effect on plant survival and recovery growth. Rock phosphate is a good source of P in flooded fields because of its slow release, which is further stimulated in acid soils. Ramakrishnayya *et al* (1999) reported that addition of P to floodwater during submergence reduced rice plant survival by 35%. The adverse effects of high P concentration in floodwater were mainly attributed to enhanced growth of algae that competed with the submerged plants for CO_2 and light. Application of P should, therefore, be considered both in the nursery and as basal rather than in floodwater.

The response of rice to post-flood nutrient management options in flood-prone ecosystems was least studied so far, though it has a strong bearing on regeneration growth and yield of rice plants after the floods, and suitable nutrient management strategies were highly demanded. Farmers in flood-prone areas mostly broadcast small amounts of urea without any scientific recommendations. Possibilities of recurrent submergence during the season are one of the reasons for avoiding nutrient application. Hence, a complete package of post-flood nutrient management is essential to enhance the productivity of flood-prone rice areas. Yamada (1959) and Ella and Ismail (2006) reported that seedling enrichment with N before submergence adversely affected plant survival, later on Gautam *et al* (2014a) confirmed that pre-submergence N application enhanced succulence of plants resulted in poor survival. In a study, Gautam *et al* (2014c) revealed that foliar spray of post-submergence N resulted in higher plant survival, and maintained higher level of chl a & b content and NSC with encouraging effects in plants submerged under turbid water. This might be due to the fact that urea spray removed the adhering silt from the leaf surface and enable plants to photosynthesize and survive. Pande *et al* (1979) suggested that use of N through foliar spray after flash flooding resulted in better recovery and higher productivity of rice under waterlogged conditions. Basal P and post-flood N application either though broadcasting or foliar spray resulted in significantly higher tiller regeneration, biomass, leaf area, specific leaf weight, Pn rate and ultimately yield. Beneficial effect of N fertilizer on greater tiller survival was due to better initial crop vigour and carbohydrate accumulation, leading to increased tolerance of plants to submergence (Palada and Vergara, 1972). The recovery of plants subjected to simulate flash flooding was better with top dressing of N fertilizer after floods, as some of the partially damaged tillers regained growth and produced more dry matter (Sharma, 1995). Studies conducted in Bangladesh revealed that NPK application during the post-flood period have positive effects on growth and grain yield of rice. Nitrogen alone applied at a rate of 50 kg ha^{-1} resulted in maximum tillers and grain yield, whereas N and K applied together at 20 kg ha^{-1} each was most effective, showing a two-fold increase in grain yield over unfertilized control. However, further studies are needed to test different nutrient combinations, doses, and the proper time for their application after the water recedes. Valid recommendations can then be developed for different target sites based on local flood conditions, particularly the speed of water recession, possibility of subsequent floods, and water depth in the field following complete submergence.

Apart from nutrient management in flood prone areas, there is an urgent need to redesign flood-prone rice varieties to improve efficiency in nutrient uptake and utilization. To redesign the plant type for better nutrient use efficiency, we should focus on modifying root-shoot interaction to optimize and prolong root activity, which is strongly related to the system of carbohydrate supply to the roots during regeneration and recovery after the floods. Nodal roots also have an important role to play in nutrient assimilation from floodwater and can help sustain the growth of rice plants under flooded conditions.

3. Methodology for recording phenotypic and biochemical observations

3.1 Flood water characteristics

Light transmission, that is photosynthetically active radiation (PAR) through the flood water, water temperature, dissolved oxygen (DO), pH, electrical conductivity (EC), redox potential (ORP), total dissolved salts (TDS) were determined at 5, 50 and 75 cm of water depth. Light intensity was measured at 12:00 h using LICOR light meter (LI-COR, Lincoln, NE, USA), whereas temperature (06:30 h and 14:00 h) and other water quality parameters (11:00 h) were determined by using U-50 Multi parameter water quality meter (HORIBA, Kyoto, Japan).

3.2 Measurement of silt deposition on leaf surface

Leaves of three plants from each treatment were harvested carefully just after draining of submergence water and placed in a beaker containing 250 mL of distilled water and left overnight. Plants were then washed thoroughly till there was no trace of silt deposition. The turbid water in the beaker was then filtered using pre-weighed Whatman filter paper no. 42. The filter papers with silt deposition were then dried in an oven at 70°C for 3 days and then reweighed. Area of leaves was also calculated with length and breadth of leaf, and expressed in cm^2. Silt deposition was determined as the difference in weight of the filter papers and expressed in mg cm^{-2} of leaf area.

3.3 Shoot elongation

Extent of elongation of the plant shoots was determined by subtracting plant height before submergence (BS) from that after submergence (AS) and expressing it as percentage of plant height before submergence.

$$\text{Shoot elongation (\%)} = \frac{\text{plant height AS - plant height BS}}{\text{plant height BS}} \times 100$$

3.4 Plant survival

Plant survival was determined by counting the number of plants that were able to

Fig. 1 Measurement of under water PAR and water quality parameters

produce at least one new leaf after 7 or 10 days of desubmergence and was expressed as percentage of the initial number before submergence.

$$\text{Plant survival (\%)} = \frac{\text{No. of green leaves after 7-10 days of desubmergnce}}{\text{Total no. of leaves before submergence}} \times 100$$

3.5 Chlorophyll concentration in leaves

Chlorophyll concentration was determined colorimetrically following the procedure of Porra (2002). Chopped fresh leaf tissue of 0.1 g was transferred to a capped measuring tube containing 25 mL of 80% acetone, and kept inside a refrigerator (4°C) for 48 h before measurements were made using a spectrophotometer (SICAN 2301 Double Beam Spectrophotometer, Incarp Instruments, Hyderabad, India). The Chl *a* and Chl *b* concentrations were calculated using the following equations:

$$\text{Chlorophyll a (mgmL}^{-1}) = 12.25\,(A_{663.6}) - 2.55\,(A_{646.6})$$
$$\text{Chlorophyll b (mgmL}^{-1}) = 20.31\,(A_{646.6}) - 2.55\,(A_{663.6})$$

3.6 Non-structural carbohydrates (sugar and starch levels)

Soluble sugar and starch content of the shoots were estimated before submergence and after 14 days of complete submergence following the procedure of Yoshida *et al* (1976). Briefly, for each measurement, shoot samples were dried and ground to a fine powder and extracted using 80% ethanol (v/v). The extract was then used for soluble sugar analysis after addition of anthrone reagent, followed by a measurement of absorbance at 630 nm using a spectrophotometer (SICAN 2301 Double Beam Spectrophotometer, USA). The residue remaining after soluble sugar extraction was dried and extracted using perchloric acid and then analyzed for starch (as glucose equivalent) using the anthrone reagent as for soluble sugars.

3.7 Ethylene accumulation

Leaves of two individual plants from each treatment were cut in 2 cm length segments and placed in 30 mL test tubes with 2 mL of distilled water. The tubes were stoppered with serum vial caps and kept horizontally. Ethylene was sampled by first injecting 1 mL of air into each tube with a tuberculin syringe, pumping the syringe several times, and then withdrawing 1mL for analysis. In calculating the amount of ethylene in the tubes, a correction was applied for the successive injections of air and removals of gas samples. Ethylene was determined by Gas Chromatography (Model Chemito Ceres 800 plus) according to Kende and Hanson (1976).

3.8 Measurement of photosynthetic rate

Net photosynthetic rate and stomatal conductance of rice plants were measured before submergence and after submergence with an infrared gas analyzer (LI-6400, LI-COR) around 11:00 h. The conditions in the assimilation chamber were kept as follows: air humidity, 70%; leaf temperature, 35°C; light intensity (PAR), 1200 μmolm^{-2} s^{-1}. Measurement was carried out using middle portion (3 cm long) of the fully expanded and not senescent leaf blade. Net photosynthetic rate was taken at the CO_2 concentration of 380 μmol CO_2 mol^{-1}.

Fig. 2 Measurement of photosynthetic rate and stomatal conductance

3.9 Allometry

Number of leaves was recorded daily after desubmergence, starting from the day of desubmergence to 10 days after desubmergence. Length of each individual leaf was measured daily to derive the leaf elongation rate prior to submergence and upto ten days of desubmergence after each stage of submergence. Plants were randomly chosen and gently uprooted, all leaves were detached and the senescence portion was removed. Four plants were harvested per replicate and total green leaf area measured using a leaf area meter (LI-3100, LiCor Inc, Lincoln, NE, USA). Leaf area was recorded by putting each fresh leaves of one plant from each treatment in digital leaf area meter and then leaves were dried to constant weight at 70°C for determination of specific leaf weight (mg dry weight cm^{-2} leaf area). Specific leaf area (SLA) was calculated as the ratio of leaf area to leaf weight. Shoot and root growth were assessed on the same four plants. Roots and shoots were gently separated and rinsed for few minutes with distilled water to remove adhering silt and salt. Dry matter weights were determined after drying the plant samples to a constant weight in an oven set at 70°C. Leaf senescence was assessed immediately after desubmergence on hill basis using a visual scale of 1 to 10. This visual score was based on the proportion of leaves that were yellow: 1 = all leaves green and 10 = all leaves completely yellow or degenerated. A chlorophyll meter (Minolta SPAD-502) was used to confirm the leaf senescence score based on the amount of chlorophyll or

Fig. 3 Recording of SPAD and CLCC value

greenness of leaves. SPAD values (SPAD units) from the five upper fully expanded functional leaves on each plant at interval of approximately 10 days. A total of 10 readings were measured in each hill and three SPAD readings per leaf expressed as mean of one reading around the mid-point of leaf blade and two readings at points three cm apart from the mid-point. The degree of lodging after one day of desubmergence was scored from one to six (1 = no plants lodged and 6 = complete lodged).

3.10 Extraction and assay of antioxidant enzymes

A 500 mg sample of leaves was homogenized in 10 mL of grinding medium prepared for each enzyme, as mentioned below. The extract was centrifuged at 4°C at 15000 g for 20 min, and the supernatant was used for assays. All operations were performed under a dim green light.

3.10.1 Estimation of Superoxide dismutase (SOD)

For the determination of SOD activity, the enzyme was extracted in 0.1 *M* potassium phosphate buffer (pH 7.8) containing 1% (w/v) insoluble PVPP. The enzyme activity was determined by measuring its ability to inhibit photochemical reduction of nitro blue tetrazolium (NBT) following Giannopolitis and Ries (1977) with modifications suggested by Choudhury and Choudhury (1985). The 3 mL reaction mixture contained 0.05 *M* Na_2CO_3, 0.1 m*M* EDTA, 63 l*M* NBT, 13 l*M* methionine, 0.2 mL enzyme extract and 1.3 l*M* riboflavin. The riboflavin was added last. The test tubes were placed under two 40 W fluorescent lamps at a distance of 30 cm at 25°C. After 15 min, the light was switched off and the absorbance at 560 nm (A560) was noted. The non-irradiated sample served as a control and was deducted from A560. The reaction mixture without the enzyme developed maximum colour due to maximum photoreduction of NBT. The reduction of NBT was inversely proportional to the enzyme activity. Thus, to obtain the activity, the A560 of a particular set was deducted from the A560 of the blank set (without enzyme).

3.10.2 Estimation of Catalase (CAT)

The CAT activity was measured in a reaction mixture containing 25 m*M* phosphate buffer (pH 7.0), 10 m*M* H_2O_2 and the enzyme extract. The degradation of H_2O_2 was followed at 240 nm (Cakmak and Marschner, 1992).

3.10.3 Estimation of peroxidase (PER)

The total PER activity was measured in a reaction mixture consisted of 0.2 mL of enzyme, 5 mL phosphate buffer (0.05 *M*, pH 6.0), 1 mL H_2O_2 (46.9 m*M*) and 1 mL catechol (0.5%). PER was assayed by the method of Chance and Maehly (1955), whereby colorimetric determination of the change in the colour intensity of oxidized catechol at 420 nm was recorded.

3.11 Estimation of Malondialdehyde (MDA)

Lipid peroxidation was measured as the amount of MDA produced by thiobarbituric acid (TBA) reaction, as described by Heath and Packer (1968). A 500 mg sample of leaves was extracted with 1% (w/v) trichloroacetic acid (TCA), and MDA content was determined by adding an equal aliquot of 0.5% TBA in 20% TCA to an aliquot of the extract. The solution was heated at 95 °C for 25 min. Absorbance was measured at 532 nm, corrected for nonspecific turbidity by subtracting the absorbance at 600 nm. The amount of MDA was calculated by using an extinction coefficient of 155 mM^{-1} cm^{-1}.

3.12 Phenology and yield

Days to flowering (80% of panicles reaching heading) was recorded. At maturity (90% of spikelets turning yellow), the number of surviving plants and panicles was counted on hill basis and grain yield was determined. Plants were separated into straw and panicles. Panicles from all the hills were hand-threshed and filled spikelets were separated from unfilled spikelets by submerging them in water. The number of filled spikelets and unfilled spikelets was determined to calculate spikelets/florets per panicle and grain filling percentage. Samples were oven-dried at 80°C for 72 h. Grain yield was determined and adjusted to moisture content of 14%.

3.13 Nutrient content

Plant samples were harvested before submergence, after desubmergence and harvest, dried and grinded for the analysis. Nitrogen, phosphorus, potassium and silica content in shoots were determined by Kjeldahl method (Subbiah and Asija, 1956), Vanado-molybdate method (Bray and Kurtz, 1945), flame photometric method (Piper, 1966), and colorimetry method, respectively.

3.13 Statistical Analysis

The data for all the parameters were analyzed using SAS version 9.3 (2012) software. Treatment means were compared at the $P<0.05$ (sometimes $P<0.01$ level) using least significance difference (LSD). Association between parameters were studied using correlation and linear regression.

4. Submergence tolerance in relation to application time of nitrogen and phosphorus during different crop growth stages

Nitrogen (N) is the most essential element in determining rice grain yield, and N fertilizer is one of the major inputs to paddy fields, with favorable effect of promoting tillering and yield. Nitrogen management is an important strategy in regulating the rice growth and photosynthetic efficiency. Improving photosynthesis is of greatest agronomic importance as the most plausible route toward enhanced biomass production, even under complete submergence. It is unclear if some nutrient elements can actually increase submergence tolerance if applied at rates above balanced applications. In particular, nitrogen has been reported to be the only possible limiting nutrient for rice production in flood-prone areas. However, nutrient recommendations have not been specially developed for flood-prone areas and farmers often avoid using inputs as a risk aversion strategy. Some of the farmers in these areas usually broadcast a small amount of only nitrogenous fertilizers without following any solid recommendation. Possibilities of recurrent submergence during the season are one of the reasons for avoiding nutrient application. The availability of tolerant varieties provides more opportunities for developing and validating proper management options effective in flood-prone areas, which could further boost and stabilize the productivity of these varieties (Ella and Ismail, 2006; Gautam et al, 2014b). Under variable and unpredictable natural flood conditions, farmers grow photosensitive long duration traditional tall varieties, rarely with fertilizer application because of the inherent risk involved. However, semi-dwarf rice cultivars having submergence tolerance developed in the recent years have been found to respond to N application and thus withstand excess water stress better (Reddy and Mitra, 1985a). In this context, several studies were conducted with submergence tolerant and intolerant rice varieties for harnessing the productivity potentials and minimizing flood damages in rice production through alteration in time and method of nitrogen application.

The experiment was conducted under natural conditions during 2012 with two Indica rice, cultivars IR64 *Sub1* and IR64; six schedules of nutrient application viz. N$_0$PK, NP$_0$K(BS), NP$_0$K (AS), NPK (BS), NPK (AS), and N$_0$P$_0$K (details of N and P application schedule given in Table 1) and three different stages of submergence viz. 21, 45 and 65 days after transplanting (DAT). The experiment was arranged in a factorial randomized block design with four replications. One 15 day old

Table 1 Fertilizer application schedule followed in the experiment

Nutrient combination	Submergence time (DAT)	Nutrient application time					
		Nitrogen*				Phosphorus	Potassium
		1st split	2nd split	3rd split	4th split		
N$_0$PK	-	-	-	-	-	Basal	Basal
NP$_0$K (BS)	21	Basal	19 DAT	PI stage	H stage	-	Basal
	45	Basal	AT stage	43 DAT	H stage	-	Basal
	65	Basal	AT stage	PI stage	63 DAT	-	Basal
NP$_0$K (AS)	21	Basal	36 DAT	PI stage	H stage	-	Basal
	45	Basal	AT stage	61 DAT	H stage	-	Basal
	65	Basal	AT stage	PI stage	81 DAT	-	Basal
NPK (BS)	21	Basal	19 DAT	PI stage	H stage	Basal	Basal
	45	Basal	AT stage	43 DAT	H stage	Basal	Basal
	65	Basal	AT stage	PI stage	63 DAT	Basal	Basal
NPK (AS)	21	Basal	36 DAT	PI stage	H stage	Basal	Basal
	45	Basal	AT stage	61 DAT	H stage	Basal	Basal
	65	Basal	AT stage	PI stage	81 DAT	Basal	Basal
N$_0$P$_0$K	-	-	-	-	-	-	Basal

DAT: days after transplanting, AT: active tillering, PI: panicle initiation, H: heading, BS: before submergence, AS: after submergence

*Nitrogen was applied in equal amounts (25% of total dose) in each split

Table 2 Submerged water characteristics during submergence at 21, 45 and 65 days after transplanting (DAT).

Time of submergence	Water Depth (cm)	Temperature (^{0}C)		Light intensity (µ mol m^{-2} s^{-1})	DO (mg L^{-1})	pH	EC (d S m^{-1})	ORP (mV)	TDS (g L^{-1})
		06:30 h	14:00 h						
21 DAT	5	22.1±1.0	24.7±1.1	829.5±112	8.2±0.6	8.4±0.5	0.270±0.02	146±14	0.171±0.01
	50	22.7±1.1	24.4±1.2	529.9±88	8.0±0.4	8.4±0.6	0.272±0.02	141±12	0.169±0.00
	75	22.9±1.1	24.3±1.2	312.7±45	7.9±0.4	8.4±0.6	0.271±0.03	138±12	0.167±0.01
	Mean	**23**	**24**	**557**	**8**	**8**	**0.271**	**142**	**0.169**
45 DAT	5	23.4±0.8	27.6±0.7	914.3±118	7.8±0.8	8.4±0.4	0.253±0.04	131±16	0.164±0.02
	50	23.8±0.5	27.1±0.8	612.6±96	7.6±0.7	8.5±0.4	0.253±0.03	130±13	0.163±0.02
	75	24.1±0.6	26.8±0.6	329.6±61	7.6±0.7	8.5±0.3	0.254±0.03	128±11	0.163±0.01
	Mean	**24**	**27**	**619**	**8**	**8**	**0.253**	**130**	**0.163**
65 DAT	5	26.5±0.7	29.9±0.6	961.9±115	7.6±0.5	8.3±0.2	0.247±0.05	124±17	0.160±0.02
	50	27.2±0.6	29.5±0.5	617.3±92	7.3±0.6	8.3±0.3	0.246±0.05	123±15	0.158±0.00
	75	27.6±0.7	29.1±0.5	350.8±52	7.3±0.5	8.3±0.3	0.246±0.004	123±15	0.158±0.00
	Mean	**27**	**29**	**643**	**7**	**8**	**0.246**	**123**	**0.159**

Values are means of measurements made every day during 14 d complete submergence ± standard error.

seedling of each cultivar was transplanted in the pots containing 8 kg of farm soils (Sandy clay loam, pH 6.4, EC – 0.079 dS m^{-1}, available N, P and K – 58.9, 4.5 and 64.7 mg kg^{-1} of soil, respectively). About 1.75 g $(NH_4)_2SO_4$, 0.95 g KH_2PO_4 and 0.52 g KCl were applied to each pot as per the treatments as N, P and K source, respectively. Plants were irrigated with fresh water to maintain 2 cm standing water except during the period when rice plants were subjected to submergence. Potted plants were completely submerged in a concrete tank containing fresh and clear water and the water depth was maintained at 30 cm above the top of the plant canopy for 14 days at three different times of crop duration i.e. 21, 45 and 65 DAT, which coincides with active tillering, panicle initiation and heading stages of crop. After desubmergence, the plants were allowed to recover for seven days, and plant survival was recorded. Plant samples were collected 48 h before submergence and then seven days after desubmergence for various measurements.

4.1 Water quality parameters

The temperature of flood water during the crop growth period ranged from 22.6 to 27.1°C in the morning (06:30 AM) and from 24.5 to 29.5°C in the afternoon (02:00 PM). The temperature was considerably lower during submergence at the active tillering stage (21 DAT), as compared to 45 and 65 DAT. pH of the flood water ranged from 8.3 to 8.5, did not vary much with water depth and time of submergence. The dissolved oxygen (DO), electrical conductivity (EC), oxidation reduction potential (ORP) and total dissolved solid (TDS) values were slightly higher during submergence at 21 DAT (Table 2) than other two stages. Water depth did not have significant effects on the DO, but substantially affect light penetration. When calculated as the percentage of total incidence irradiance above the water surface, underwater light intensity decreased by 35.9% at five cm below the water surface, and by an additional 53.7% decrease at 50 cm and 77% at 75 cm. The underwater light intensity increased from 21 DAT to 65 DAT because of increased incidence solar radiations due to natural weather conditions.

4.2 Shoot elongation

Submergence enhanced the shoot elongation of both the cultivars (Table 3), increase in shoot length was greater in IR64 (89.9%) as compared to IR64 *Sub1* (63.1%). Time of submergence significantly influenced shoot elongation, which was highest after submergence at 21 DAT (111.5%). The application of P substantially suppressed underwater shoot elongation in both the cultivars with a greater effect on IR64 *Sub1.* Underwater shoot elongation was enhanced to a greater extent by addition of pre-submergence N application (48 h before submergence). It was increased by 83.7% where no basal P was applied and 76.7% where basal P was applied. Shoot elongation was lowest (68.7%) in the treatment with basal P application as compared to other treatments. Interactive effect of variety, time of submergence and nutrient application on shoot elongation (Table 3) was found significant. Underwater shoot elongation was less when IR64 *Sub1* was subjected to submergence at 65 DAT and supplied with basal P, whereas, it was maximum when IR64 subjected to submergence at 21 DAT and supplied with pre-submergence N.

4.3 Plant survival and metabolic changes

Submergence reduced plant survival percentage of both the cultivars (Table 3) with significantly greater effects on IR64 (29.9%) than IR64 *Sub1*. The plant survival after submergence at different stages followed the same trend as in case of shoot elongation; highest plant survival (45.9%) was recorded at active tillering stage.

Plant survival was highest (43%) when N was top dressed 48 h after desubmergence along with basal P followed by post-submergence N (48 h after desubmergence) with no P application (39.6%), whereas plant survival was substantially decreased (30.1%) with pre-submergence N application. Interactive effect of variety, time of submergence and nutrient application on plant survival (Table 3) was significant. Pre-submergence N application in IR64 showed lowest plant survival percentage at the heading stage, whereas, IR64 *Sub 1* recorded highest plant survival percentage, with post-submergence N application along with basal P application.

4.4 Growth after recovery and photosynthesis

Nitrogen application seems to affect assimilate partitioning, increased leaf-stem ratio, higher increase in leaf blade biomass (due to underwater low light intensity) but this could be at the expense of carbohydrate reserves (Ella and Ismail, 2006) leading to less number of phyllochron, low SLA and poor survival. Post-submergence N and basal P application maintained higher level of chlorophyll after submergence. Nitrogen treated plants had higher chlorophyll concentration before submergence (Table 3), but maintained much lower levels of chlorophyll than no N receiving plants after desubmergence with a greater decrease in IR64 than IR64 *Sub 1*. This suggests greater chlorophyll degradation during submergence in N enriched plants. Non-structural carbohydrates before and after submergence are important for providing energy needed for maintenance and metabolism during submergence and for fast recovery after submergence (Das *et al*, 2005; Panda *et al*, 2008). N application before submergence resulted in more damage in terms of NSC, this might be attributed to two facts: (1) N application enhanced shoot growth over root growth with the subsequent depletion of soluble carbohydrates stored in shoots (Ella and Ismail, 2006); and (2) N application might be resulted in more succulence/softness of shoots, leading in more shoot elongation and plant mortality. However, post-submergence N application when combined with basal application of P seems to be beneficial, as application of P reduces the stem elongation, which ultimately helps in conserving carbohydrates and increasing submergence tolerance.

Further, N application before submergence (48 h before submergence) resulted in highest percentage change in LAI (96%) which indicates that before submergence N application contributed to more chlorophyll degeneration, damage was more severe when basal P was not applied. Minimum percentage change in LAI (23.1%) was observed when N applied after desubmergence (48 h after submergence). N application before submergence resulted in

Table 3 Shoot elongation, plant survival, pre and post-submergence chlorophyll concentration as affected by variety, time of submergence and nutrient application

Treatments	Shoot elongation (%)	Plant survival (%)	Chlorophyll content (mg g^{-1} Fresh weight)		
			BS	AS	% Change
Variety (V)					
IR64	89.9^{a}	29.9^{b}	4.31^{a}	2.90^{b}	48.6^{a}
IR64 *Sub-1*	63.1^{b}	39.2^{a}	4.41^{a}	3.60^{a}	22.5b
Mean	76.5	34.5	4.36	3.25	35.6
Time of Submergence (T)					
21 DAT	111.5^{a}	45.9^{b}	4.29^{a}	3.17^{a}	35.3^{a}
45 DAT	78.6^{b}	38.5^{b}	4.41^{a}	3.31^{a}	33.2^{a}
65DAT	39.5^{c}	19.2^{c}	4.39^{a}	3.26^{a}	34.7^{a}
Mean	76.5	34.5	4.36	3.25	34.4
Nutrient application (N)					
N$_0$PK	72.6^{c}	36.2^{c}	4.28^{c}	3.28^{b}	30.5^{a}
NP$_0$K(Apply BS)	83.7^{a}	30.1^{e}	4.52^{bc}	3.04^{c}	48.7^{a}
NP$_0$K(Apply AS)	74.9^{bc}	39.6^{b}	4.46^{c}	3.33^{b}	33.9^{b}
NPK(Apply BS)	76.7^{b}	32.4^{d}	4.83^{a}	3.32^{b}	45.5^{a}
NPK(Apply AS)	68.7^{d}	43.0^{a}	4.62^{abc}	3.64^{a}	25.9^{c}
N$_0$P$_0$K	79.9^{b}	31.5^{e}	3.97^{d}	3.09^{c}	28.5^{b}
Mean	76.4	35.1	4.45	3.28	35.7
LSD$_{0.05}$ (VxT)	6.2	2.3	ns	0.25	6.6
LSD$_{0.05}$ (VxN)	10.2	3.8	0.37	0.41	10.8
LSD$_{0.05}$ (TxN)	12.5	4.7	ns	0.51	13.3
LSD$_{0.05}$ (VxTxN)	17.7	6.6	ns	0.72	18.8

In a column, values followed by a common letter are not significantly different at $P<0.05$ using least significance difference test.

Shoot elongation was calculated as % increase in plant height during submergence and % survival was determined after 7 d of recovery to the initial number of plants before submergence

BS-Before submergence; AS-After submergence; DAT- days after transplanting higher leaf senescence and ethylene accumulation but lower photosynthetic (Pn) rate because of chlorosis and degeneration of leaves and this damage was fatal without basal P application. The Pn rate and stomatal conductance (Gs) of both cultivars was almost similar under non-submerged conditions. The leaf Pn rate decreased in both the cultivars with the progression of time of submergence but decrease was more in IR64 (44.1%) than in IR64-Sub1 (17.8%) as compared to non-submerged conditions (Fig. 4).

Higher value of leaf Gs in IR64 *Sub1* after submergence resulted in 27.7% higher Pn rate over the Pn rate of IR64. The factor controlling Pn rate varied significantly with time of submergence and stage of crop. The Pn rate remained highest when plants were submerged at AT stage, whereas minimum Pn rate and Gs were observed during harvesting (H) stage submergence. Nitrogen application after desubmergence considerably improved Pn rate and Gs, this improvement was more prominent when basal P was applied along with N. Application

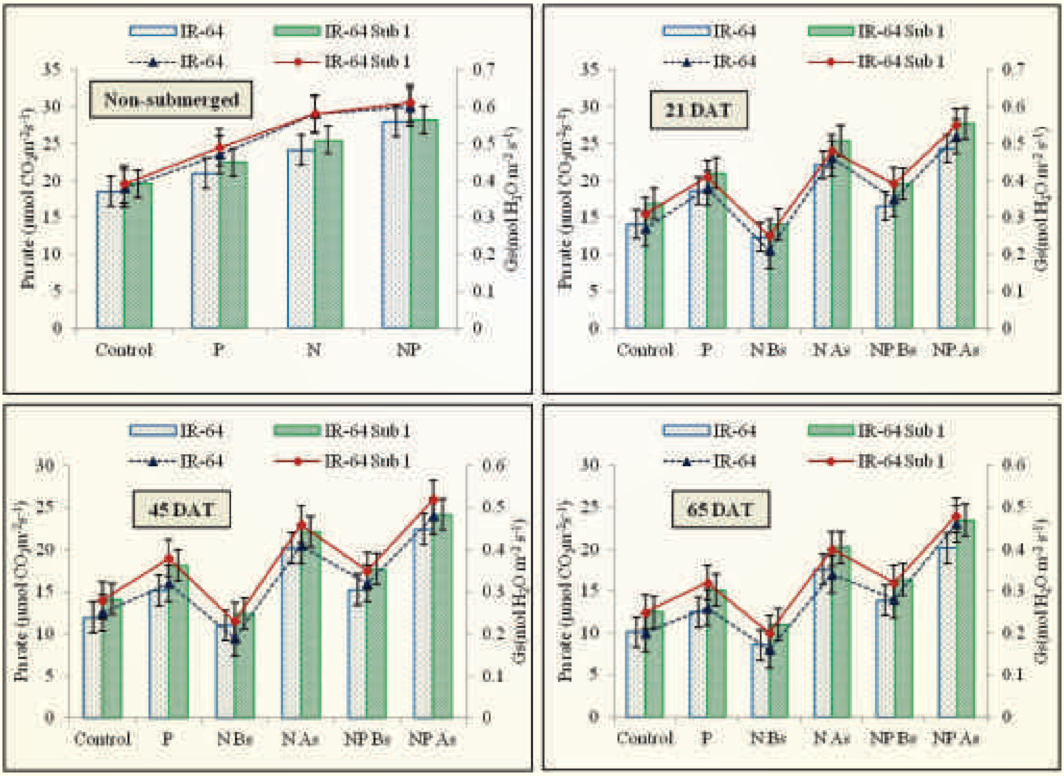


Fig. 4 Photosynthetic (Pn) rate (µmol $CO_2 m^{-2} s^{-1}$) and stomatal conductance (Gs) (mol H_2O m^{-2} s^{-1}) of IR64 and IR64 Sub 1 under non-submerged conditions and submergence at 21, 45 and 65 days after transplanting (vertical bars in each line and column represents standard error). Vertical bars on primary axis represent Pn rate and lines on secondary axis represent stomatal conductance. Control - no N and P application, P - basal P application only, N Bs - pre-submergence N application, N As - post-submergence N application, NP Bs - Basal P and pre-submergence N application and NP As - Basal P and post-submergence N application.

of N after desubmergence along with basal P resulted in 78.7% higher Pn rate as compared to control. Pn rate was lowest when N was applied before submergence and much less when no basal P was applied with N.

Nitrogen application after desubmergence along with basal P contributed to fast recovery of surviving plants leading to early flowering and maturity and this is of particular relevance to actual field condition in some areas (Gautam *et al*, 2014b; Lal *et al*, 2014). Reddy and Mittra (1985b) reported that the N requirement being more during vegetative stage, the N and P fertilization resulted in higher number of tillers and ultimately the number of panicle bearing tillers. This indicates the crop fertilized with these two nutrients could withstand better the onslaught of complete plant submergence than that of unfertilized crop and resulted in the grain yield 9–14% over N alone under deepwater conditions. Submergence at the reproductive stage caused more damage in terms of lowest LAI, Pn rate, biomass, survival and productivity but maximum LS and ethylene accumulation of rice as compared to active tillering and panicle initiation stage.

4.5 Phenology and grain yield

Under non-submerged condition, IR64 and IR64 *Sub1* flowered at the same time. Delay in flowering was observed after submergence in both the cultivars and more in IR64. Flowering was substantially delayed for both the genotypes, by about 30 days in IR64 and 18 days in IR64 *Sub1* (Fig. 5). The apparent delay in maturity was mostly because of the delay in flowering, which was more affected when submergence induced at reproductive stage. Submergence for 14 days delayed flowering and maturity by 29, 20 and 10 days when submergence induced at H, PI (panicle initiation) and AT stage, respectively. This suggest that the reproductive stage is more sensitive to complete submergence because plants are hardy and at later stages of crop growth and contain less NSC and take more time to recover and regenerate.

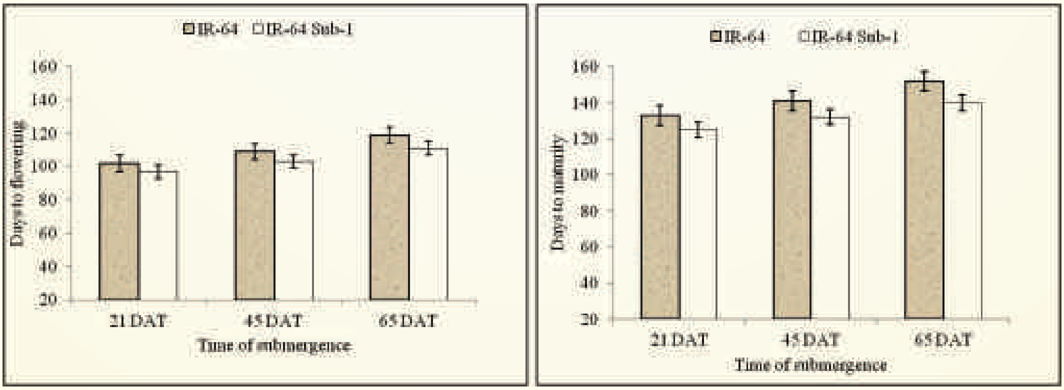

(a)
(b)

Fig. 5 a) Days to flowering, b) days to maturity of rice genotypes influenced by submergence at active tillering (21 DAT), panicle initiation (45 DAT) and heading stage (65 DAT) for 14 days (vertical bars in each column represents standard error.)

Application of N prior to submergence substantially delayed flowering (27 d) and delay was more (33 d) when basal P was not applied with N. Flowering and maturity in both the cultivars were took less time (18 d) when N was applied after desubmergence with basal P compared to control (Fig. 5).

Yield of the *Sub 1* introgression line were similar to that of its recurrent parent under non-submerged condition. The exposure to submergence had left detrimental effects on yield of both the cultivars, grain yield decreased up to 35.5 and 13.8% in IR64 and IR64 *Sub1*, respectively as compared to non-submerged condition (Table 4). The *Sub 1* genotype did not have any apparent negative effect on grain yield under controlled conditions, but considerably a yield advantage of 21.8% over IR64 under submerged condition. Submergence at different stages of crop growth caused a significant reduction in grain yield in both the genotypes. Fourteen days complete submergence at H, PI and AT stage resulted in 58.7, 29.8 and 7.6% yield loss, respectively, as compared to non-submerged conditions. Grain yield was influenced by application of both N and P, positive influence was reflected in case of basal P and post-submergence N application

Table 4 Grain yield (g plant^{-1}) influenced by interaction effect of cultivar, time of submergence and nutrient application

Treatment	**IR64**			**IR64 *Sub1***		
	AT	**PI**	**H**	**AT**	**PI**	**H**
Control	86.5^{cd}	67.6^{c}	54.3^{d}	104.4^{d}	94.5^{c}	74.8^{d}
P	96.7^{c}	78.6^{c}	69.8^{bc}	113.7^{c}	104.2^{c}	91.2^{c}
N_{BS}	79.7^{d}	52.4^{d}	43.3^{d}	103.0^{d}	89.2^{d}	71.3^{d}
N_{AS}	109.8^{b}	93.4^{b}	79.6^{b}	127.7^{b}	119.8^{b}	102.3^{b}
NP_{BS}	94.5^{c}	75.6^{c}	65.4^{c}	110.1^{cd}	101.3^{c}	88.7^{c}
NP_{AS}	121.6^{a}	112.4^{a}	92.3^{a}	143^{a}	131.2^{a}	118.4^{a}
Mean	98.1	80.0	67.4	116.9	106.7	91.1
$LSD_{0.05}$ (VxS)	9.68					
$LSD_{0.05}$ (VxN)	15.81					
$LSD_{0.05}$ (SxN)	19.36					
$LSD_{0.05}$ (VxSxN)	27.38					

In a column, values followed by a common letter are not significantly different at $P<0.05$ using least significance difference test.

BS- before submergence, AS-after submergence, N-nitrogen, P- phosphorus, V-variety, S-stage of submergence, N-nutrient whereas, negative effect was seen when N applied before submergence. When N applied after desubmergence without basal P resulted in 16.7% yield reduction but when it combined with basal P then yield loss before submergence along with basal P resulted in yield reduction of 35.3% but when no basal P was supplied, yield subdued up to 44.7% indicating maximum damage was only 4.2% as compared to non-submerged condition. Reddy and Mittra (1985a) reported that decrease in grain yield on submergence at reproductive stage was due to impaired anthesis causing high sterility as evidenced by higher number of unfilled spikelets. Improper grain filling following submergence might attribute to a reduction in source capacity to provide sufficient carbohydrate, as well as a reduced translocation of assimilates to the sink (Palada and Vergara, 1972). This study suggests that productivity could be enhanced in areas where untimely flooding is anticipated by applying basal P and adjusting the time of N application, if combined with tolerant germplasm, this approach could contribute to enhanced productivity and production of rice in flood-prone lowlands.

5. Effect of water turbidity and nutrient management on submergence tolerance

Rice is adapted to aquatic environments because of its aerenchyma tissues, which facilitate gas diffusion and leaf gas film, which enables internal aeration between submerged tissues and water (Raskin and Kende, 1984; Colmer and Pedersen, 2008). Aerenchyma formation is an important plant trait for improving mainly waterlogging tolerance. However, it interacts strongly with another trait, which becomes relevant when entire plant is covered with water. The response that is frequently observed in rice plants under complete submergence is shoot elongation, adverse effect of which is an increase in carbohydrate consumption for cell division, cell elongation and leaf elongation maintenance (Setter and Laureles, 1996; Ito *et al*, 1999; Voesenek *et al*, 2006). The plant hormone ethylene also accumulates in plants during submergence because its diffusive escape is inhibited while its synthesis is promoted by flooding (Jackson *et al*, 1987; Xu *et al*, 2006). Enhanced ethylene concentration in submerged plants could promote: (a) underwater elongation during submergence as observed in rice (Jackson 2008) and (b) chlorophyll degradation and leaf senescence (Jackson *et al*, 1987; Ella et al, 2003) that may reduce photosynthetic rate during and after submergence. Leaf photosynthesis decreases under complete submergence because of CO_2 depletion and low irradiation, resulting in decreased supply of carbohydrates following degradation of photosynthesizing tissues. Both elongation growth and a reduction in photosynthesis during submergence can result in a depletion of carbohydrate reserves with a consequent increase in plant mortality. Another important factor in submergence is poor light transmission through floodwater, particularly in the presence of water turbidity (Whitton *et al*, 1988). Light reaching the leaves of submerged plants is attenuated by water, dissolved organic matter and silt suspended in the floodwater. When floodwater is turbid, only a scanty amount of solar radiation reaches the canopy level and thus limits the capacity of plants for underwater photosynthesis (Setter *et al*, 1995). Sediment load in flood water and water depth also affect light transmission and the extent of shading of submerged plants (Das *et al*, 2009).

The experiment was conducted during 2012-13 to study the effects of nitrogen and phosphorus application and their application time, on four Indica rice cultivars viz, IR64 *Sub1*, Swarna *Sub1*, Savitri *Sub1* and IR20 and their tolerance to submergence under turbid and clear floodwater. N was applied as basal, before submergence and after submergence, whereas P was applied as basal; clear and turbid water was used for seedling submergence. Approximately 80 pre-soaked seeds of each cultivar were directly sown in plastic tray (37x35x25cm) containing 10 kg of farm soil (Sandy clay loam, pH 6.4, EC–0.089 dSm^{-1}, available N, P and K–58.9, 4.5 and 64.7 mg kg^{-1} of soil, respectively), and later on plants were thinned up to 50 per tray. About 5.92 mg urea, 22.75 mg single super phosphate (SSP) and 4.55 mg potassium chloride (MoP) were applied to each tray as N, P and K source, respectively as per the treatments. The floodwater of the Mahanadi River and the tap water were used as source of turbid and clear water, respectively. The silt particles remained suspended in the water; to prevent the settling of silt in floodwater, the water was stirred manually twice a day during 08:00 h in the morning and 15:30 h in the afternoon for 10 min. Twelve days old seedlings were submerged in a concrete tank filled with clear and turbid water and the water depth was maintained at 30 cm above the top of the plant canopy for 12 d.

5.1 Floodwater conditions

The temperature ranged from 26.1°C to 30.2°C in clear water and from 25.2°C to 29.4°C in turbid water in the morning (06:30 h) and afternoon (14:00 h), respectively during the crop growth period. Water depth did not have significant effect on the temperature, but substantially affect light penetration in both clear and turbid water. Light intensity was substantially decreased in turbid water up to 32.9%, 90.6% and 234.7% at 5, 50 and 75 cm, respectively, in comparison to clear water (Table 2). pH, DO, EC and TDS of the flood water did not vary much with water depth and turbidity, except DO, which was considerably lower in turbid water. Floodwater conditions were assessed in experiment to evaluate the response of cultivars and nutrients to variable conditions of floodwater. This is because plant survival after submergence is dependent on various aspects of floodwater, including depth, duration of submergence, turbulence and extent of water turbidity (Ram *et al*, 2002; Das *et al*, 2009). Oxygen concentration in water is known to be important for waterlogging tolerance of plants and growth of rice roots during submergence (Waters *et al*, 1989). In this study, oxygen concentration in water decreased with increased duration of submergence, the magnitude was more pronounced under turbid water (Fig. 6).

5.2 Silt deposition, shoot elongation and plant survival

Silt deposition on leaf surface was determined to assess the genotypic or treatment differences, which can explain the response of plants when submerged under turbid water. The silt deposition on the leaf surface was greater (P =0.05) in IR20 followed by Swarna *Sub 1*, and the differences were not significant for all the genotypes (Fig. 7). The amount of silt

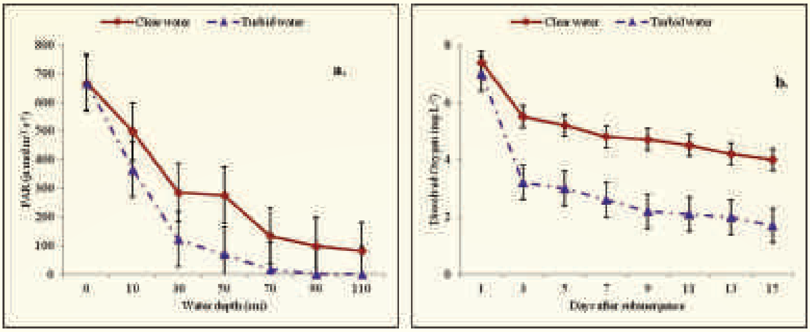


Fig. 6 (a) Photosynthetically active radiation (PAR mmolm^{-2} s^{-1}) and (b) dissolved oxygen (mg L^{-1}) of clear and turbid water during submergence; PAR values are means of measurements made every day during 12 d complete submergence (vertical bars in each column represent s.d.).

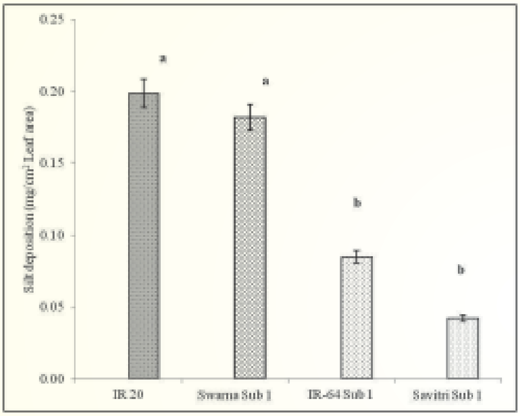


Fig. 7 Deposition of silt on leaves of different rice cultivars submerged with turbid floodwater (vertical bars in each column represents standard error), in the figure, common letter over bars are not significantly different at P<0.05.

deposition on the submerged plant also depends upon the leaf inclination, the higher the leaf angle from main stem, the more will be the silt deposition.

Submergence enhanced the shoot elongation of all the cultivars (Fig. 8), the extent of shoot elongation was much lower in the tolerant genotypes, Savitri *Sub 1* and IR64 *Sub 1* followed by Swarna *Sub 1* and was highest in the sensitive genotype, IR20. In turbid water, shoot elongation was 20.8, 21.7, 25.1 and 29.1% less ($P =0.05$) in IR20, Swarna *Sub 1*, IR64 *Sub 1* and Savitri *Sub1*, as compared to clear water, respectively.

Application of P prior to sowing resulted in suppressed underwater shoot elongation in all the cultivars with greater effects on *Sub1* cultivars. Underwater shoot elongation was enhanced ($P =0.05$) to a greater extent by addition of N before submergence followed by basal application of N and elongation was greater (up to 20.1%) when basal P was not applied. Interactive effect of variety, floodwater and nutrient application on shoot elongation was found significant (Fig. 8). Under water shoot elongation was significantly less ($P =0.05$) when Savitri *Sub1* was submerged in turbid water and supplied with basal P (21.7%), whereas it was maximum when IR20 was subjected to submergence in clear water and supplied with pre-submergence N without basal P (80.1%).

Plant survival of all the cultivars was decreased significantly under submergence, and the reduction was considerably greater in turbid water as compared to clear water (Fig. 9). Savitri *Sub1*, IR64 *Sub 1* and Swarna *Sub 1* showed 179.5, 144.3, 101.7%, higher

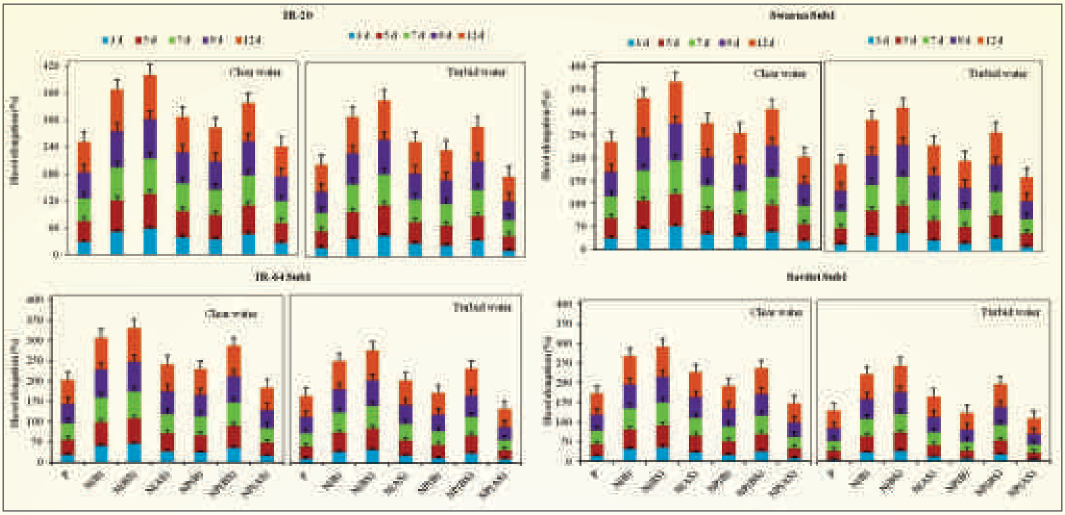


Fig. 8 Effect of N and P application on underwater shoot elongation of IR20, Swarna *Sub1*, IR64 *Sub1* and Savitri *Sub1* after 3, 5, 7, 9 and 12 d of complete submergence in clear and turbid water (vertical bars in each column represents standard error). B- Basal, BS-Before submergence; AS-After submergence, in the figure, common letter over bars are not significantly different at *P<0.05*.

survival (*P =0.05*), respectively, as compared to sensitive genotype, IR20. Plant survival was highest (72.2%) when foliar spray of urea was applied AS along with basal P followed by basal N and P application (68.9%), The plant survival was substantially decreased (45.5%) with pre-submergence N application, especially in turbid water (34%). This decrease might be due to continuous utilization of DO by plants and was more in turbid water because of lack of PAR under water, forcing plants not to photosynthesize. Rapid exposure to anoxia prevents plants from acclimating O_2 deficiencies and has severe effects on growth and metabolism. In turbid water, plant survival was significantly lower as compared to clear water irrespective of the genotypes, although shoot elongation was also lower in turbid water. The greater reduction in survival in turbid water could, therefore, be partially attributed to the greater impairment of light transmission at the canopy level (Das *et al*, 2009). Panda *et al* (2006) reported that submergence under darkness (due to turbidity) decreased the survival percentage in rice due to the degradation of chloroplast, structural and functional integrity and severe inhibition of photosynthesis. The extent of survival is dependent on the genotypic differences of cultivars and not on the silt deposition on the shoot surface. However, the silt deposition was higher on the shoots of sensitive cultivar, because of higher elongation and leaf extension due to submergence. Interactive effect of variety, flood water and nutrient application on plant survival was significant (Fig. 9). Nitrogen application before submergence without basal P in IR20 showed the lowest (*P =0.05*) plant survival percentage in turbid water (2.0%), whereas, Savitri *Sub 1* recorded highest plant survival percentage, with foliar spray of N after submergence along with basal P application in clear water (93.6%).

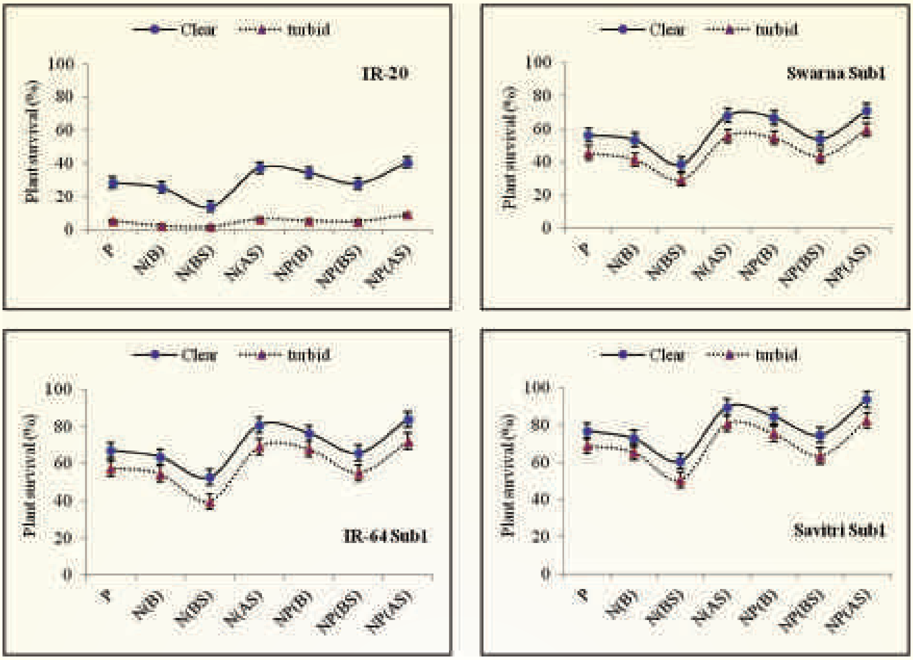


Fig. 9 Effect of N and P application on Plant survival of IR20, Swarna *Sub1*, IR64 *Sub1* and Savitri *Sub1* after 7 d of desubmergence in clear and turbid water (vertical bars in each line represents standard error). B- Basal, BS-Before submergence; AS-After submergence, in the figure, common letter over bars are not significantly different at *P<0.05*.

5.3 Photosynthetic rate, stomatal conductance and ethylene accumulation

The leaf photosynthetic rate decreased in all the cultivars under both clear and turbid water submergence and reduction was more in IR20 than in other cultivars (Fig. 10). Higher stomatal conductance of tolerant cultivars resulted in higher photosynthetic rate than in sensitive ones. Photosynthetic rate was decreased (*P =0.05*) up to 23.1%, 18.1%, 13.7% and 11.9% in IR20, IR64 *Sub 1*, Swarna *Sub 1* and Savitri *Sub1*, respectively when submerged in turbid water. Foliar spray of post-submergence N considerably improved Pn rate and Gs; this improvement was more prominent when basal P was applied with N. Photosynthetic rate was decreased (*P =0.05*) when N was applied as pre-submergence and decrease was greater when it was not combined with basal P (Fig. 10).

Clear flood water resulted in higher underwater photosynthesis than turbid water because of better light conditions and greater chlorophyll retention in clear water. The reduction in chlorophyll a and b concentration upto 12 d of submergence was greater in turbid water and regain in chlorophyll a and b concentration (20 d) was significantly higher in clear water. The reduction due to submergence was greater in chl a content as compared to chl b in all the cultivars. However, chl b reduced drastically in turbid water submergence irrespective of the cultivars. The high amount of water turbidity created lower oxygen

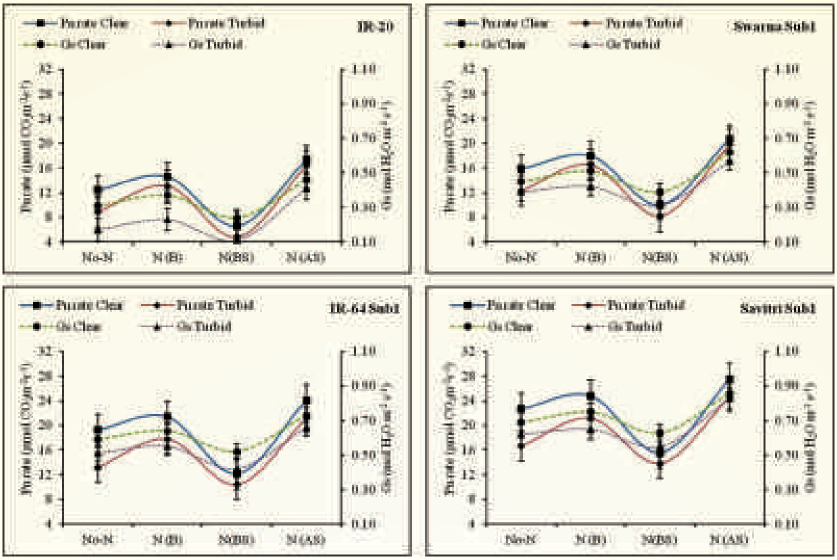


Fig. 10 Effect of N application on photosynthetic rate (Pn rate) and stomatal conductance (Gs) of IR20, IR64 *Sub1*, Swarna *Sub1* and Savitri *Sub1* after seven days of desubmergence in clear and turbid water, in the figure, common letter over bars are not significantly different at *P<0.05.*

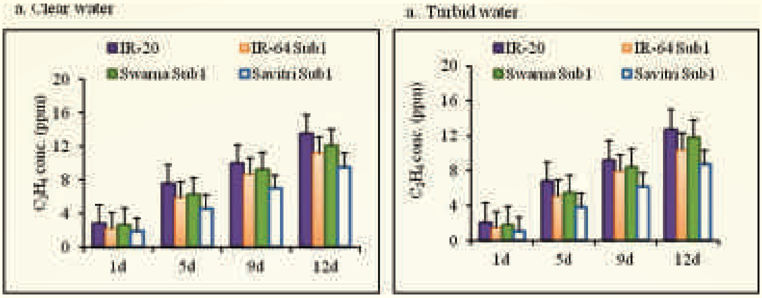
concentrations, probably because light intensity at the canopy level was extremely low (Das *et al*, 2009). Silt deposition might be another reason for decreased photosynthesis in turbid water, as silt particles on the leaf surface clogged the stomata and hampered the stomatal conductance reflecting in lower Pn rate.

Submergence enhanced the ethylene (C_2H_4) accumulation in all the cultivars (Fig. 11), the extent of accumulation followed the order Savitri *Sub1*<IR64 *Sub1*<Swarna *Sub 1*<IR20. Submergence resulted in a continuous increase (*P=0.05*) in ethylene accumulation from the day of submergence to 12 d after submergence in both clear as well as turbid water, irrespective of the cultivars. In turbid water, ethylene accumulation was lower as compared to clear water but not significant irrespective of the genotypes. The ethylene accumulation under submergence was considerably influenced (*P =0.05*) by P application in all the cultivars with a greater effect on flood tolerant genotypes. Basal P application resulted in 15.7%, 19.7%, 18.1% and 24.5% reduction in ethylene accumulation in IR20, IR64 *Sub 1*, Swarna *Sub 1* and Savitri *Sub1*, respectively, over no P application (Fig. 11).

5.4 Effect of submergence on antioxidant enzymes

Submergence caused significant reduction in the activities of antioxidant enzymes in both tolerant and susceptible cultivars, however, after exposure to air, the activity of enzymes increased (Table 5). The activity of enzymes increased on transfer of plants from submerged to aerated conditions, although it remained lower than that of control. The activities of antioxidant enzymes, namely SOD, CAT and PER were more or less similar before submergence but significant differences were noticed among

With P application



Without P application

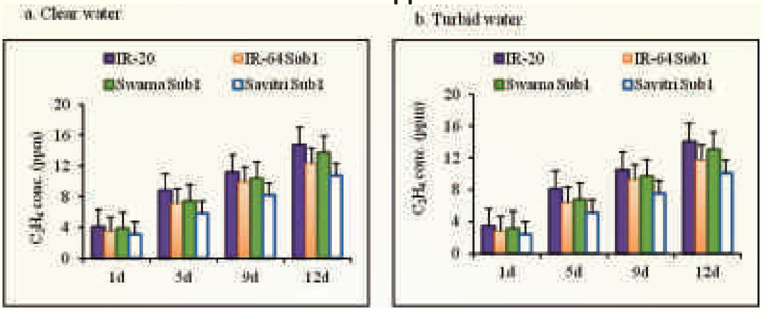


Fig. 11 Effect of P application on Ethylene (C_2H_4) accumulation of IR20, IR64 *Sub1*, Swarna *Sub1* and Savitri *Sub1* after 1, 5, 9 and 12 d of complete submergence in clear and turbid water (vertical bars in each column represents standard error), in the figure, common letter over bars are not significantly different at *P<0.05*.

the varieties during the submergence and after, re-aeration. The antioxidant enzyme activities were greater up to 101% (SOD), 66.1% (CAT) and 132% (PER) in *Sub1* cultivars on re-aeration after submergence as compared to IR20. The floodwater influenced the activities of antioxidant enzymes; activities of SOD, CAT and PER were 46.2, 55.5 and 42.5%, respectively, higher in clear water submergence as compared to turbid water. Activities of SOD, CAT and PER were significantly affected by N and P application. Enzymatic activities before submergence were higher in the treatments receiving basal N and P; however, after submergence and re-aeration, these activities were significantly higher with the application of basal P. Pre-submergence N application without P resulted in significant reduction of SOD, CAT and PER activities after submergence (Table 5). Post-submergence N application with basal P resulted in 97.3, 114.8 and 167.4% increase in activities of SOD, CAT and PER, respectively, after subsequent re-aeration over pre-submergence N application.

Malondialdehyde (MDA) is one of the products of plant lipid peroxidation, and its level reflects the level of lipid peroxidation resulting from flood stress. The MDA content after submergence and subsequent re-aeration found to be increased in all the cultivars, more in susceptible cultivar than tolerant ones. The increase in MDA level was 22.3% higher in IR20 after re-aeration over *Sub1* cultivars.

Table 5 Changes in Superoxide dismutase, Catalase, Peroxidase and Malondialdehyde activities in rice leaves due to submergence and subsequent re-aeration

Treatments	**SOD (Unit g^{-1} FW)**			**CAT (Unit min^{-1} g^{-1} FW)**			**PER (Unit min^{-1} g^{-1} FW)**			**MDA (µmol g^{-1} FW)**		
	C	**S**	**R**	**C**	**S**	**R**	**C**	**S**	**R**	**C**	**S**	**R**
Variety (V)												
IR20	168^{a}	59^{b}	68^{b}	15.8^{a}	3.1^{b}	5.6^{b}	19.3^{a}	3.3^{b}	4.6^{b}	17.5^{a}	16.4^{a}	25.8^{b}
IR64 *Sub1*	164^{a}	97^{a}	139^{a}	13.2^{a}	6.6^{a}	9.1^{a}	17.2^{a}	7.2^{a}	10.5^{a}	19.3^{a}	18.2^{a}	21.6^{a}
Swarna *Sub1*	165^{a}	95^{a}	128^{a}	14.4^{a}	5.9^{a}	8.6^{a}	18.7^{a}	6.3^{a}	10.3^{a}	18.9^{a}	17.6^{a}	21.2^{a}
Savitri *Sub1*	163^{a}	102^{a}	143^{a}	12.4^{a}	7.2^{a}	10.3^{a}	17.4^{a}	8.1^{a}	11.2^{a}	19.1^{a}	18.7^{a}	20.4^{a}
Flood Water (W)												
Clear water	163^{a}	119^{a}	136^{a}	13.1^{a}	6.7^{a}	9.8^{a}	18.6^{a}	6.8^{a}	10.4^{a}	19.2^{a}	17.4^{a}	25.7^{a}
Turbid water	163^{a}	79^{b}	93^{b}	12.8^{a}	4.3^{b}	6.3^{b}	18.6^{a}	5.1^{b}	7.3^{b}	19.5^{a}	18.2^{a}	23.4^{a}
Nutrient application (N)												
P	164^{bc}	118^{a}	132^{b}	12.5^{bc}	5.8^{ab}	7.6^{c}	14.4^{bc}	5.3^{c}	6.2^{c}	17.9^{a}	16.3^{bc}	21.8^{d}
N (Basal)	168^{ab}	84^{d}	105^{e}	13.6^{ab}	5.1^{bc}	9.8^{b}	18.2^{a}	4.9^{c}	8.9^{b}	17.6^{a}	16.0^{bcd}	23.3^{bc}
N(BS)	161^{cd}	61^{e}	73^{f}	13.1^{bc}	2.7^{d}	5.4^{d}	15.7^{b}	2.6^{d}	4.3^{d}	18.1^{a}	17.2^{b}	25.4^{a}
N(AS)	157^{d}	79^{d}	112^{d}	11.2^{c}	5.2^{ab}	8.6^{bc}	13.3^{c}	7.3^{b}	10.2^{ab}	19.3^{a}	18.4^{a}	21.2^{de}
NP (Basal)	171^{a}	101^{b}	124^{c}	15.6^{a}	6.3^{a}	10.1^{ab}	20.6^{a}	6.8^{b}	9.1^{b}	16.2^{a}	15.3^{d}	22.5^{c}
NP (BS)	165^{bc}	92^{c}	109^{de}	14.4^{ab}	4.6^{c}	7.7^{c}	16.1^{b}	5.7^{c}	5.8^{cd}	16.6^{a}	15.8^{cd}	23.9^{b}
NP (AS)	164^{bc}	121^{a}	144^{a}	12.3^{bc}	6.8^{a}	11.6^{a}	14.6^{bc}	8.6^{a}	11.5^{a}	17.8^{a}	16.3^{bc}	20.6^{e}
LSD $_{0.05}$ VxW	ns	4.8	5.4	ns	1.1	1.9	ns	0.5	1.6	ns	ns	Ns
LSD $_{0.05}$ VxN	6.2	6.4	6.7	4.5	1.7	2.2	4.1	0.8	1.7	ns	0.3	0.4
LSD $_{0.05}$ WxN	5.1	5.5	4.9	4.8	1.8	2.4	3.9	0.9	1.4	ns	0.5	0.5
LSD $_{0.05}$ VxWxN	ns	11.2	13.4	ns	2.6	3.1	ns	1.6	2.8	ns	ns	Ns

In a column, mean values followed by a common letter are not significantly different at $P<0.05$. BS-Before submergence; AS-After submergence C-controlled condition, S-after 15 days of submergence, R-subsequent re-aeration of one day after desubmergence.

The level of MDA increased in both clear and turbid water submergence. Prior to submergence, N and P application did not affect the MDA content significantly but after submergence the level of MDA increase significantly with the application of pre-submergence N without P. The MDA level was increased up to 16.5% with pre-submergence N application, whereas it was 5.8% lower with post-submergence N application over no nitrogen application (Table 5). When floodwater recedes, submerged rice plants should change anaerobic metabolism to acclimatize to aerobic conditions. Lethal damages developing after desubmergence is caused by failure of adaptation to aerobic condition (Palada and Vergara, 1972). Therefore, plants have their own systems for scavenging active oxygen species through some enzymes and abundant antioxidants. High level of some antioxidant enzymes such as SOD, CAT and PER are important to survive oxidative stress after the plants are subjected to different levels of water logging (Panda and Sarkar, 2013). Antioxidant enzymes, namely, SOD, CAT and PER were decreased during submergence in all the cultivars, and reduction was greater in IR20. Possible cause of reduction of these enzymes is the decrease of production or the activity of ROS (Panda and Sarkar, 2012) and due to higher absorption of O_2 from surrounding water by tolerant cultivar (Sarkar *et al,* 2001). Sarkar and Bera (1997) reported that tolerant cultivars had higher root activity and hence could transport more O_2 through the shoot to the roots under complete submergence. After floodwater recedes, plants were re-aerated only *Sub1* cultivars showed a significant increase in the activities of antioxidant enzymes. The increased activities of these enzymes in tolerant cultivars emphasize better physiological adaptation under hypoxic condition, so as to maintain the structural integrity of the enzymes better and when needed upon exposure to air, detoxify the toxic oxygen species and protect the system effectively (Das *et al,* 2004). Besides O_2, another environmental constraint that plant has to encounter during submergence is the low irradiance, which may be resulted from water turbidity. Basal N and P application resulted in higher enzymatic activities before submergence, whereas after submergence and re-aeration, these activities were significantly higher with the application of basal P only. Pre-submergence N application led to significant reduction of SOD, CAT and PER activities after submergence. Rios-Gonzalez *et al* (2002) reported that CAT activity was increased with ammonium nutrition, but SOD and PER remained unaffected. Basal P application suppressed the production of MDA, whereas pre-submergence N application enhanced the MDA content, leading to leaf senescence and chlorophyll breakdown. Crafts-Brandner (1992) also reported that N deficiency can induce leaf senescence and production of ROS, which leads to degradation of some leaf macromolecules which can oxidize some pigments and lipid peroxidation may be happen. Lipid per oxidation is linked to the activity of antioxidant enzymes, for example with the increase of SOD, CAT and PER, oxidative stress tolerance is enhanced and MDA is decreased.

Foliar application of post-flood N was better than broadcast application of post-flood N. Basal P and post-flood N application either

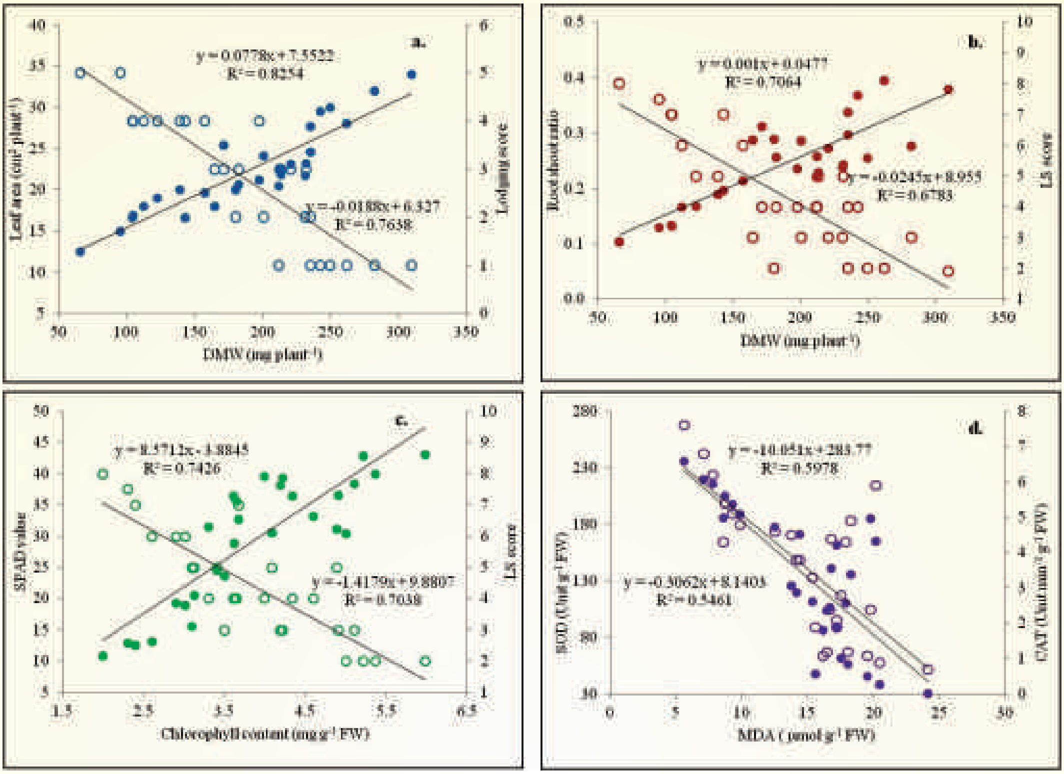


Fig. 12 Relation between **(a)** dry matter weight (mg plant^{-1}), leaf area (cm^{2} plant^{-1}) and lodging score; **(b)** dry matter weight (mg plant^{-1}), root shoot ratio and leaf senescence score; **(c)** chlorophyll concentration (mg g^{-1} FW), SPAD value and leaf senescence score; **(d)** activities of superoxide dismutase (g^{-1} FW), Catalase (min^{-1} g^{-1} FW) and malondialdehyde (µmol g^{-1} FW) as affected by nutrient application. Correlations on primary axis are represented by closed symbols and correlations on secondary axis are represented by open symbols. FW : fresh weight

though broadcasting or foliar spray resulted in significantly higher tiller regeneration, DMW, LA, SLW, and ultimately the yield. Beneficial effect of N fertilizer on greater tiller survival was due to better initial crop vigor and carbohydrate accumulation, leading to increased tolerance of plants to submergence.

6. Submergence tolerance in relation to method of post-flood nitrogen application

When rice plants subjected to flash floods, they should adapt themselves to two drastic environmental changes: the changes from aerobic to hypoxic condition during complete submergence and the subsequent changes from hypoxic to aerobic condition when the floodwater recedes. The visual damage caused by the submergence is generally not apparent immediately but develops soon after the water level recedes after complete submergence. Several new varieties have been developed by the introgression of the *Sub1A* gene and these varieties can ensure rice production in flood-prone areas because of their tolerance to complete submergence (Mackill *et al,* 2012). The availability of tolerant varieties provides more opportunities for developing and validating proper management options effective in flood-prone areas, which could further boost and stabilize the productivity of these varieties (Ella and Ismail, 2006). However, nutrient recommendations have not been specially developed for flood-prone areas and farmers often avoid using inputs as a risk aversion strategy. Therefore, improving plant health through nutrient management may lead to better crop establishment. It is unclear if some nutrient elements can actually increase submergence tolerance if applied at rates above balanced applications. Post-submergence nutrient management particularly N can also contribute substantially towards increasing productivity in flood-prone areas. There exists vast possibility for increasing rice production and harnessing the productivity potentials of submergence-affected areas with the use of submergence-tolerant varieties, particularly when combined with the best nutrient management practices specific for these areas. Basal fertilization of N (Reddy et *al*,1985) and NP (Gautam *et al*, 2014b; Lal *et al*, 2014) improves initial vigor of rice plants for better tolerance to submergence at later growth stages. Post-submergence nutrient application can also contribute substantially towards increasing productivity in flood-prone areas (Gautam *et al*, 2014a; Lal *et al*, 2014). In this context, the study is conducted with submergence tolerant rice varieties for harnessing the productivity potentials and minimizing flood damages in rice production through alteration in method of post-flood N application with basal P.

The experiment was conducted during 2012–13 with three Indica rice cultivars IR64 *Sub1* and Swarna *Sub1* and IR20; six schedules of N and P application including control (details of N and P application schedule given in Table 6); clear and turbid water was used for submergence. One 15-day-old seedling of each cultivar was transplanted in the pots containing 10 kg of farm soils (Sandy clay loam, pH 6.4, EC-0.079 dSm^{-1}, available N, P and K-58.9, 4.5 and 64.7 mg kg^{-1} of soil, respectively). A total of 80 mg urea, 114 mg SSP and 30 mg MOP was applied to each pot as per the treatments as N, P and K sources, respectively. Nitrogen was applied in three equal splits at basal, maximum tillering and panicle initiation stage, second split of N at maximum tillering was applied after de-submergence as urea foliar spray and broadcasting as per the treatments (Table 6). Leaves of rice seedlings were sprayed on their ad axial surface with 2.0% (w/v) urea solution through a backpack sprayer in a water carrier until they were completely wetted. The plants were submerged at maximum tillering stage in a concrete tank filled with clear and turbid water and the water depth was maintained at 30 cm above the top of the plant canopy for 15 d. The experiment was arranged in a factorial randomized block design with five replications. After de-submergence, the plants were allowed to recover for seven days, and plant survival was recorded. Plant samples were collected 48 h before submergence and then seven days after de-submergence for various measurements.

Table 6 Nutrient application schedules used in experiment

Nutrient combination	Treatment symbols	Method of nutrient application				
		1st split of N	2nd split of N	3rd split of N	Phosphorus	Potassium
Control	-	-	-	-	-	-
P	P	-	-	-	Basal	Basal
N	N (S)	Urea broadcasting	Urea foliar spray	Urea broadcasting	-	Basal
	N(B)	Urea broadcasting	Urea broadcasting	Urea broadcasting	-	Basal
NP	NP (S)	Urea broadcasting	Urea foliar spray	Urea broadcasting	Basal	Basal
	NP(B)	Urea broadcasting	Urea broadcasting	Urea broadcasting	Basal	Basal

6.1 Shoot elongation and leaf senescence

Submergence enhanced (*P = 0.05*) the shoot elongation of all the cultivars (Fig. 13), the extent of shoot elongation was much lower in the tolerant genotypes, Swarna *Sub1* and IR64 *Sub1* and it was highest in the sensitive genotype, IR20. In turbid water, shoot elongation was 20.8, 41.8, and 28.8% less in IR20, IR64 *Sub1* and Swarna *Sub1* compared with clear water, respectively.

Basal application of P resulted in suppressed (*P = 0.05*) underwater shoot elongation in all the cultivars with greater effects on *Sub1* cultivars. Underwater shoot elongation was enhanced (*P = 0.05*) to a greater extent in the control treatments. Shoot elongation was 80.7 and 76.3% less with the application of basal P over control in turbid and clear water, respectively. Interactive effect of variety, flood water and nutrient application on shoot elongation was found significant (*P = 0.05*) (Fig. 13). Underwater shoot elongation was significantly less when Swarna *Sub1* was submerged in turbid water and supplied with basal P (16.8%), whereas it was maximum when IR20 was subjected to submergence in clear water and plants grown without nutrients (89.4%).

Leaf senescence occurred when plants were exposed to submerged conditions for 15 days complete submergence, especially under turbid flood water (Fig. 13). Leaf senescence score increased (*P = 0.05*) after submergence and effect was higher in IR20. More green leaves or less senescence in *Sub1* genotypes was also confirmed by the SPAD meter (chlorophyll meter) values, which was higher in *Sub1* cultivars (Table 2). Clear water submergence resulted in 25.9, 33.3 and 40.1% higher number of green leaves and less (*P = 0.05*) senescence in IR20, IR64 *Sub1* and Swarna *Sub1*, respectively, compared with turbid water. Leaf senescence was accelerated (*P = 0.05*) when nutrients was not applied, whereas basal P application suppressed (*P = 0.05*) the leaf senescence and it was also evident from higher SPAD values.

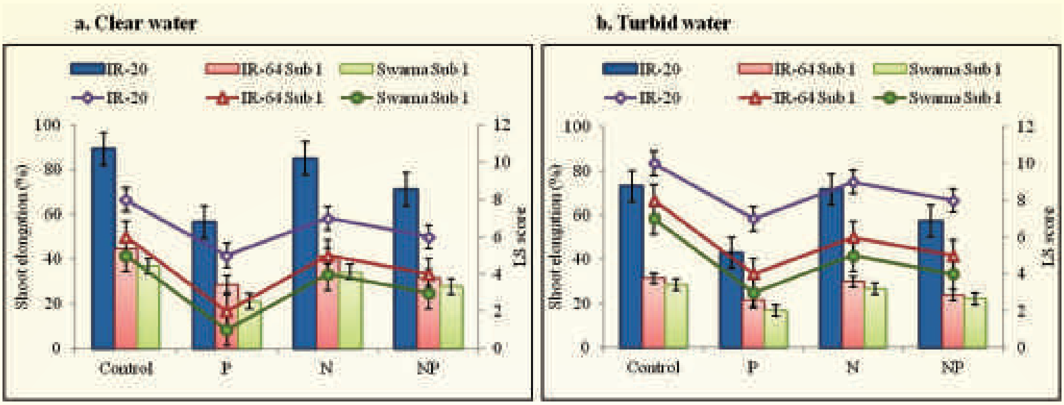


Fig. 13 Effect of N and P application on underwater shoot elongation (%) and leaf senescence score of IR20, IR64 *Sub1* and Swarna *Sub1* after 15 d of complete submergence in clear and turbid water (vertical bars in each column and line represents standard error). N- Nitrogen, P- Phosphorus, LS- leaf senescence. Column bars on primary axis represents shoot elongation and lines on secondary axis represents leaf senescence score.

6.2 Plant survival and ethylene accumulation

Tolerant cultivars with *Sub1* gene showed greater re-growth measured in terms of emergence of new leaves and higher survival percentage (Fig. 14). Thus, a plant's ability to survive for long periods under submergence is related to the storage organs able to survive and regenerate new shoots and roots after the flood water recedes (Crawford, 2003). Plant survival of all the cultivars was decreased significantly under submergence, and the reduction was considerably greater in turbid water compared with clear water (Fig. 14). IR20, IR64 *Sub1* and Swarna *Sub1* showed 25.7, 77.1 and 77.5% higher plant survival in clear water compared with turbid water respectively. Plant survival was highest when foliar spray of urea was applied after de-submergence along with basal P followed by basal N and P application, irrespective of the cultivars. Basal application of P ensured less shoot elongation, leaf senescence, ethylene accumulation and plant mortality, which reflected in higher plant survival because the added P contributes to the submergence tolerance of plants. According to Jackson and Ram (2003), addition of P to soil before seed sowing can stimulate overall growth in height, dry mass and carbohydrate content. However, Rama Krishnayya *et al,* (1999) reported that application of P to the soil at planting, rather than to flood water, could be beneficial in higher plant survival and productivity. One of the reasons for the increased survival due to basal P application may be lower ethylene accumulation in shoots. Complete submergence enhances the accumulation of ethylene due to increased synthesis which triggers chlorophyll degradation and leaf senescence of the submerged plants through suppression of abscisic acid synthesis but enhanced synthesis and sensitivity to gibberellins (Fukao and Bailey-Serres, 2008) resulting in lower survival.

Table 7 SPAD reading of rice leaves after 15 d of complete submergence at maximum tillering stage

	IR20		**IR64 *Sub 1***		**Swarna *Sub1***	
	Clear	Turbid	Clear	Turbid	Clear	Turbid
Control	10.9^{de}	8.6^{d}	18.7^{e}	13.9^{d}	22.4^{d}	18.1^{d}
P	15.6^{c}	12.8^{c}	26.8^{c}	21.2^{c}	33.2^{c}	28.1^{c}
N(S)	12.6^{d}	10.2^{d}	24.5^{cd}	20.1^{c}	31.2^{c}	27.2^{c}
N(B)	13.2^{cd}	10.9^{d}	23.7^{d}	19.8^{c}	30.8^{c}	26.6^{c}
NP (S)	23.6^{a}	20.5^{a}	39.6^{a}	36.5^{a}	43.1^{a}	40.3^{a}
NP (B)	21.5^{b}	16.3^{b}	35.6^{b}	31.7^{b}	39.4^{b}	35.8^{b}

In a column, mean values followed by a common letter are not significantly different at *P<0.05*.

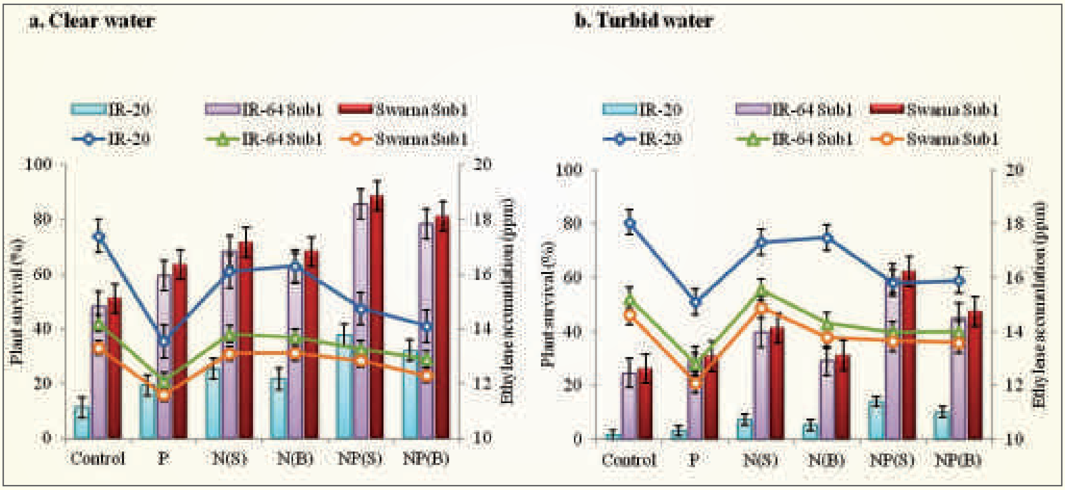


Fig. 14 Effect of N and P application on plant survival (%) after seven days of desubmergence and ethylene accumulation (ppm) of IR20, IR64 *Sub1* and Swarna *Sub1* after 15 d of complete submergence in clear and turbid water (vertical bars in each column and line represents standard error). N- Nitrogen, P- Phosphorus, S- Urea spray, B- Urea broadcasting. Column bars on primary axis represents plant survival and lines on secondary axis represents ethylene accumulation.

Effect of foliar spray was more evident in turbid water, plant survival was 10.4 and 32.6% higher over urea broadcasting in clear and turbid water respectively might be due to the fact that urea spray removed the adhering silt from the leaf surface and enable the plants to photosynthesize and survive. The interactive effect of variety, flood water and nutrient application on plant survival was significant (Fig. 14). IR20 showed lowest plant survival in turbid water when no nutrient was applied (1.2%), whereas Swarna *Sub1* recorded highest plant survival with foliar spray of N and basal P application in clear water (88.5%).

Submergence enhanced the ethylene (C_2H_4) accumulation in all the cultivars ($P = 0.05$), the extent of accumulation followed the order Swarna *Sub1* < IR64 *Sub1* < IR20 (Fig. 16). In turbid water, ethylene accumulation was higher ($P = 0.05$) compared with clear water but not significant irrespective of the genotypes. The ethylene accumulation under submergence was considerably influenced ($P = 0.05$) by P application in all the cultivars with a greater effect on flood tolerant genotypes. Basal P application resulted in 15.1, 18.9 and 25.2% reduction in ethylene accumulation over control in IR20, IR64 *Sub1* and Swarna *Sub1*, respectively (Fig. 14).

6.3 Chlorophyll content

Submergence resulted in significant reduction of Chl a and b content both in the susceptible and tolerant cultivars (Fig. 15). The reduction in Chl a and b concentration was greater ($P = 0.05$) in turbid water and regain in Chl a and b concentration after desubmergence (20 d) was significantly higher in clear water, reflecting in lower % change in Chl a and b. The reduction due to submergence was greater ($P = 0.05$) in Chl a content compared with Chl b in all the cultivars. But Chl b reduced drastically in turbid water submergence irrespective of the cultivars. Basal P application reduced ($P = 0.05$) the chlorophyll damage under submergence over no P application in all the cultivars. Foliar spray of urea contributed most to the regain in Chl a and b after de-submergence (20 d), up to the extent of 5.2, 5.9 and 6.4% higher ($P = 0.05$) in IR20, IR64 *Sub1* and Swarna *Sub1*, respectively, compared with urea broadcasting. The interactive effect of variety, flood water and nutrient application on Chl a and b content was significant (Fig. 15). IR20 showed highest ($P = 0.05$) decrement and percentage change in chlorophyll content in turbid water under control treatment (Chl a 84.3 and Chl b 243.6%), whereas, Swarna *Sub1* recorded highest ($P = 0.05$) recuperation of chlorophyll, and lowest % change with foliar spray of urea along with basal P in clear water (Chl a 18.5 and Chl b 15.1%).

6.4 Non-structural carbohydrate levels

A significant depletion of NSC was observed after submergence in the shoots of all the cultivars, resulted in change in percentage of NSC, which was considerably higher ($P = 0.05$) in IR20. Plants submerged under turbid water resulted in 41.3, 36.9 and 34.4% lower ($P = 0.05$) NSC concentrations in IR20, IR64 *Sub1* and Swarna *Sub1*, respectively, than clear water (Fig. 16). The depletion of NSC in all the cultivars was enhanced and the % change was higher when no N and P were applied. The concentration of NSC after de-submergence was significantly higher ($P = 0.05$) in the treatments with basal P over no P application, irrespective of the cultivars mainly in tolerant cultivars. Foliar spray of urea in combination with basal P resulted in a higher concentration ($P = 0.05$) of NSC after submergence (5.3, 7.9 and 9.5% higher in IR20, IR64 Sub1 and Swarna Sub1 respectively) than N applied as urea broadcast (Fig. 16).

A strong positive correlation was also observed among survival, chlorophyll and NSC concentration (Fig. 17). These correlations suggest that maintenance of chlorophyll as well as maintenance of NSC in plants after submergence was more important for survival under submerged

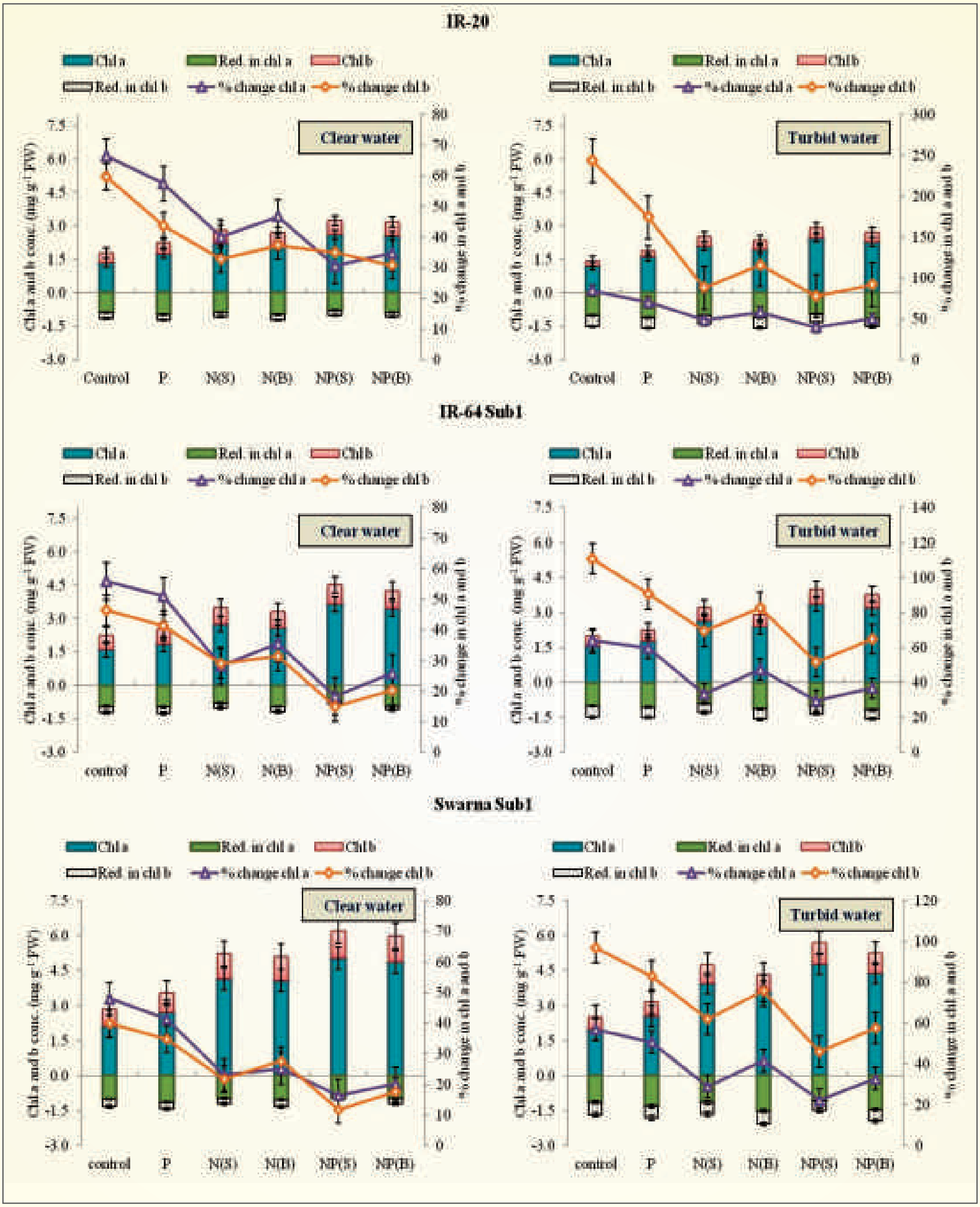


Fig. 15 Effect of N and P application on concentration of chlorophyll a and b (mg g^{-1} FW) after 7 d of desubmergence, reduction and % change in chlorophyll a and b after desubmergence over pre-submergence of IR20, IR64 *Sub1* and Swarna *Sub1* in clear and turbid water submergence (vertical bars in each column and line represents standard error). N-Nitrogen, P-Phosphorus, S-Urea spray, B-Urea broadcasting, chl-Chlorophyll. Column bars on primary axis represents chlorophyll a and b concentration and lines on secondary axis represents percentage change in chlorophyll.

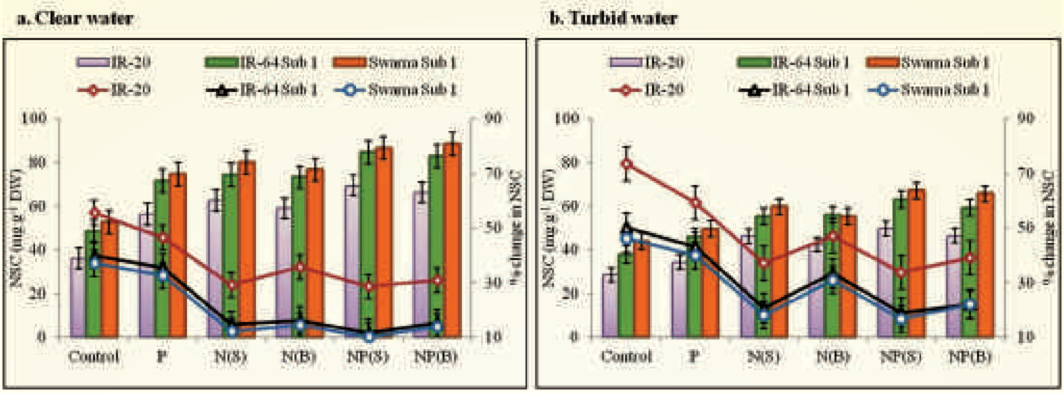


Fig. 16 Effect of N and P application on non-structural carbohydrate concentration (mg g^{-1} DW) after seven days of desubmergence and percentage change in non-structural carbohydrate concentration (sugar + starch) after desubmergence over pre-submergence of IR20, IR64 *Sub1* and Swarna *Sub1* in clear and turbid water submergence (vertical bars in each column and line represents standard error). N-Nitrogen, P-Phosphorus, S-Urea spray, B-Urea broadcasting, NSC-non-structural carbohydrate concentration. Column bars on primary axis represents non-structural carbohydrate concentration and lines on secondary axis represents percentage change in NSC.

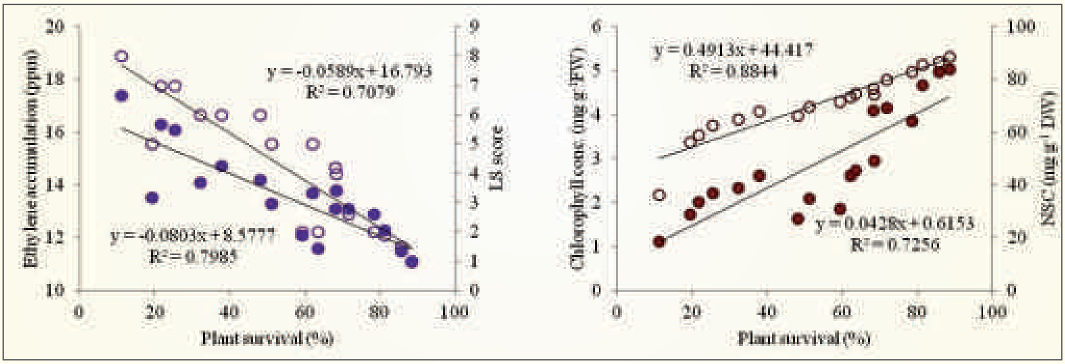


Fig. 17 Relation between (a) plant survival (%), ethylene accumulation (ppm) and leaf senescence score; (b) plant survival (%), chlorophyll content (mg g^{-1} FW) and non-structural carbohydrates (mg g^{-1} DW), as affected by nutrient application and flood water. Correlations on primary axis are represented by closed symbols and correlations on secondary axis are represented by open symbols.

conditions. Foliar spray of post-submergence N with basal P seems to be beneficial, as application of P contributes to submergence tolerance, reduces the ethylene accumulation which ultimately helps in conservation and maintenance of carbohydrates while N spray helps in regeneration and survival, ultimately increasing submergence tolerance.

6.5 Tiller mortality and regeneration

Submergence caused drastic (*P=0.05*) reduction in number of tillers of all the cultivars, and reduction was greater under turbid water submergence. Under non-submerged conditions, number of tillers in Swarna *Sub1* was higher as compared to other cultivars, due to its genotypic characteristics (Fig. 18). Turbid water submergence resulted in significant (*P=0.05*) tiller mortality, and tillers remained lower throughout the growth period as compared to clear water submergence. On zero day of desubmergence, reduction in tillers was comparatively low but after subsequent re-aeration, number of tillers decreased remarkably (Fig. 18). The decrease in number of tillers was in the order IR20<IR64 *Sub1*<Swarna *Sub1* both under clear and turbid water. After third day of desubmergence, regeneration of tillers started, with more prominent effect in *Sub1* cultivars.

Nitrogen application after desubmergence favored the regeneration and growth of tillers after third day of desubmergence and beyond. With the application of P, plants

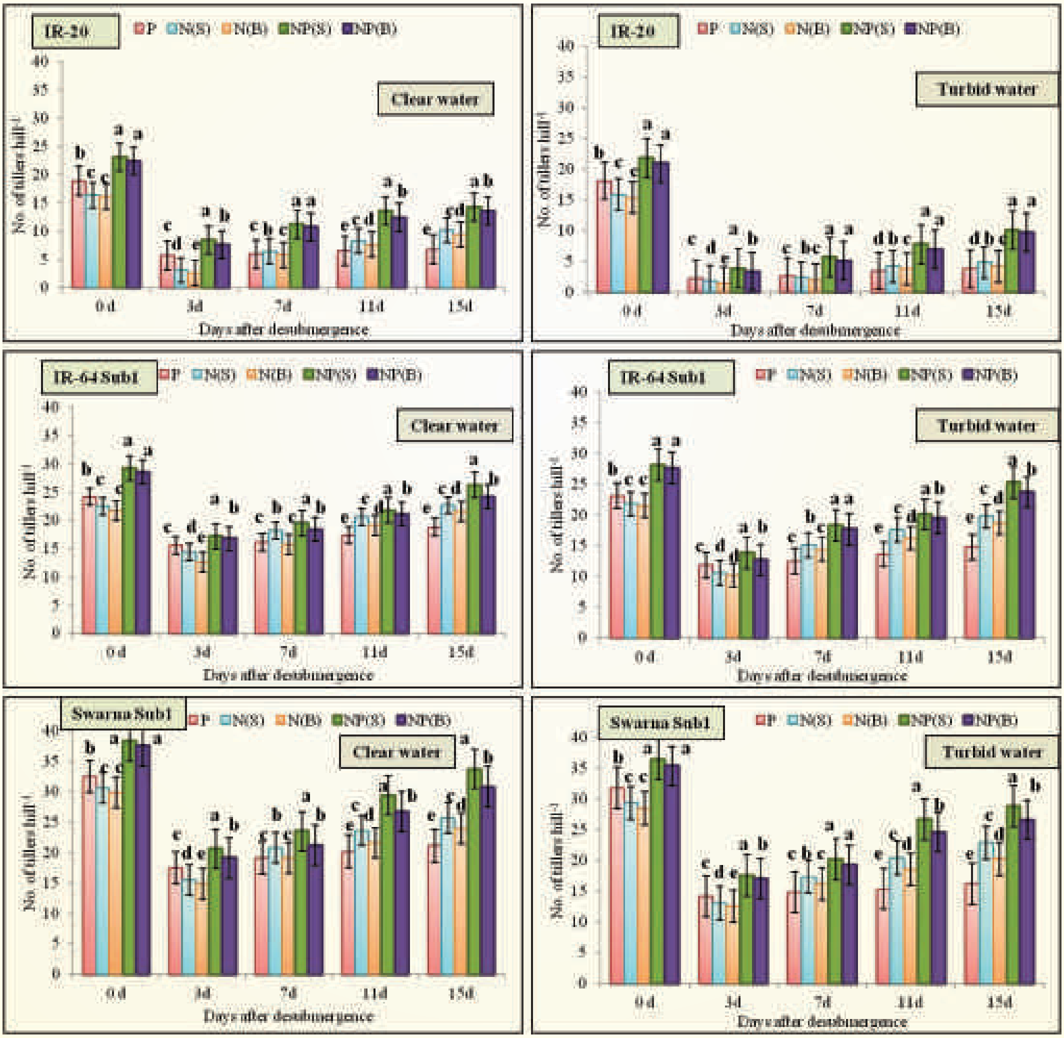


Fig. 18 Number of tillers per hill after 0, 3, 7, 11 and 15 d after desubmergence in IR20, IR64 *Sub1* and Swarna *Sub1* under clear and turbid water submergence (vertical bars in each column represents standard error). P-phosphorus, N-nitrogen, B- broadcasting, S-spray

resist the damage of tillers, whereas N application contributed significantly (*P = 0.05*) for recovery of tillers and regeneration. After 15 d of desubmergence, highest (*P = 0.05*) number of tillers were observed in the treatment receiving basal P and foliar spray of post-submergence N (Fig. 18). With the application of basal P alone 110.3, 39.9 and 59.7 % less tillers were observed in IR20, IR64 *Sub1* and Swarna *Sub1*, respectively, over basal P and foliar spray of urea.

6.6 Leaf nitrogen concentration

The leaf N concentration was recorded prior to submergence (maximum tillering stage) as well as immediately (0^{th} d), 1 week (7^{th} d) and 2 week (15^{th} d) after desubmergence (Fig. 19). Leaf N concentration decreased significantly (*P = 0.05*) after submergence irrespective of the cultivars; on the progression of survival, N content of leaves gradually increased. Nitrogen concentration of tolerant cultivars was significantly (*P = 0.05*) higher as compared to IR20 at every sampling starting from 0^{th} d. In terms of percentage change in N content (worked out as N content before submergence over N content after 15 d of desubmergence); *Sub1* cultivars proved their superiority over IR20, as percentage change in leaf N was recorded up to 86.6, 43.1 and 35.8% in IR20, IR64 *Sub1* and Swarna *Sub1*, respectively. Turbid water submergence led to greater (*P = 0.05*) loss of leaf N as compared to clear water. Leaf N content was 86.3, 32.6 and 20.8% lower in IR20, IR64 *Sub1* and Swarna *Sub1*, respectively, under turbid water over clear water submergence. Nitrogen content before submergence was recorded significantly (*P = 0.05*) higher in the treatments receiving nitrogen over control and basal P alone. On progression of submergence, N content decreased in all the treatments, but post-flood N application resulted in increased N content of leaves in all the cultivars. However, foliar spray of urea with basal P recorded significantly (*P = 0.05*) highest leaf N content during all the sampling days (Fig. 19). After desubmergence, leaf N content was three times lower in IR20 and almost two times in IR64 *Sub1* and Swarna *Sub1*, in non-fertilized treatments over foliar spray of urea and basal P. After 15 d of desubmergence, urea broadcasting resulted in 7.7, 1.3 and 1.1% lower leaf N content in IR20, IR64 *Sub1* and Swarna *Sub1*, respectively, in comparison to urea foliar spray.

6.7 Grain yield and effective tillers

The exposure to submergence had left detrimental effects (*P = 0.05*) on number of effective tillers and yield of all the cultivars, grain yield was decreased up to 55.8 and 161.7% in IR20, 14.2 and 26.7% in IR64 *Sub1*, 9.7 and 21.2% in Swarna *Sub1* under clear and turbid water submergence, respectively, as compared to non-submerged condition (Fig. 8). Number of effective tillers decreased (*P = 0.05*) up to 2.5 times in IR20, and around 0.8 times in IR64 *Sub1* and Swarna *Sub1* due to submergence as compared to non-submerged conditions. Grain yield and effective tillers was positively influenced (*P = 0.05*) by post-flood N application and influence was more when basal P was applied. When N was applied alone, grain yield was 25.8, 17.8 and 17.1% lower as compared to basal P application with post-flood N. The crop fertilized with urea foliar spray and P yielded 87.6, 53.1 and 47.6% higher grains in IR20, IR64 *Sub1* and Swarna

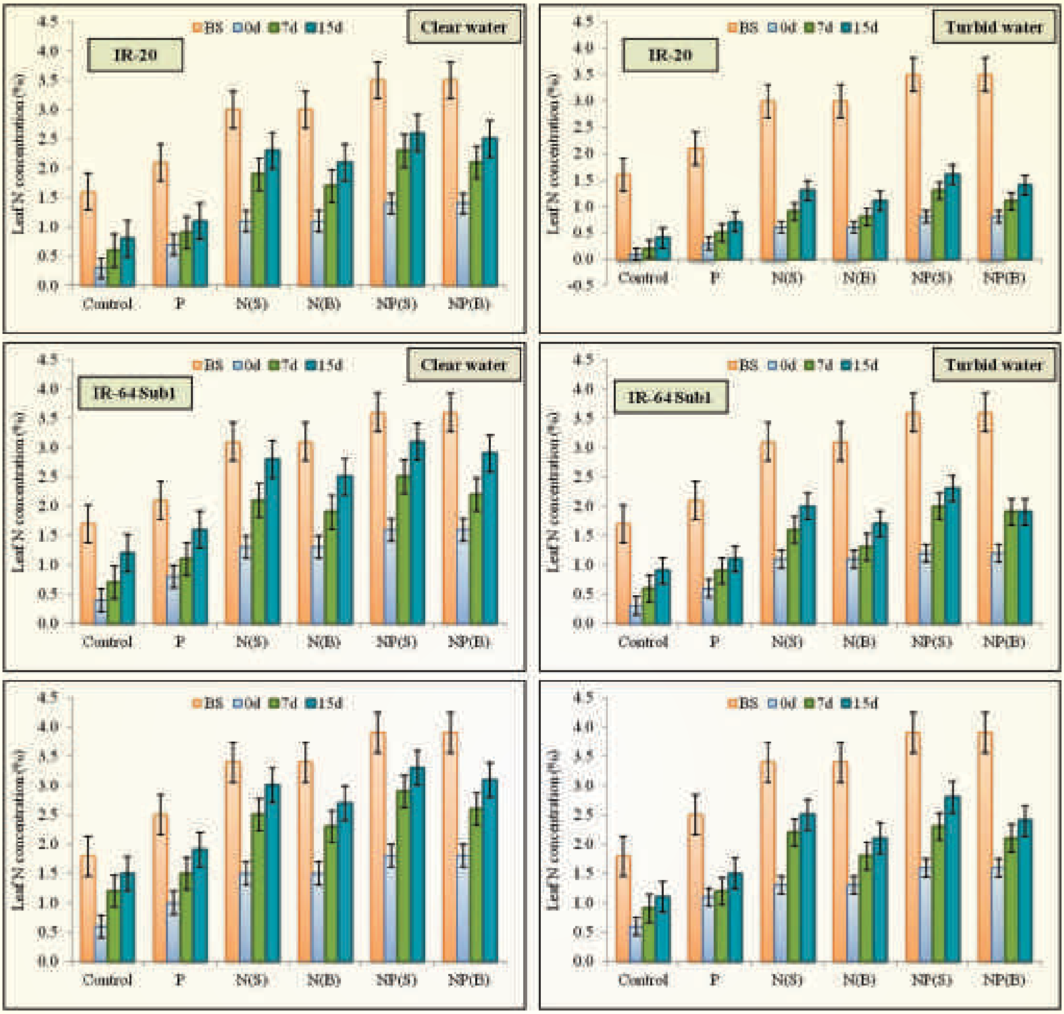


Fig. 19 Nitrogen concentration (%) in rice leaves prior to submergence and 0, 7, 15 d after desubmergence in IR20, IR64 *Sub1* and Swarna *Sub1* under clear and turbid water submergence (vertical bars in each column represents standard error). P-phosphorus, N-nitrogen, B-broadcasting, S-pray

Sub1, respectively, than the unfertilized crop (Fig. 20). Further, urea foliar spray had additive beneficial effect on grain yield and proved superior to urea broadcasting.

Alteration in time and method of N application can enhance the yield of submerged rice, if combined with *Sub1* varieties, enhancement in yield will be greater. The turbidity of the floodwater affected plant response in terms of photosynthesis during submergence because of low light intensity at crop canopy level and less concentrations of dissolved oxygen. Application of basal P helped in improving the phenology, photosynthetic rate and effective tillers. Foliar spray of post-flood N application with basal P proved its superiority in terms of better photosynthesis

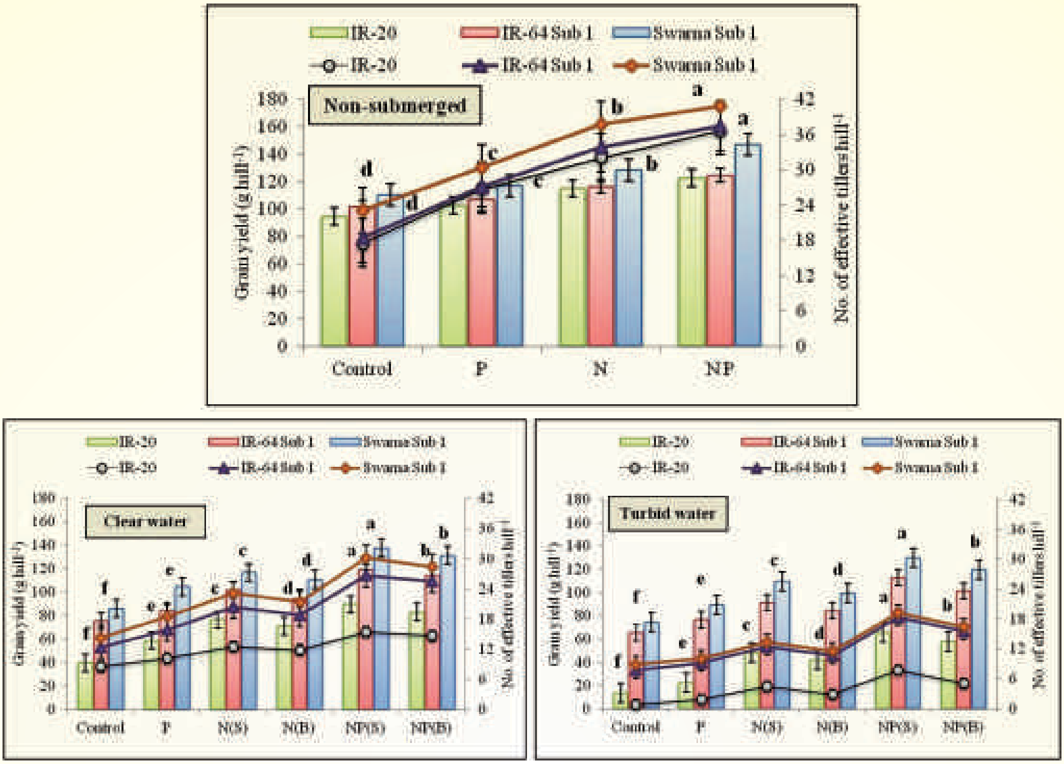


Fig. 20 Grain yield (bars, on primary axis) and number of effective tillers (lines, on secondary axis) of IR20, IR64 *Sub1* and Swarna *Sub1* at harvest under non-submerged, clear and turbid water submergence (vertical bars in each column and line represents standard error).

and higher N concentration led to higher grain yield. Our results open up new opportunity for enhancing the productivity of rice cultivars by manipulating the N application time and method in flood-prone areas.

7. Role of potassium application in submergence tolerance of rice

Waterlogging/flooding treatment induces a "physiological drought", reducing stomatal conductivity by several fold (Pang *et al*, 2004; Polacik and Maricle, 2013) and the low energy status under hypoxic conditions results in a substantial depolarization of plasma membrane potential (Shabala, 2011) thus affecting nutrients delivery to the shoot. On a shorter time scale, root growth is arrested immediately upon onset of hypoxia, while shoot growth still persists (Malik *et al*, 2002). This may result in a significant reallocation of root K^+ towards the expending shoot and, in the light of reduced root capacity to take up K^+, may lead to severe K^+ deficiency in the root. Out of all the mineral nutrients, potassium (K) plays a critical role in plant growth, metabolism and activation of several enzymes and it contributes greatly to the survival of plants that are under various biotic and abiotic stresses. The regulation of K^+ homeostasis is essential for plant adaptation to biotic and abiotic stresses. This adaptation is associated with the wide range of functions in which K^+ participates (Demidchik, 2014; Shabala and Pottosin, 2014). Exogenous application of K could effectively ameliorate the adverse effects of waterlogging on plants by not only higher plant growth, photosynthetic pigments and photosynthetic capacity, but also by improving plant nutrient uptake as a result of higher K^+, Ca^{2+}, N, Mn^{2+} and Fe^{2+} accumulation (Ashraf *et al*, 2011). Potassium deprivation leads to large reductions of the growth and yield of plants, and the addition of K is required for the optimal yields. Therefore, studying plant responses to K deficiency together with N and P deficiency is of significant agricultural importance as these are the nutrients that are added to soils in the largest quantities to ensure high yields. Although certain aspects of how plants respond to K deprivation and high K application under complete submergence are poorly characterized. A study is conducted with the graded dose of K varying from 0 to 150% of recommended dose of fertilizer (RDF) with N and P for submergence tolerance in rice.

The experiment was conducted to study the submergence tolerance in response to K with respect to allometry, changes in metabolic activities and antioxidant enzymes during oxidative stress induced by submergence in IR64, IR64 *Sub1*, Swarna and Swarna *Sub1* cultivars. Best evaluated N and P application treatments of our previous study i.e. post-submergence urea foliar spray and basal P (Gautam *et al*, 2014a) were combined with basal application of graded K i.e. 50, 100 and 150% of recommended dose. Approximately 100 pre-soaked seeds of each cultivar were directly sown in plastic tray (37x35x25 cm) containing 10 kg of farm soil and later on plants were thinned upto 80 per tray. About 0.49 g urea and 1.14 g single super phosphate was applied to each tray as N and P was kept constant. In this study, 50, 100 and 150% of recommended dose of K (K_{50}, K_{100} and K_{150}) was applied as 0.16, 0.31 and 0.47 g MoP, respectively as per the treatments. Nitrogen was applied as post-submergence urea foliar spray, whereas P and K were applied as basal after six days of seeding. Twenty days old seedlings (15 d after basal P and K application) were submerged in a concrete tank filled with clear and turbid water and the water depth was maintained at 30 cm above the top of the plant canopy for 12 d.

7.1 Plant survival and shoot elongation

Survival of the cultivars during complete submergence was measured as the ability of plants to grow during desubmergence (i.e. under natural irrigated conditions) after 12 d of submergence (Table 8). *Sub1* cultivars proved their superiority ($P = 0.001$) in plant survival over their recurrent parents. On an average, *Sub1* cultivars recorded 92.2% higher plant survival as compared to non-*Sub1*. Plant survival of the cultivars without nutrient application was recorded 31%, addition of K significantly ($P = 0.001$) enhance the survival in the order of $K_{150} > K_{100} > K_{50}$ of all the cultivars. Application of K at low level increased the plant survival up to 60% and at high level, survival enhanced up to >80% (Table 8). Thus, higher concentrations of K application led to 5-fold increase in the survival of all the cultivars to that of the control. Interaction effect of variety and nutrient for plant survival was found significant (*P = 0.001*); IR64 *Sub1* resulted in greatest survival with the application of 150% K (Table 8). Shoot elongation of the cultivars was calculated as the difference in plant height after desubmergence over plant height prior to submergence (0 d). Plant height after desubmergence varied among cultivars and with nutrient application resulting in variable shoot elongation (Table 8). An excessive (*P = 0.001*) elongation was observed on non-*Sub1* cultivars; although *Sub1* cultivars elongate but to less extent. On an average, *Sub1* cultivars recorded 84.2% less shoot elongation than their recurrent parents. The highest shoot elongation was observed on the plants grown on unfertilized soils; addition of K significantly (*P = 0.001*) decreased the elongation. Higher levels of K application proved beneficial for less elongation, which leads to better survival.

7.2 Non-structural carbohydrates (sugar and starch concentration) in shoots

Variations in inherent carbohydrate contents were observed after desubmergence among the cultivars and with the nutrient application (Table 47). All the genotypes showed reduction in stem soluble sugar and starch from the last measured at pre-submergence. Reduction (*P = 0.001*) in carbohydrate of *Sub1* lines was significantly lower compared to that of non-*Sub1* lines, resulting in less percentage of change in carbohydrates. After 12 d of submergence and 10 d of desubmergence significant (*P = 0.001*) differences in carbohydrates were observed between control and levels of K application (Table 9). At low levels of K, sugar and starch content was 56.7 and 27.5% higher; and at high levels of K, sugar and starch content was 87.8 and 55.0% higher over control. Higher NSC concentrations after desubmergence with K application led

Table 8. Effect of potassium application on shoot elongation (immediately after desubmergence) and plant survival (after 10 days of desubmergence) of IR64, IR64 *Sub1*, Swarna and Swarna *Sub1*

	Shoot elongation (%)				**Plant survival (%)**			
	IR64	IR64 *Sub1*	Swarna	Swarna *Sub1*	IR64	IR64 *Sub1*	Swarna	Swarna *Sub1*
Control	57.77±4.6	41.99±2.7	60.67±5.1	44.54±3.2	17.34±2.1	35.76±1.4	16.22±2.3	32.42±1.7
No K	38.16±3.7	31.68±2.1	79.21±4.2	28.27±2.5	26.22±2.5	52.76±1.8	23.36±2.8	50.02±2.1
K_{50}	32.54±3.1	23.56±1.8	84.27±3.6	21.36±2.1	33.96±2.8	61.42±2.2	31.68±3.1	57.78±2.4
K_{100}	29.67±2.7	21.77±1.5	68.59±3.2	20.72±1.9	45.18±3.1	76.46±2.5	40.52±3.4	74.50±2.7
K_{150}	24.19±2.1	14.55±1.1	44.83±2.7	16.47±1.4	46.44±3.3	82.60±2.7	42.22±3.7	78.22±2.8
$LSD_{P=0.05}$	4.36				5.12			

For each parameter, 5 replicates were used for each sample.

Table 9 Effect of potassium application on sugar and starch concentration of IR64, IR64 Sub1, Swarna and Swarna *Sub1* measured before submergence, 10 d after desubmergence and % change in sugar and starch after desubmergence over before submergence

	Sugar content (mg g^{-1} DW)			**Starch content (mg g^{-1} DW)**		
	BS	**AS**	**%change**	**BS**	**AS**	**%change**
Variety (V)						
IR64	34.2^{a}	23.0^{b}	48.6^{b}	38.3^{a}	27.2^{b}	41.5^{b}
IR64 *Sub1*	34.2^{a}	27.2^{a}	28.2^{d}	38.3^{a}	30.7^{a}	25.3^{d}
Swarna	35.4^{a}	22.4^{b}	59.0^{a}	39.5^{a}	26.0^{b}	52.6^{a}
Swarna *Sub1*	35.4^{a}	26.1^{a}	37.3^{c}	39.5^{a}	29.9^{a}	33.1^{c}
LSD$_{P=0.05}$	ns	2.18	4.29	ns	1.85	3.56
Nutrient application (N)						
Control	30.5^{d}	19.3^{d}	58.8^{a}	35.9^{d}	23.5^{d}	53.3^{a}
K$_0$	33.7^{c}	22.7^{c}	49.2^{b}	37.8^{c}	26.7^{c}	42.4^{b}
K$_{50}$	35.1^{b}	25.0^{bc}	41.4^{c}	39.1^{bc}	29.1^{b}	35.8^{c}
K$_{100}$	36.9^{a}	27.2^{ab}	36.4^{d}	40.6^{a}	30.9^{ab}	31.7^{d}
K$_{150}$	37.7^{a}	29.1^{a}	30.4^{e}	41.0^{a}	32.3^{a}	27.5^{e}
LSD$_{P=0.05}$	1.62	2.44	4.48	1.28	2.08	3.88
LSD$_{P=0.05}$ (VxN)	ns	2.63	5.06	ns	2.53	4.03

to lower percentage of change ($P = 0.05$), with more prominent effects in *Sub 1* genotypes (19.9%). Interaction effect of variety and nutrient for NSC concentrations was found significant ($P = 0.05$); IR64 *Sub1* resulted in maintenance of sugar and starch levels along with less percentage of change with the application of 150% K (Table 10).

7.3 Allometric characteristics after desubmergence

The study also focused on allometric characteristics especially dry weight changes of root and shoots, leaf development after submergence, leaf decay during submergence and lodging, and all these characters varied significantly among cultivars and with the levels of K application (Table 11).

Leaf chlorosis was quantified in terms of LS score and the score was high in non-*Sub1* cultivars. Application of K significantly helped in retaining the leaf greenness of all the cultivars. Along with LS score, standing ability of plants after desubmergence was determined using lodging score; as per the score, it is evident that increasing dose of K significantly reduce the lodging of all the

Table 10 Effect of potassium application on Leaf senescence and Lodging of IR64, IR64 *Sub1*, Swarna and Swarna *Sub1* after 12 d of submergence

	Leaf senescence score				**Lodging score**			
	IR64	IR64 *Sub1*	Swarna	Swarna *Sub1*	IR64	IR64 *Sub1*	Swarna	Swarna *Sub1*
Control	5±0.1	4±0.2	5±0.1	4±0.5	6±0.1	4±0.2	6±0.1	4±0.5
K_0	4±1.1	3±0.8	4±1.2	3±1.1	5±0.6	3±0.6	5±0.8	3±0.8
K_{50}	4±1.3	2±1.0	4±1.5	3±1.3	4±1.0	2±0.9	4±1.2	2±1.1
K_{100}	3±1.4	2±0.3	3±1.6	2±0.6	4±1.1	2±0.8	4±1.5	2±1.2
K_{150}	2±1.5	1±1.2	2±1.8	1±1.4	3±0.9	1±1.1	3±1.3	1±1.6

In leaf senescence; 1= all leaves green; 5= all leaves completely yellow or degenerated; In lodging; 1= No lodging; 6= >80% plants lodged; For each parameter, 5 replicates were used for each sample. Values are means of measurements made after 12 d complete submergence ± standard deviation

cultivars (Table 11). Dry matter, leaf development and R:S ratio was significantly affected by submergence and effect was more visible on intolerant cultivars (Table 11). Increasing levels of K significantly improved the allometry of plants irrespective of the cultivars. IR64 and Swarna recorded lowest growth after desubmergence under control resulting in lowest values of DMW, LA, SLW and R:S ratio. Although *Sub1* cultivars showed better growth after desubmergence but high levels i.e. 150% of K contributed significantly to the greatest values of all the allometric characters of Swarna Sub1 followed by IR64 *Sub1* (Table 11).

7.4 Nutrient content in shoots before and after submergence

Nutrient content (NPK) in shoot was also influenced by varieties and K application. Before submergence, the content was almost similar in all the cultivars but after desubmergence, nutrient content was significantly higher in IR64 *Sub1* and Swarna *Sub1* (Table 12). The nutrient content was significantly improved due to K application before and after submergence. The content of NPK was lowest in control, but interaction of NP and K led to higher K content even in 50% K application. Concentration of N, P and K in shoots after desubmergence was increased upto 11-fold, 30-fold and, 7-fold, respectively with 150% K application over control treatment.

It can be said that *Sub1* cultivars proved their superiority in plant survival over their recurrent parents. *Sub1A* protects plants in submerged fields in two ways: (1) inhibition of elongation growth, thereby preventing exhaustion of carbohydrates and an energy crisis during submergence; and (2) up-regulation of the ROS-scavenging system, thereby providing protection against cell damage upon de-submergence (Fukao *et al*, 2011). Higher K concentrations in the shoot may be an important trait for increasing survival under lowland conditions, where flooding is common during the early stage of crop establishment because reactive oxygen species (ROS) production is a major reason

Table 11 Mean values and ANOVA of allometric data of *Sub1* cultivars and their recurrent parents after 10 d of desubmergence influenced by potassium application

	Control	NP	NPK_{50}	NPK_{100}	NPK_{150}	V	N	V x N
IR64								
DMW (g $plant^{-1}$)	164.5	200.1	217.3	229.3	244.4	***	***	*
LA (cm^2 $plant^{-1}$)	11.6	18.3	22.6	26.1	28.4	***	***	**
SLW (mg cm^{-2} leaf)	1.33	1.38	1.54	1.78	2.11	**	**	*
R:S ratio	0.111	0.140	0.147	0.153	0.167	*	*	ns
IR64 *Sub1*								
DMW (g $plant^{-1}$)	225.6	286.0	299.3	318.1	323.1	***	***	*
LA (cm^2 $plant^{-1}$)	17.5	31.0	34.5	38.7	45.2	***	***	**
SLW (mg cm^{-2} leaf)	1.54	1.67	1.90	2.18	2.40	**	**	*
R:S ratio	0.149	0.165	0.192	0.200	0.218	*	*	ns
Swarna								
DMW (g $plant^{-1}$)	190.0	255.1	264.7	283.7	295.4	***	***	*
LA (cm^2 $plant^{-1}$)	14.7	21.1	24.3	26.8	31.7	***	***	**
SLW (mg cm^{-2} leaf)	1.42	1.48	1.79	2.06	2.23	**	**	*
R:S ratio	0.132	0.153	0.160	0.172	0.181	*	*	ns
Swarna *Sub1*								
DMW (g $plant^{-1}$)	273.7	297.3	323.9	344.5	358.0	***	***	*
LA (cm^2 $plant^{-1}$)	20.1	33.2	38.6	45.4	48.6	***	***	**
SLW (mg cm^{-2} leaf)	1.65	1.85	2.08	2.25	2.55	**	**	*
R:S ratio	0.159	0.186	0.201	0.216	0.229	*	*	ns

Mean values represents average of n= 3 replicate measurements, ANOVA significance levels: *, *P<0.05*; **, *P<0.01*; ***, *P<0.001*; ns, not significant.

DMW, dry matter weight; LA, leaf area; SLW, specific leaf weight; R: S ratio, root: shoot ratio

for excessive K losses from the shoots of rice plants during submergence. Potassium when applied with basal P, effect was more beneficial as role of P to resist the damage during submergence is well documented (Gautam *et al*, 2014a). Basal K and P application along with post-flood N significantly improved the survival by regaining chlorophyll, NSC that contributed to higher photosynthesis, leaf and plant growth after submergence (Gautam *et al*, 2014b). The maintenance of a sufficient K

Table 12 Effect of potassium application on nutrient content (%) of IR64, IR64 *Sub1*, Swarna and Swarna *Sub1* before submergence after 10 d of desubmergence

	Before submergence			10 days after desubmergence		
	N	P	K	N	P	K
Variety (V)						
IR64	2.98^{b}	0.36^{a}	2.19^{a}	2.26^{b}	0.20^{b}	1.37^{b}
IR64 *Sub1*	3.02^{b}	0.39^{a}	2.23^{a}	3.03^{a}	0.27^{a}	1.65^{a}
Swarna	3.33^{a}	0.35^{a}	2.11^{a}	2.33^{b}	0.16^{b}	1.31^{b}
Swarna *Sub1*	3.36^{a}	0.37^{a}	2.17^{a}	3.41^{a}	0.24^{a}	1.73^{a}
$LSD_{P=0.05}$	0.221	ns	ns	0.478	0.09	0.212
Nutrient application						
Control	2.08^{c}	0.23^{c}	1.76^{c}	1.51^{d}	0.08^{c}	1.06^{d}
K_0	3.15^{b}	0.35^{b}	1.98^{c}	2.87^{bc}	0.18^{b}	1.37^{c}
K_{50}	3.38^{ab}	0.39^{b}	2.23^{b}	3.02^{b}	0.26^{ab}	1.59^{b}
K_{100}	3.54^{a}	0.44^{a}	2.42^{a}	3.18^{a}	0.29^{ab}	1.73^{ab}
K_{150}	3.68^{a}	0.48^{a}	2.57^{a}	3.24^{a}	0.32^{a}	1.85^{a}
$LSD_{P=0.05}$	0.384	0.049	0.258	0.519	0.116	0.256
$LSD_{P=0.05}$ (VxN)	0.396	ns	ns	0.582	0.148	0.308

supply can be an effective nutritional strategy to minimize submergence stress related losses in crop production especially in rainfed lowlands.

8. Role of silica application in submergence tolerance of rice

8.1 Application of silica with nitrogen and phosphorus alleviates the damage of flooding stress

Silicon (Si) is the second most abundant element in the earth's crust, with soils containing ~32% Si by weight (Lindsay, 1979). In agronomy, Si is generally not considered an essential element, mainly because there is no evidence to show that Si is involved in the metabolism of plants, which is one of the three criteria of essentiality as established by Arnon and Stout (1939). Rice is considered a 'silicon accumulator' because of the ability of its roots to take up Si (Mitani and Ma, 2005; Ma and Yamaji, 2006), and it tends to actively accumulate Si to tissue concentrations of around 5%. Recently, Si has been regarded as a quasi-essential element (Epstein, 1999), especially for rice. Application of nitrogenous fertilizers is an important practice for increasing rice yields. However, when applied in excess, N may limit yield, because of lodging, and promote shading and susceptibility to insects and diseases. These effects could be minimized by the use of Si (Ma *et al*, 1989; Munir *et al*, 2003). Through a synergistic effect, the application of Si has the potential to raise the optimum N rate, thus enhancing productivity of existing lowland rice fields (Ho *et al*, 1980). Silicon has been reported to raise the optimum level of N in rice. It is also suggested to play a crucial role in preventing or minimizing lodging in cereal crops (Munir *et al*, 2003), providing culm sturdiness and increased leaf erectness, a matter of great importance in terms of agricultural productivity. Basal fertilization of N (Reddy *et al*, 1985), and of N+ P (Gautam *et al*, 2014a; Lal *et al*, 2014), improves initial vigour of rice plants for better tolerance to submergence at later growth stages. Post-submergence N application can also contribute substantially to increasing productivity in flood-prone areas (Gautam *et al*, 2014b; Lal *et al*, 2014). However, the role of Si in the growth and survival of rice under submergence is unknown and information on the combined effect of Si, N and P on completely submerged rice is negligible. In this context, the present study was undertaken to evaluate the effects of Si, N and P on survival, crop establishment, and chlorophyll and nonstructural carbohydrate contents of submerged rice.

The experiment was carried out during 2013–14, under clear and turbid water submergence: IR64 *Sub1*, Swarna *Sub1*, Savitri *Sub1* and IR20. The best treatments from our previous study (i.e. post-submergence urea foliar spray and basal P; Gautam *et al*, 2014b) were combined with basal Si application. Approximately 100 pre-soaked seeds of each cultivar were directly sown in plastic trays and later, plants were thinned to 70 per tray. Urea (0.49 g), SSP (1.14 g), MOP (KCl, 0.31 g), and calcium silicate (3.35 g) were applied to each tray, as per the treatments, as the N, P, K and Si sources. The P, K and Si fertilizers were applied as basal at six days after seeding. Urea (2.0%, w/v in a water carrier) was applied as a post-submergence foliar spray. Tap water was used as the source of clear water, and turbidity was created by mixing silt into the water at a concentration of 0.4%, based on a survey of silt concentration of the Mahanadi River, which was found to be 0.4% (Das *et al*, 2009). Twelve-day-old seedlings were submerged in a concrete tank filled with clear and turbid water and the water depth was maintained at 30 cm above the top of the plant canopy for 12 d.

8.1.1 Shoot elongation

The introduction of submergence resulted in shoot elongation of all cultivars, and elongation was significantly higher in IR20 than in *Sub1* cultivars. Among the *Sub1* cultivars, elongation was slightly higher in Swarna *Sub1*, followed by IR64 *Sub1* (Table 13). Shoot elongation also varied significantly with water turbidity, and comparatively lower elongation of shoots was observed in turbid water. Elongation of IR20, IR64 *Sub1*, Swarna *Sub1* and Savitri *Sub1*, respectively, was 34.4%, 49.5%, 48.6% and 66.7% lower after 12 d of complete submergence in turbid water than clear water. Basal application of Si and P suppressed ($P < 0.05$) underwater shoot elongation, either in combination or individually, in all of the cultivars. Combined application of basal Si and P reduced shoot elongation relative to the control by 104.5%, 209.3%, 166.2% and 241.1% in IR20, IR64 *Sub1*, Swarna *Sub1* and Savitri *Sub1*, respectively, in both clear and turbid water (Table 13).

8.1.2 Plant survival

Plant survival recorded at 7 and 15 d after de-submergence showed that submergence resulted in considerable decrease ($P < 0.05$) in the survival of all of cultivars, and the reduction was higher in turbid water (Table 14). Basal Si and P application in combination with post-flood N contributed to highest plant survival, followed by basal Si and N application. Although the effect of Si application alone was not apparent in survival, when combined with N and P, Si resulted in significantly higher survival and better recovery. IR20, IR64 *Sub1*, Swarna *Sub1* and Savitri *Sub1* recorded 124.1%, 77.6%, 66.8% and 60.7% greater survival, respectively, when supplied with Si, N and P than in the control treatment (Table 14).

8.1.3 Leaf senescence, lodging and allometry

Complete submergence under clear and turbid water enhanced the leaf senescence and lodging of rice seedlings, and after desubmergence, the DM, LA and SLW were decreased. Lodging and leaf senescence score were higher in IR20 and in turbid water, which was reflected in significantly ($P < 0.05$) lower SPAD values, ultimately leading to lower recovery and growth. At the end of the submergence period, plants grown with P and Si showed significantly ($P < 0.05$) lower leaf senescence and lodging. In addition, when post-flood N was applied, recovery and growth were enhanced, resulting in higher DM, LA and SLW (Table 15). When rice seedlings enriched with Si and P were submerged, and supplied with N after de-submergence, they had higher DM, LA and SLW, by 128.8%, 64.1% and 63.5%, respectively, than the control. There was a

Table 13 Shoot elongation (%) of IR20, IR64 *Sub1*, Swarna *Sub1* and Savitri *Sub1* influenced due to basal phosphorus (P) and silica (Si) application under clear and turbid water submergence

Treatments	**IR20**		**IR64 *Sub1***		**Swarna *Sub1***		**Savitri *Sub1***	
	Clear	**Turbid**	**Clear**	**Turbid**	**Clear**	**Turbid**	**Clear**	**Turbid**
Control	94.3^{a}	77.1^{a}	66.2^{a}	51.4^{a}	68.4^{a}	52.2^{a}	63.1^{a}	50.5^{a}
P	80.6^{b}	64.5^{b}	51.6^{b}	40.9^{b}	55.3^{b}	42.5^{b}	45.2^{b}	33.8^{b}
Si	78.6^{b}	63.1^{b}	48.7^{b}	37.1^{b}	50.3^{b}	40.1^{b}	41.7^{b}	29.7^{b}
P x Si	46.1^{c}	34.3^{c}	21.4^{c}	14.3^{c}	25.7^{c}	17.3^{c}	18.5^{c}	11.1^{c}

Shoot elongation of the plant shoots was determined by subtracting plant height before submergence (BS) from that after desubmergence (AS) and expressing it as percentage of plant height before submergence.

Table 14 Plant survival (%) of IR20, IR64 *Sub1*, Swarna *Sub1* and Savitri *Sub1* after 7 and 15 d of desubmergence influenced due to basal phosphorus (P), silica (Si) and post-flood nitrogen (N) application under clear and turbid water submergence

Treatments	**IR20**		**IR64 *Sub1***		**Swarna *Sub1***		**Savitri *Sub1***	
	Clear	**Turbid**	**Clear**	**Turbid**	**Clear**	**Turbid**	**Clear**	**Turbid**
Plant survival (%) after 7 days of desubmergence								
Control	14.7^{e}	4.1^{e}	31.1^{f}	17.9^{f}	38.7^{f}	27.1^{f}	41.2^{f}	29.8^{f}
N	20.1^{cd}	7.3^{cd}	42.6^{d}	33.5^{d}	52.4^{d}	47.1^{d}	55.6^{d}	47.9^{d}
P	16.5^{e}	6.4^{d}	36.9^{e}	28.8^{e}	48.7^{e}	39.8^{e}	50.8^{e}	43.5^{e}
Si	17.4^{de}	6.6^{d}	37.4^{e}	29.5^{e}	49.3^{e}	41.3^{e}	51.1^{e}	44.2^{e}
N x Si	27.8^{b}	11.9^{b}	53.4^{b}	44.1^{b}	61.2^{b}	54.1^{b}	63.1^{b}	57.4^{b}
P x Si	22.3^{c}	9.8^{bc}	49.1^{c}	41.2^{c}	55.4^{c}	50.2^{c}	58.4^{c}	52.8^{c}
NP x Si	31.4^{a}	17.5^{a}	58.7^{a}	52.3^{a}	65.1^{a}	58.7^{a}	68.5^{a}	61.1^{a}
Plant survival (%) after 15 days of desubmergence								
Control	17.5^{e}	5.7^{e}	35.2^{f}	21.4^{f}	41.3^{f}	30.4^{f}	44.1^{e}	32.5^{f}
N	28.6^{c}	8.5^{cd}	46.9^{d}	37.3^{d}	56.2^{d}	49.1^{d}	58.7^{c}	51.4^{d}
P	22.1^{d}	7.2^{d}	40.5^{e}	32.8^{e}	51.9^{e}	43.2^{e}	53.2^{d}	45.6^{e}
Si	23.3^{d}	7.6^{d}	41.1^{e}	33.5^{e}	53.2^{e}	44.8^{e}	53.9^{d}	47.1^{e}
N x Si	35.6^{b}	13.7^{b}	57.8^{b}	48.7^{b}	64.5^{b}	59.8^{b}	66.3^{b}	59.9^{b}
P x Si	29.1^{c}	11.2^{c}	52.7^{c}	44.6^{c}	59.7^{c}	53.7^{c}	61.6^{c}	56.3^{c}
NP x Si	39.2^{a}	19.5^{a}	62.5^{a}	55.1^{a}	68.9^{a}	61.5^{a}	70.9^{a}	64.2^{a}

Plant survival was determined by counting the number of plants that were able to produce at least one new leaf after 7 and 15 d of desubmergence and was expressed as percentage of the initial number before submergence.

significant ($P < 0.05$) variety x floodwater x nutrient application interaction; application of N, P and Si to Savitri *Sub1* resulted in significantly lower leaf senescence and lodging, whereas highest SPAD value, DM, LA and SLW occurred after clear water submergence.

8.1.4 Soluble carbohydrate concentration

Depletion of soluble carbohydrates in the shoots of all of the cultivars was observed after submergence, resulting in a change in NSC% that was significantly higher ($P < 0.05$) in IR20. Submergence in turbid water resulted in considerable depletion of NSC in all cultivars (Fig. 22). With the introduction of submergence, soluble carbohydrates were depleted, but the extent of depletion was considerably lower with Si application. When Si was applied in combination with N and P, the regain in carbohydrates after submergence was also higher, with less depletion during submergence. Considering the combined effect of variety, floodwater and nutrient application, Savitri *Sub1* had the highest ($P < 0.05$) concentration of NSC after desubmergence (smallest percentage change) when supplied with post-flood N and basal Si and P application under clear water submergence (Fig. 22).

8.1.5 Ethylene accumulation

Ethylene accumulation was enhanced and photosynthetic rate decreased during submergence, irrespective of cultivar and floodwater. The extent of ethylene accumulation was in the order Savitri *Sub1* <

Table 15 Leaf senescence, lodging, and growth attributes of IR20, IR64 *Sub1*, Swarna *Sub1* and Savitri *Sub1* influenced due to basal phosphorus (P), silica (Si) and post-flood nitrogen (N) application under clear and turbid water submergence

Treatments	LS score	SPAD value	Lodging score	DMW (mg plant^{-1})	Leaf area (cm^2 plant^{-1})	SLW (mg cm^{-2} leaf)
Varieties						
IR20	5±0.8	20.7±3.5	6±0.8	158.2±17.5	10.5±3.1	1.18±0.05
IR64 *Sub1*	3±0.7	36.2±2.4	3±0.4	226.6±10.5	14.5±2.4	1.58±0.03
Swarna *Sub1*	2±0.3	40.4±1.4	2±0.3	292.2±11.6	16.8±1.6	1.74±0.03
Savitri *Sub1*	2±0.2	37.2±1.7	2±0.3	258.5±8.8	15.3±1.7	1.69±0.02
Floodwater						
Clear water	3±0.5	35.5±3.7	4±0.7	171.3±16.2	11.4±3.5	1.62±0.05
Turbid water	5±0.9	21.7±4.1	6±0.9	123.7±21.4	7.1±4.9	1.28±0.06
Nutrient application						
Control	4±0.7	18.7±1.7	5±0.6	126.6±12.7	10.6±2.0	1.12±0.04
N	4±0.5	33.9±2.1	5±0.4	178.2±9.4	13.1±1.3	1.34±0.03
P	3±0.6	27.4±1.9	3±0.5	166.4±9.1	12.5±1.1	1.27±0.04
Si	3±0.4	28.8±1.3	2±0.3	171.2±8.5	13.4±1.1	1.30±0.04
N x Si	3±0.3	37.3±2.1	2±0.3	232.9±7.2	16.8±0.7	1.46±0.02
P x Si	2±0.2	32.1±1.8	1±0.2	189.5±8.3	14.7±0.5	1.40±0.02
NP x Si	2±0.1	40.5±2.2	1±0.2	289.7±4.6	17.4±0.3	1.83±0.01

LS-leaf senescence, DMW-dry matter weight, SLW-specific leaf weight

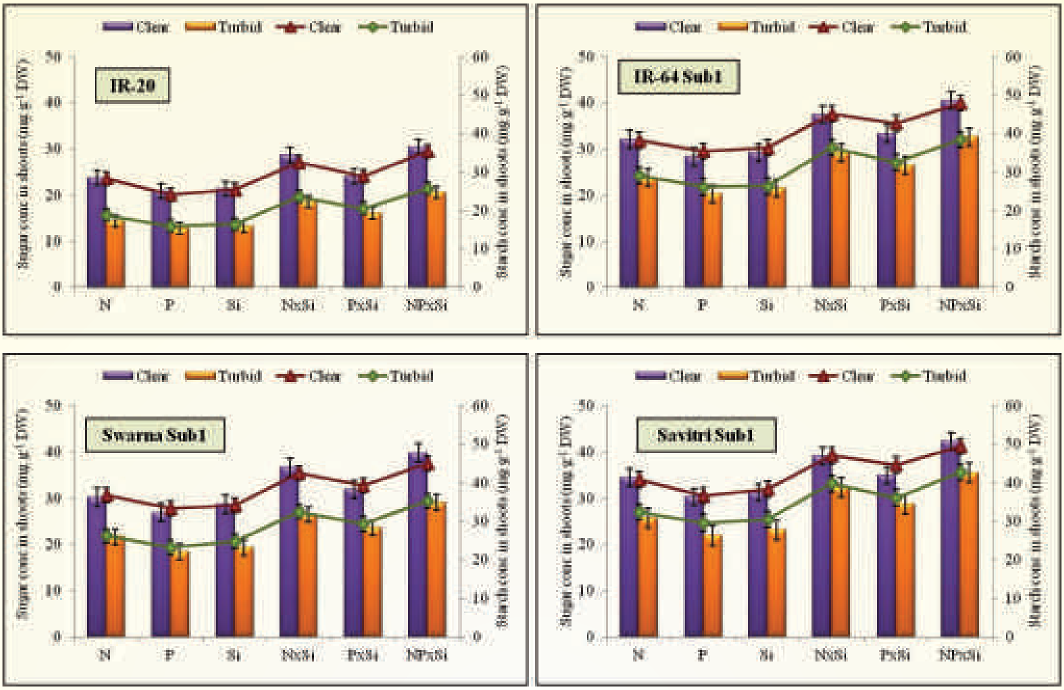


Fig. 21 Sugar concentration in shoots (bars, on primary axis) and starch concentration in shoots (lines, on secondary axis) of IR20, IR64 *Sub1*, Swarna *Sub1* and Savitri *Sub1* after 12 d of desubmergence in clear and turbid water submergence influenced by nutrient application (vertical bars in each column and line represents standard error). N-nitrogen, P-phosphorus, Si-silica

Swarna *Sub1* < IR64 *Sub1* < IR20, and higher accumulation was recorded under turbid water (Fig. 22). Nutrient application, especially Si and P, significantly ($P < 0.05$) influenced ethylene accumulation in shoots of all of the cultivars. Basal Si application reduced the ethylene accumulation, and the effect was more prominent when combined with basal P application. Basal Si and P application resulted in lower accumulation of ethylene than the control by 60.4%, 93.5%, 93.7% and 101.5% in IR20, IR64 *Sub1*, Swarna *Sub1* and Savitri *Sub1*, respectively.

Submergence enhances the production of ethylene (Raskin and Kende, 1984; Cohen and Kende, 1987) in the shoots or in tissues of internodes of all of the cultivars, and more so in sensitive cultivars (Gautam *et al*, 2015). Ethylene is an endogenous regulator of leaf senescence (Bleecker and Kende 2000; Lim *et al*, 2007). Greater underwater accumulation of ethylene leads to greater shoot elongation and leaf senescence in all the cultivars. Application of Si and P reduces the damage, and post-flood N enhances the recovery after desubmergence, the combined effect of all the three nutrients resulting in greater photosynthesis, stomatal conductance, and leaf area and weight, ultimately contributing to greater plant survival under both clear and turbid water. Silicon tends to maintain erectness of rice leaves and clumps, thereby increasing photosynthesis because of better light interception. Application of silicate not only augmented its absorption by rice plant but

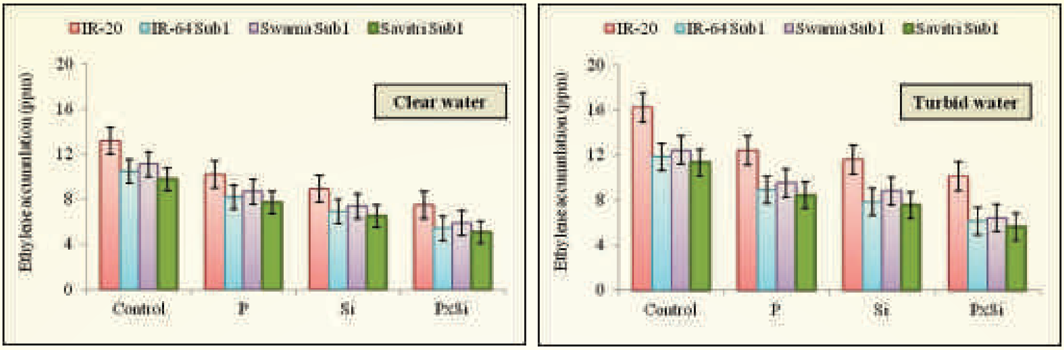


Fig. 22 Ethylene accumulation (ppm) in IR20, IR64 *Sub1*, Swarna *Sub1* and Savitri *Sub1* immediately after desubmergence in clear and turbid water submergence influenced by nutrient application (vertical bars in each column and line represents standard error). N-nitrogen, P-phosphorus, Si-silica

also had a significant interrelationship with the other nutrients. With adequate silicon, the uptake of N was increased (Okamoto, 1969; Sadanandan and Verghese, 1969).

8.2 Silica and nitrogen interaction for enhancing submergence tolerance of rice

Nitrogen (N) is the important and primary element in determining rice grain yield, and N fertilizer is one of the key inputs to paddy fields, with favorable effect in stimulating tillering and increasing spikelet number per panicle (Qiao *et al*, 2011). Semi-dwarf rice cultivars having submergence tolerance developed in the recent years have been found to respond to nitrogen fertilization and withstand flooding stress better (Reddy *et al*, 1986). Basal fertilization of N (Reddy *et al*, 1985) and NP (Gautam *et al*, 2014a) provides initial vigor to rice plants for higher tolerance to submergence at later crop growth stages. Post-submergence nutrient application can also contribute considerably towards increasing production in flood-prone areas (Gautam *et al*, 2014b). The effect of Si has been traditionally accredited to its role in decelerating abiotic and biotic stresses, as well as in imparting resistance to lodging and enhancing the erectness of leaves; these effects allow higher light transmittance in and above plant canopies and thus obliquely improve photosynthesis (Tamai and Ma, 2008). Due to a synergistic effect, the application of Si has the potential to raise the optimum N rate, resulting in enhancing productivity of lowland rice fields (Ho *et al*, 1980) of Eastern India. In a study, Lal *et al*. (2015a) studied the combined effects of Si, P and N in rice nursery but the interaction of Si and N in respect to their application time and methods is unknown to the rice crop when flooding occurs in the main field. An experiment was designed to study the interaction effect of N and Si, with respect to their application time and method. The study is conducted with submergence tolerant rice varieties for harnessing the productivity potentials and minimizing flood damages in rice production through interaction of N x Si.

In the experiment, 15 d old seedlings of uniform appearance of IR64, Swarna, IR64 *Sub1* and Swarna *Sub1* were selected and transplanted to plastic pots containing 10 kg of alluvial soil with two seedlings per pot. 0.89 g urea, 1.24 g SSP, 0.37 g MoP and 120 gm of Si were applied to each pot as per the treatments (application time of N and Si is given in Table 16). Nitrogen was applied as basal and post-submergence urea foliar spray, Si as basal (Na_2SiO_3) and pre/post submergence spray (H_4SiO_4), whereas P and K were applied as basal at the time of transplanting. Plants were completely submerged at maximum tillering stage for 14 days in concrete tanks (plants were approximately 30 cm below the water surface) under following conditions: (i) water temperatures of 27.2– 30.8°C; (ii) dissolved oxygen (DO) of 2.5–5.39 mg L^{-1}; (iii) pH ranged from 6.5 to 8.1; and (iv) photosynthetically active radiation (PAR) was 586.9 µmol m^{-2} s^{-1} at surface and 353.4, 105.2, 12.3 µmol m^{-2} s^{-1} at 10, 30 and 50 cm of water depth.

However, the *Sub1* cultivars which tolerated flood did not show the high elongation and resulted in better survival and growth after recovery. Application of silica (Si) as basal resulted in lower elongation (*P = 0.01*) either alone or with P and K, whereas Si spray proved fatal in terms of elongation and survival leading to poor growth post recovery. Basal Si application resulted in 39.1% lower elongation and 41.4% greater survival irrespective of cultivars, additional 21.5% advantage in survival was recorded with N application with Si (Fig. 24). Higher percentage of plant survival was recorded

Table 16 Details of nutrient application followed in the experiment

Nutrient	**Treatment symbols**	**Method of Nutrient application**				
		N			**Si**	**P and K**
		1st split	**2nd split**	**3rd split**		
Nitrogen	N_B	urea broadcasting	urea broadcasting after desubmergence	urea broadcasting	-	Basal
	N_S	urea broadcasting	urea spray after desubmergence	urea broadcasting	-	Basal
Silica	Si_B	-	-	-	Basal; broadcasting	Basal
	Si_S	-	-	-	Pre and post submergence spray	Basal
Nitrogen x Silica	N_B x Si_B	urea broadcasting	urea broadcasting after desubmergence	urea broadcasting	Basal; broadcasting	Basal
	N_S x Si_S	urea broadcasting	urea spray after desubmergence	urea broadcasting	Pre and post submergence spray	Basal
	N_B x Si_S	urea broadcasting	urea broadcasting after desubmergence	urea broadcasting	Pre and post submergence spray	Basal
	N_S x Si_B	urea broadcasting	urea spray after desubmergence	urea broadcasting	Basal; broadcasting	Basal

Fig. 23 Plant growth after a) basal Si application and b) basal Si and post-flood N application

with significant interaction effect of N spray as post-flood and basal Si application led to maximum growth during recovery period. Interaction effect of nutrient and variety was found significant (*P = 0.01*), IR64 *Sub1* recorded highest survival and lowest elongation with the application of basal Si and N spray.

8.2.2 Chlorosis and photosynthesis

Chlorophyll content of leaves immediately after submergence stress seems to be an important parameter in the photosynthesis and recovery. Before submergence, chlorophyll concentration was similar in *Sub1* cultivars with their respective recurrent parents. Submergence resulted in significant

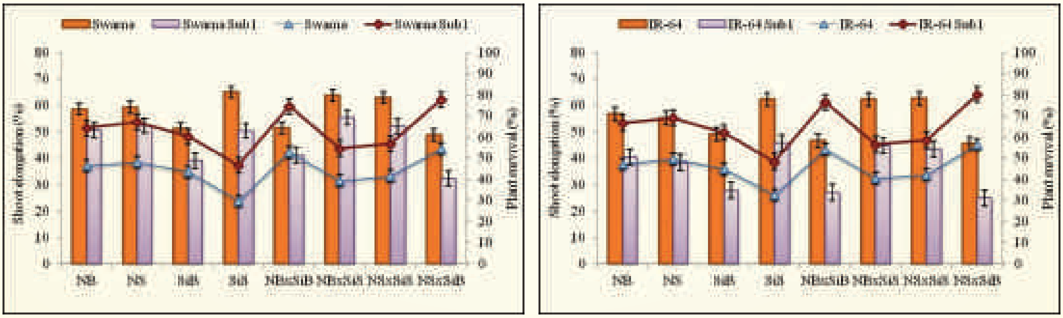


Fig. 24 Shoot elongation (%) after 14 d of submergence and plant survival (%) after 10 d of desubmergence of a) Swarna and Swana *Sub1* and b) IR64 and IR64 *Sub1*, influenced by nutrient application (vertical bars in each column and line represents standard error). Columns on primary axis represents shoot elongation and lines on secondary axis represent plant survival.

reduction ($P = 0.05$) of chlorophyll content in all the cultivars, but reduction was lower in tolerant cultivars and regain after desubmergence was also significantly higher (Fig. 25). Basal Si application significantly suppressed the chlorosis and N application as post-flood resulted in maximum regain of chlorophyll after desubmergence. It can be said that basal Si and N spray interaction was beneficial ($P = 0.05$) for maintaining higher chlorophyll content and lowest % change (36.8%) irrespective of the cultivars (Fig. 25).

Photosynthetic (Pn) rate and stomatal conductance (Gs) was more or less similar in all the cultivars, a little effect of nutrient application was visible only (Fig. 26). After 1d of submergence, both type of cultivars showed a significant reduction ($P = 0.01$) in the Pn rate which was about 90% irrespective of the nutrient application and cultivars. However, basal Si application resisted the decrease in Pn rate, around 48.7% lower reduction was observed. Post-flood N application led to gradual increase in Pn rate and conductance, after 10d recovery, it was noticed that maximum photosynthesis and conductance ($P = 0.05$) was recorded in the plants treated with both basal Si and N application (Fig. 26). As per the interaction effect of nutrient and variety, maximum photosynthesis was in Swarna *Sub1* supplied with Si x N.

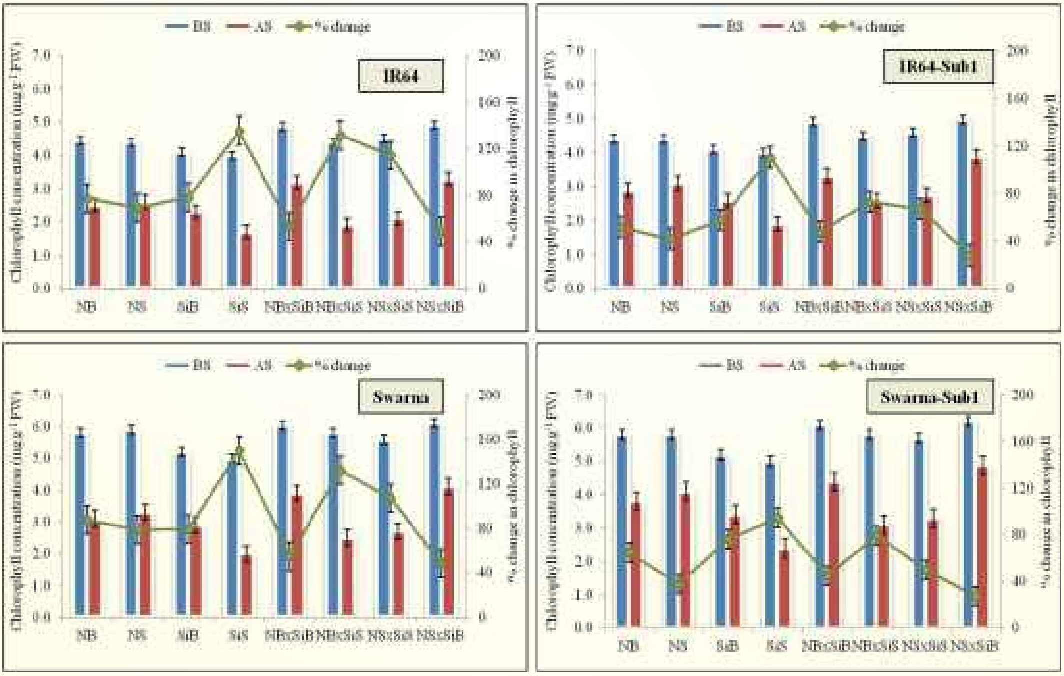


Fig. 25 Chlorophyll concentration (mg g^{-1} FW) before submergence (BS), after desubmergence (AS) and % change in chlorophyll (calculated as change in chlorophyll concentration after desubmergence over pre-submergence) of a) IR64, b) IR64 *Sub1*, c) Swarna and d) Swarna *Sub1*, as influenced by nutrient application (vertical bars in each column and line represents standard error). Columns on primary axis represent chlorophyll concentration before and after submergence and lines on secondary axis represent % change in chlorophyll concentration.

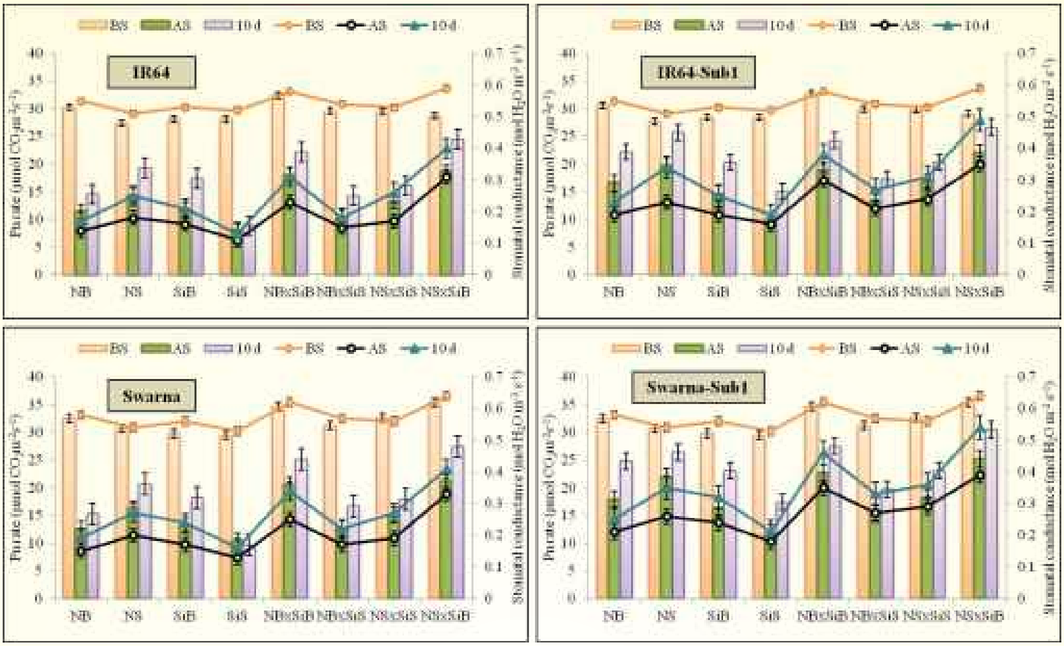


Fig. 26 Effect of submergence on photosynthetic rate (µmol CO$_2$m^{-2}s^{-1}) and stomatal conductance (mol H$_2$O m^{-2} s^{-1}) before submergence (BS), after 14 d of submergence (AS) and after 10 d of desubmergence (10d) as influenced by nutrient application (vertical bars in each column and line represents standard error). Symbols a, b, c and d denotes the photosynthetic rate and stomatal conductance of IR64, IR64 *Sub1*, Swarna and Swarna *Sub1*, respectively; columns on primary axis represents photosynthetic rate and lines on secondary axis represents stomatal conductance.

8.2.3 SOD activity

Fourteen days complete submergence resulted in decreased ($P = 0.05$) activity of all the antioxidant enzymes i.e. SOD, CAT and PER in all the cultivars and more in intolerant ones. However, basal Si application resists the decrement ($P = 0.05$) up to some extent whereas, Si sprays affected negatively. At 2d after desubmergence, the SOD activity was increased upto 150% in *Sub1* cultivars, also basal Si and N spray contributed to higher SOD activity, which was around 126.7, 28.2 and 20.3% higher over Si spray, basal Si and N spray alone, respectively (Fig. 27).

8.2.4 Plant allometry

Different allometric characters were studied viz, leaf senescence, lodging, leaf area, number of leaf emergence; these parameters were confirmed with SPAD and LCC value. IR64 *Sub1* and Swarna *Sub1* proved their superiority in terms of lower leaf senescence and lodging and higher number of green leaves and leaf area after desubmergence which was reflected in high SPAD value.

Application of basal Si significantly reduced the lodging and senescence, whereas, pre-submergence Si spray increased the lodging and senescence resulting in higher plant mortality. Leaf senescence was 44.8% and lodging was 55.6% higher with Si spray over basal Si application, irrespective of the cultivars (Fig. 28). Post-flood N spray

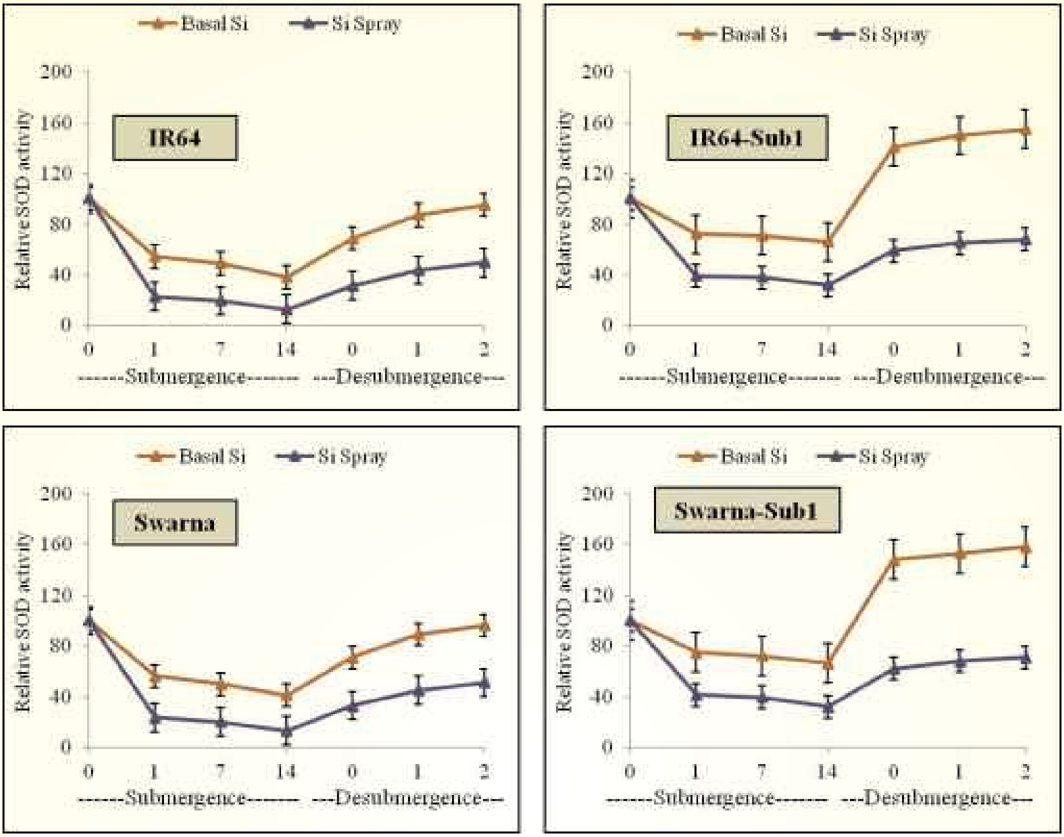


Fig. 27 Effect of submergence on relative superoxide dismutase (SOD) activity (%) of a) IR64, b) IR64 *Sub1*, c) Swarna and d) Swarna *Sub1*, during submergence and after desubmergence as influenced by silica application (vertical bars in each line represents standard error). The average values of the enzyme activity due to basal Si was 70 (IR64), 107 (IR64 *Sub1*), 72 (Swarna) and 110 (Swarna *Sub1*); for Si spray was 40 (IR64), 57 (IR64 *Sub1*), 41 (Swarna) and 59 (Swarna Sub1), respectively.

contributed significantly to the number of green leaves (*P = 0.01*) after desubmergence; results were more promising when it combined with basal Si application.

It can be said that basal Si and N application resulted in approximately 5-fold higher green leaves as compared to Si spray (Fig. 29). On an average, number of leaf emergence was nil or minimum after 10 d in the treatments of Si spray and basal N. The leaf emergence pattern was illustrated with SPAD value which was significantly higher with N spray and basal Si application after 10d of desubmergence. Leaf area was decreased (*P = 0.01*) due to submergence in all the cultivars and nutrient application (Table 17) but depletion was lower with basal Si application (13-fold), whereas it was very high (*P = 0.01*) with Si spray (50-fold). The leaf area gradually increased as regeneration of plants started in the recovery phase and the increased was higher with post-flood N spray and more prominently when combined with basal Si application (74.2%).

Table 17 SPAD, LCC value and Leaf area before submergence (BS), after 14 d of submergence (AS) and after 10 d of desubmergence (10d) as influenced due to nutrient application (Values are means of measurements in replicates ± standard deviation)

Treatments	SPAD value			LCC value (AS)	Leaf area (cm^2 plant^{-1})		
	BS	AS	10 d		BS	AS	10 d
Varieties							
IR64	36.4±2.1	16.5±4.2	28.6±4.8	1±0.8	2146±71	316±29	525±48
IR64 *Sub1*	36.9±1.3	26.6±3.2	38.2±2.4	2±0.5	2130±42	798±24	1402±40
Swarna	40.3±1.9	20.7±2.5	32.6±2.9	2±0.5	2452±62	589±36	792±39
Swarna *Sub1*	40.7±1.1	30.4±2.1	41.9±2.1	3±0.3	2468±56	984±32	1697±32
Nutrient application							
N_B	37.9±1.8	21.7±2.4	35.7±3.1	2±0.4	2036±53	816±39	1365±54
N_S	38.4±2.2	23.8±1.9	38.4±2.7	2±0.4	2088±58	828±43	1789±59
Si_B	36.1±1.7	20.4±1.9	33.2±2.4	2±0.5	1885±67	790±35	1265±46
Si_S	32.7±2.6	17.8±1.3	26.1±1.9	1±0.8	1810±60	305±46	410±62
N_B x Si_B	39.6±0.8	26.4±2.1	38.4±2.2	3±0.5	2240±46	963±40	1526±51
N_S x Si_S	38.4±1.2	21.1±1.8	33.3±2.9	2±0.6	2023±42	721±32	916±47
N_B x Si_S	37.6±1.3	18.6±2.2	31.5±2.3	2±0.6	2002±52	701±44	877±53
N_S x Si_B	39.9±0.6	31.3±1.2	40.8±1.9	3±0.2	2564±46	982±27	1711±43

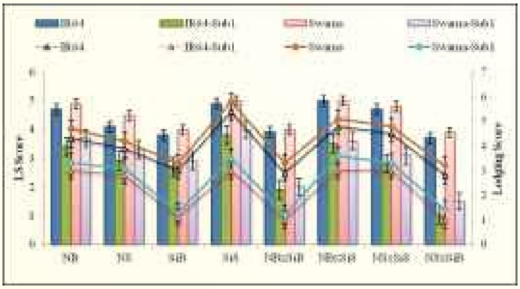


Fig.28 Leaf senescence (LS) score and lodging score of IR64, IR64 *Sub1*, Swarna and Swarna *Sub1*, measured after 14 d of submergence as influenced by nutrient application (vertical bars in each column and line represents standard error); columns on primary axis represents LS score and lines on secondary axis represents lodging score.

8.2.5 Yield attributes and yield

Submergence influenced the plant survival which was reflected in the yield attributing characters and ultimately yield, though varietal differences were there. *Sub1* cultivars, because of their higher survival after desubmergence resulted in highest ($P = 0.01$) number of panicles, florets and grain filling percentage (Table 18). The grain yield was 33.9 and 38.9% higher in Swarna *Sub1* and IR64 *Sub1* over their recurrent parents, respectively. As compared to non-submerged condition, yield of *Sub1* cultivars was decreased upto 21% and of non *Sub1* cultivars, the yield decline was around 60%.

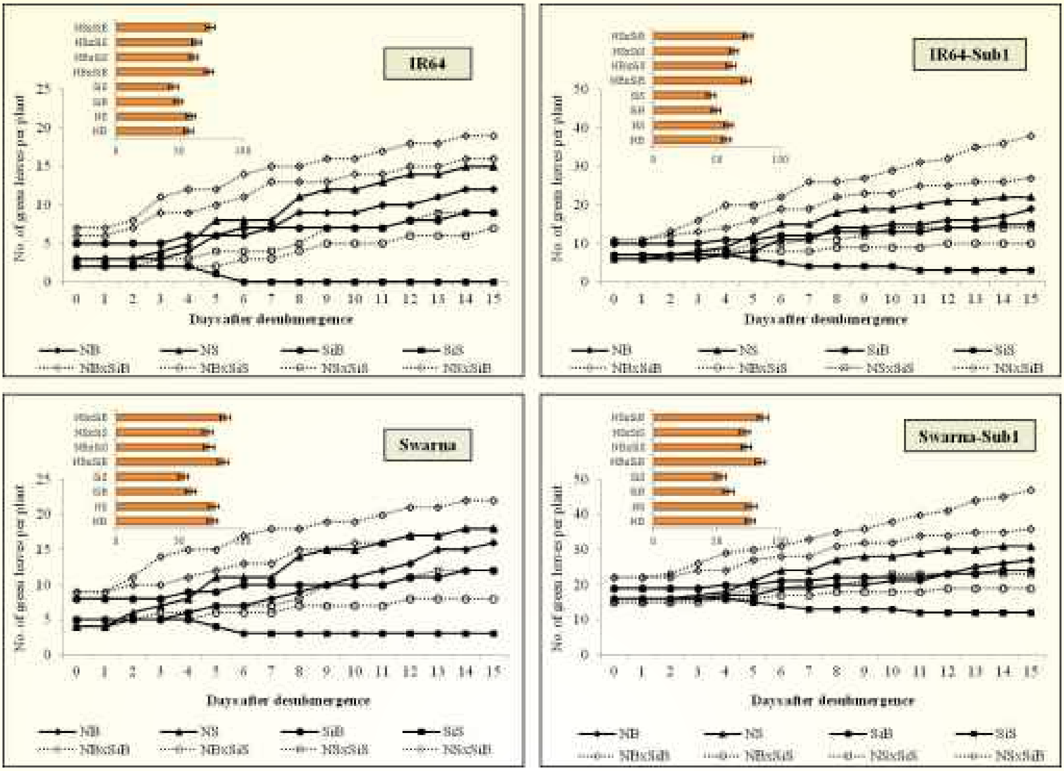


Fig. 29 Number of green leaves of a) IR64, b) IR64 *Sub1*, c) Swarna and d) Swarna *Sub1*, emerged after desubmergence as influenced by nutrient application; No of leaf emergence was measured daily after the day of desubmergence to 15 d of desubmergence. Bar graph represents the total number of leaves prior to submergence of each cultivar.

Basal Si application resists the damage of plant during submergence and helped in recovery after desubmergence, whereas Si spray showed the negative effect on growth and yield. Post-flood N spray resulted in maximum growth and highest yield attributing characters and yield ($P = 0.01$). Interaction effect of N and Si was studied and it was found that basal Si and N spray was a synergistic interaction, which resulted in 15.1 and 42.7% higher yield over N alone or Si alone, respectively.

8.2.6 Nitrogen and silica content

Nitrogen and Si content was also influenced by nutrient application and varieties, N and Si content was higher in IR64 and IR64 *Sub1* as compared to Swarna and Swarna *Sub1*, and content was slightly higher in Sub1s over their recurrent parents. Nitrogen content of IR64 varied between 1.4–2.2 and IR64 *Sub1*, it was 1.5–2.3; whereas in Swarna, it ranged from 1.2 to 1.8 and in Swarna *Sub1*, it varied from 1.3 to 2.0%, respectively. Similarly, Si content was 0.31–0.52, 0.33–0.60, 0.29–0.48 and 0.30–0.53% in IR64, IR64 *Sub1*, Swarna and Swarna *Sub1*, respectively. In *Sub1* cultivars, on an average, the N and Si content was 7.8 and 6.9% higher over non *Sub1* cultivars, respectively. Application of N and Si, alone increased their contents in grains, but as per their interaction N spray

Table 18 Yield attributes and yield of rice cultivars as influenced by nutrient application

Treatments	No of panicles plant^{-1}	No. of florets panicle^{-1}	Panicle weight (g)	Grain filling (%)	1000-grain weight (g)	Grain yield (g plant^{-1})
Variety (V)						
IR64	6.1^{d}	53.3^{d}	1.59^{b}	59.7^{d}	22.8^{a}	41.2^{d}
IR64 *Sub1*	14.3^{b}	81.3^{b}	2.05^{a}	80.3^{b}	23.3^{a}	56.1^{b}
Swarna	8.4^{c}	65.3^{c}	1.72^{b}	64.5^{c}	19.2^{b}	49.5^{c}
Swarna *Sub1*	16.8^{a}	93.7^{a}	2.12^{a}	82.5^{a}	19.5^{b}	68.4^{a}
$LSD_{P=0.05}$	2.04	4.15	0.242	2.16	1.08	3.07
Nutrient application (N)						
N_B	12.4^{cd}	76.7^{b}	1.96^{a}	75.2^{c}	21.5^{a}	54.8^{c}
N_S	14.2^{bc}	81.6^{b}	1.99^{a}	77.8^{bc}	21.6^{a}	61.1^{b}
Si_B	10.1^{de}	66.5^{c}	1.75^{ab}	66.8^{de}	20.7^{a}	49.6^{de}
Si_S	5.6^{f}	51.3^{e}	1.49^{b}	58.3^{f}	20.3^{a}	28.6^{f}
N_B x Si_B	15.9^{ab}	92.5^{a}	2.09^{a}	79.5^{b}	21.7^{a}	67.6^{a}
N_S x Si_S	8.1^{ef}	66.2^{c}	1.87^{a}	68.9^{d}	21.1^{a}	51.4^{cd}
N_B x Si_S	7.3^{ef}	57.1^{d}	1.63^{b}	64.7^{e}	20.9^{a}	48.5^{e}
N_S x Si_B	18.1^{a}	95.6^{a}	2.15^{a}	83.1^{a}	21.8^{a}	70.2^{a}
$LSD_{P=0.05}$	3.11	5.62	0.286	3.09	n s	4.3^{4}
$LSD_{P=0.05}$ V x N	5.68	11.28	0.842	6.23	n s	8.56

Values within the column showing the same letter are not significantly different at $P < 0.05$

increased the content but Si spray decreased the content of nutrients (Fig. 30).

It can be accomplished that application of basal Si and post-flood N resulted in synergistic interaction with improved phenology, photosynthetic rate, survival and yield attributes, also resulted in better leaf emergence, productive tillers, and higher anti-oxidant activities led to higher productivity.

9. Role of seedling age in submergence tolerance of rice

Germinating seeds, established seedlings, and even mature plants may be exposed to flooding. In rice, the reproductive stage is the most sensitive to complete submergence, followed by the seedling and the maximum tillering stages (Reddy and Mittra, 1985). When flooding occurs during the seedling

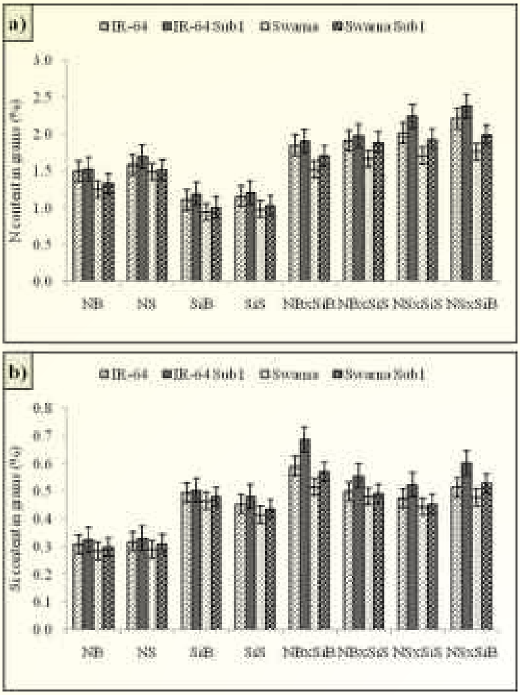


Fig. 30 Nitrogen and silica content of IR64, IR64 *Sub1*, Swarna and Swarna *Sub1* as influenced by application time and method of N and Si interaction (vertical bars in each column represents standard error).

stage, increasing the water depth inhibits the production of basal tillers and reduces tiller numbers, thereby decreasing eventual grain yield. The inhibitory effect of flooding up to 40 cm water depth 20–40 days after seeding is more pronounced in seedlings of improved rice cultivars with high tillering ability than those of traditional cultivars with low tillering ability (Kupkanchanakul, 1980). The correct age of seedlings used for transplanting is of primary importance for uniform stand and seedling establishment, as half of the success of rice cultivation depends on the seedling (Padalia, 1980). The technical know-how on rice seedlings (morphology and physiology) plays a pivotal role in transplanting due to the pronounced effect of temperature and water depths at that time. Younger seedlings lead to mortality of plants under submergence and older seedlings, which have greater submergence tolerance, also contain more carbohydrate (Chaturvedi *et al*, 1995). Late transplanting of older and taller seedlings may be another promising option for mitigating submergence damage, although too old seedlings are less productive. Damage from submergence is most likely when rice plants are small, and the damage seems higher if plant nutrition is unbalanced. Therefore, improving seedling health in the nursery through balanced nutrient management may lead to better crop establishment (Sarkar *et al*, 2006; Sarangi *et al*, 2015). An experiment was designed to study the effect of seedling age on submergence tolerance of *Sub1* and non-*Sub1* cultivars of rice in response to application of nitrogen, with the hypothesis that increasing seedling age might lead to higher survival and submergence tolerance.

The experiment was conducted during 2014–2015 under natural conditions. IR64 *Sub1*, Swarna *Sub1*, and their recurrent parents, that is, IR64 and Swarna, were the test varieties in the experiment. IR64 *Sub1* and Swarna *Sub1* are the flood-tolerant versions of the popular varieties IR64 and Swarna (MTU 7029). IR64 *Sub1* was released in 2013-14 and Swarna *Sub1* in 2009 in India. These varieties are almost identical to their counterparts (IR64 and Swarna) in terms of grain yield and grain quality, but they have an added advantage—they can survive full submergence for more than two weeks. Uniformly sprouted seeds of these varieties were sown (3–4 seeds in each pot) in pots (height 22 cm x diameter 25 cm) containing

10 kg of farm soils. To produce seedlings of various ages, that is, 15, 20, 25, 30, 35, and 40 d old, sowing was done at five days interval up to 40 d seedlings. The fertilization treatments were comprised no-N application (No-N), application of basal N (NB), post-flood N application (PF-N), and application of both basal and post-flood-N (NB + PF-N). 80 mg urea, 114 mg SSP, and 30 mg MOP were applied to each pot as per the treatments as N, P, and K sources, respectively. In NB + PF-N treatment, N was applied in three equal splits, basal, post-flood (after desubmergence), and panicle emergence. The basal dose was applied five days after seeding, and the second split, which must be applied at maximum tillering, was applied after 48 h of desubmergence. Post-flood N was applied after 48 h of desubmergence as urea foliar spray as per the treatments. In the PF-N treatment, no basal N (the first dose of N, which needs to be applied as basal) was skipped and nitrogen was applied in three equal splits, at post-flood (after desubmergence), maximum tillering, and panicle emergence. In the NB treatment, N was applied in three equal splits at basal, maximum tillering, and panicle emergence stages, and no post-flood N was applied. The plants were submerged in a concrete tank, and the water depth was maintained at 30 cm above the top of the plant canopy for 10 d.

9.1 Gain in plant height

There was a significant difference between plant height recorded prior to submergence and immediately after submergence (Table 19). Plant height before submergence was mainly because of varietal characters and age of the plant, but with the progression of submergence gain in plant height was observed. Seedling age and nutrient application significantly affected the increase in plant height or shoot elongation. Younger seedling, that is, 15 and 20 d old, resulted in high elongation as compared to older seedlings and the effect was prominent in IR64 and Swarna (Table 19). The tolerant *Sub1* introgression lines elongated at significantly slower rates compared to their recurrent parents. On an average, non-*Sub1* cultivars elongated 44.6% higher as compared to submergence tolerant cultivars. As far as seedling age is concerned, 15 d old seedlings elongated around 200% more as compared to 40 d old seedlings. Basal N application resulted in higher elongation of younger seedlings, whereas in older seedlings this impact was not visible.

9.2 Plant Survival

Survival decreased substantially following submergence, when plants were submerged for 10 d in turbid water. Survival percentage increased with the increase in age of seedlings. Among the varieties, Swarna *Sub1* had the highest plant survival followed by IR64 *Sub1* (Table 19). In in-tolerant cultivars, 15 and 20 ds old seedlings did not survive and survival was also lower among 25 and 30 d old seedlings. Across the cultivars, plant survival of 40 days old seedlings was 50-fold higher than 15 d old seedlings. Apart from seedling age, N application also significantly influenced plant survival. Post flood N application contributed positively towards higher survival; basal N along with post-flood N showed a positive response on older seedlings but the response was quite negative on younger age seedlings. Plant

Table 19 Gain in plant height, survival and chlorophyll content of rice cultivars as influenced by seedling age and nitrogen application

	Plant height (cm)		Elongation (%)	Plant survival (%)	Chlorophyll concentration (mg g^{-1} FW)		
	BS	2 h AS			BS	10 d AS	% change
Cultivars							
IR64	25.6^{a}	41.9^{a}	75.7^{a}	31.8^{b}	3.65^{b}	1.23^{d}	112.5^{b}
IR64 *Sub1*	25.8^{a}	34.8^{b}	43.9^{c}	66.1^{a}	3.68^{b}	2.71^{b}	37.6^{d}
Swarna	24.3^{a}	42.7^{a}	63.7^{b}	33.5^{b}	4.40^{a}	1.85^{c}	137.8^{a}
Swarna *Sub1*	24.6^{a}	35.4^{b}	34.9^{d}	67.7^{a}	4.42^{a}	3.08^{a}	51.2^{c}
$LSD_{P=0.05}$	ns	2.48	4.15	3.73	0.661	0.352	5.14
Seedling age (Days)							
15	13.9^{f}	28.2^{e}	97.8^{a}	11.3^{e}	3.74^{c}	1.31^{e}	153.1^{a}
20	17.6^{e}	33.1^{d}	71.3^{b}	22.1^{d}	3.85^{c}	1.82^{d}	113.8^{b}
25	22.5^{d}	35.9^{c}	54.4^{c}	47.2^{c}	3.92^{bc}	2.28^{cd}	78.6^{c}
30	28.3^{c}	42.4^{b}	42.7^{d}	65.8^{b}	4.11^{ab}	2.45^{bc}	62.5^{d}
35	32.3^{b}	44.1^{b}	32.4^{e}	74.9^{a}	4.26^{a}	2.69^{ab}	55.3^{e}
40	35.7^{a}	48.2^{a}	28.7^{e}	77.5^{a}	4.35^{a}	2.77^{a}	45.2^{f}
$LSD_{P=0.05}$	2.61	2.14	5.56	4.08	0.185	0.447	6.91
Nitrogen application							
No N	23.7^{b}	35.7^{b}	51.4^{bc}	21.1^{d}	3.22^{b}	1.43^{d}	109.9^{b}
NB	26.2^{a}	42.1^{a}	61.5^{a}	33.2^{c}	4.78^{a}	1.86^{c}	122.4^{a}
PF-N	23.8^{b}	35.2^{b}	48.5^{c}	78.2^{a}	3.27^{b}	2.27^{b}	48.2^{d}
NB+PF-N	26.7^{a}	41.6^{a}	56.6^{ab}	67.1^{b}	4.86^{a}	3.33^{a}	58.7^{c}
$LSD_{P=0.05}$	1.26	2.33	5.11	4.84	0.921	0.414	6.75

Values having common letters are not significantly different at *P=0.05*; BS- Before submergence; AS- After desubmergence; No-N: no nitrogen application, NB: application of basal nitrogen, PF-N: post-flood nitrogen application and; NB+PF-N: application of both basal and post-flood-nitrogen survival was 58.1 and 53.8% higher with the application of post flood N with basal N and without basal N, respectively, over no-N application, irrespective of the cultivar and age of seedlings (Table 19).

9.3 Chlorophyll content and photosynthesis

The pre-submergence chlorophyll level was slightly higher with older seedlings and N application treatment; older seedlings, that is, 35 and 40 d old, recorded higher chlorophyll content (Table 19). Due to submergence, chlorophyll content decreased in all the varieties but reduction was lower in tolerant cultivars and regain after desubmergence was also significantly higher resulting in lower percentage change in chlorophyll. Younger seedlings of IR64 and Swarna did not survive the submergence; therefore, the percentage change in chlorophyll is expressed as zero. Similar to survival, less chlorosis and higher regain in chlorophyll were observed in older seedlings (around 100 fold higher compared to 15 d old seedlings) (Table 19). Post-flood N application resulted in the highest regain in chlorophyll (133% higher over no-N application); results were more pronounced if combined with *Sub1* cultivars and older seedlings.

Submergence-induced stomatal closure significantly decreased the photosynthetic rate and stomatal conductance, which also varied with seedling age (Fig. 31). Among the varieties, photosynthesis was significantly higher in

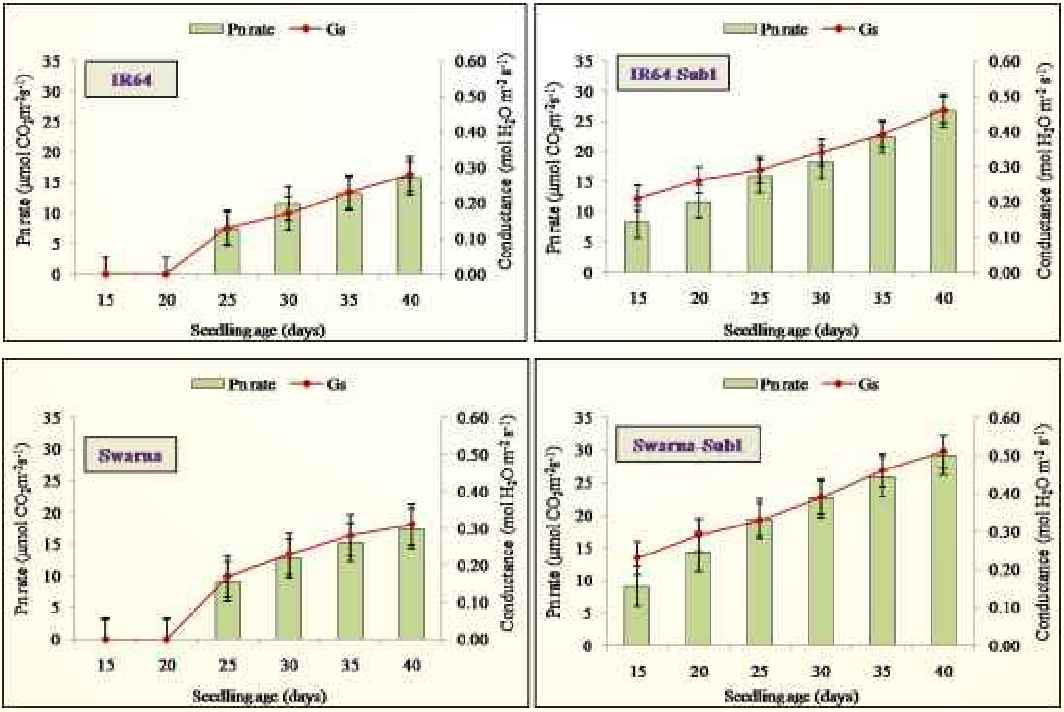


Fig. 31 Photosynthetic rate (µmol CO$_2$m^{-2}s^{-1}) and stomatal conductance (Gs) (mol H$_2$O m^{-2} s^{-1}) of IR64, IR64 *Sub1*, Swarna and Swarna *Sub1* as influenced by seedling age (vertical bars in each column and line represents standard error); columns on primary axis represents Photosynthetic (Pn) rate and lines on secondary axis represents stomatal conductance.

tolerant cultivars, but between *Sub1* no significant difference was observed. Forty days old seedlings recorded around 5.5, 1.2, 4.7, and 1.0 times higher photosynthesis in IR64, IR64 *Sub1*, Swarna, and Swarna *Sub1* over younger seedlings, respectively.

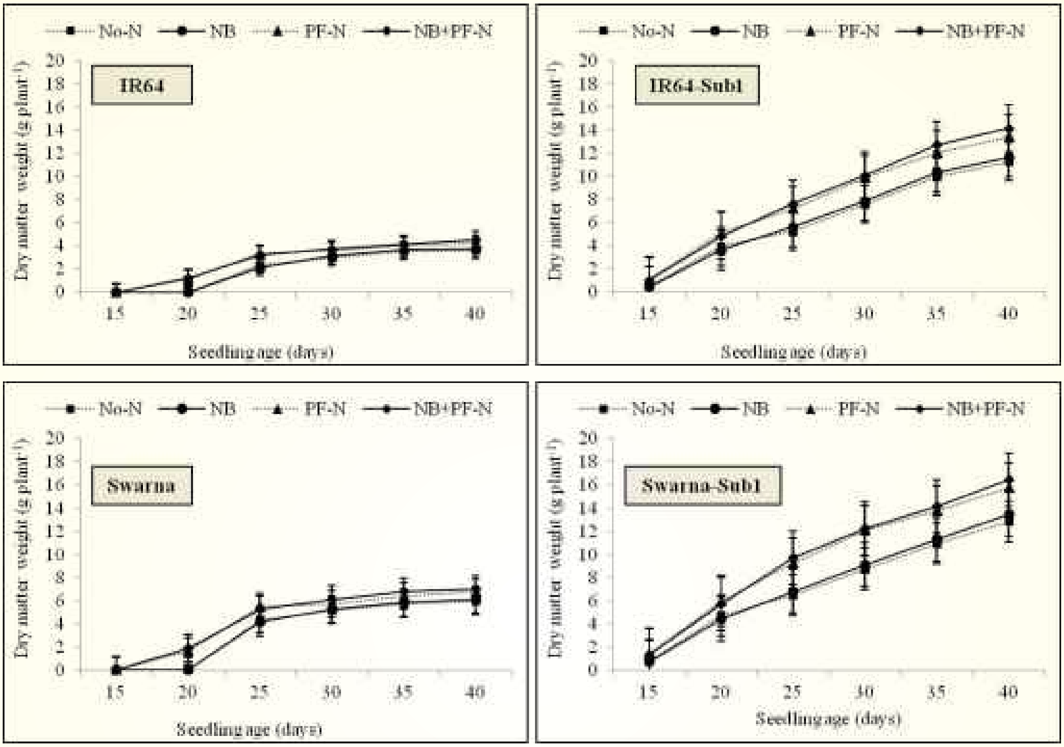


Fig. 32 Dry matter weight of plant shoots (g plant^{-1}) of IR64, IR64 *Sub1*, Swarna and Swarna *Sub1* as influenced by seedling age under different nitrogen application schedules (vertical bars in each line represents standard error)

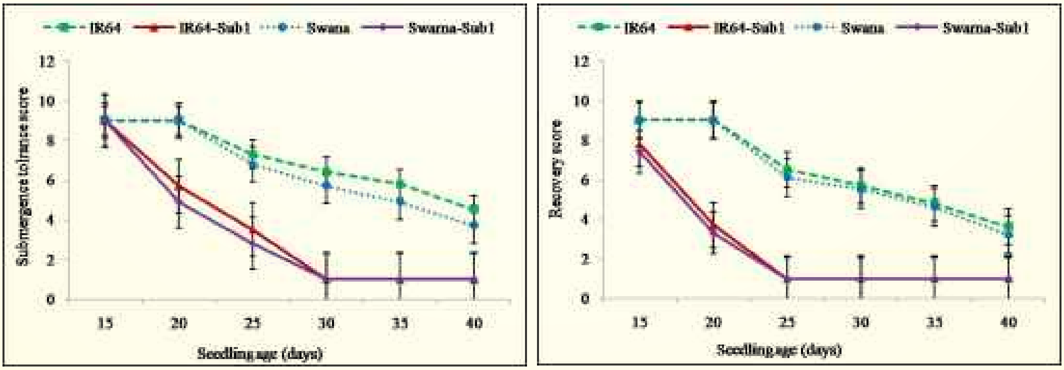


Fig. 33 Submergence tolerance and recovery score of IR64, IR64 *Sub1*, Swarna and Swarna *Sub1* as influenced by seedling age (vertical bars in each line represents standard error).

9.4 Allometric characteristics and tolerance score

Irrespective of the varieties and nutrients, older seedlings produced high dry matter during the recovery period after submergence (Fig. 32). Although the difference in dry matter weight was quite significant among the varieties, at all the seedling ages, the dry matter produced was significantly higher in Swarna *Sub1* followed by IR64 *Sub1* over their recurrent parents. Post-flood N application contributed to better recovery of rice seedlings resulting in higher dry matter production especially in older seedlings. Dry matter weight of rice seedlings was around 28.6, 33.2, 25.5, and 32.3% higher in IR64, IR64 *Sub1*, Swarna, and Swarna *Sub1* with post-flood N application, respectively (Fig. 32).

A similar trend was also observed for leaf and root growth, in terms of leaf area, root length, leaf dry weight, and root dry weight (Table 20). All these parameters were increased with increasing seedling age and were significantly higher in *Sub1* cultivars. The ability to maintain greenness in leaves and stem strength was monitored using leaf senescence and lodging score; the lower the score, the higher the tolerance of the plants. Older seedlings especially of tolerant cultivars showed the lowest score of lodging and senescence. Post-flood N application led to 2.4, 4.7, and 6.2 times higher leaf area, leaf dry weight, and root dry weight, respectively, over no-N application. Hypoxic condition of submergence also significantly affected the numbers of adventitious roots and the length of roots. Among the treatments, 40 d old seedlings of Swarna *Sub1* varieties had the highest and longest roots under the supply of post-flood N (Table 20).

Submergence tolerance and recovery scores were worked out based on the above allometric characters, irrespective of the treatments; submergence tolerance and recovery increased significantly with the increase in seedling age as indicated by their lower score (Fig. 33). As the name indicates, Swarna *Sub1* and IR64 *Sub1* had the highest level of tolerance at any seedling age.

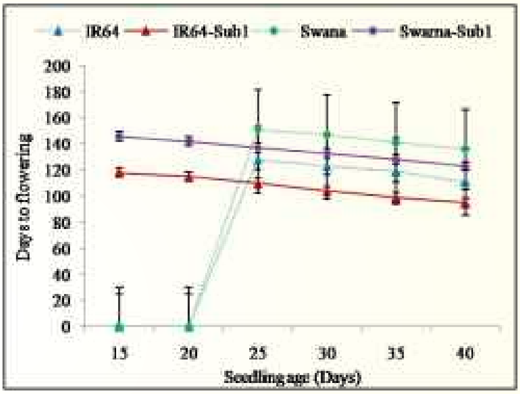


Fig. 34 Days taken to flowering in IR64, IR64 *Sub1*, Swarna and Swarna *Sub1* as influenced by seedling age (vertical bars in each line represents standard error)

9.5 Yield attributes and yield

Submergence in general delayed the flowering of rice genotypes, and the maximum effect was observed in the susceptible genotypes and younger seedlings. A delay of around 5–7 d and 11–13 d was observed in tolerant and susceptible genotypes, respectively (Fig. 34). This delay directly affected the yield attributes especially grain filling which was evident in reduced yield. Higher survival of *Sub1* cultivars reflected in better yield attributing characters and yield (Table 21).

Table 20 Allometric parameters of rice cultivars, recorded 10d after desubmergence (AS) as influenced by seedling age and nitrogen application

	Leaf senescence	Lodging	LA (cm^2 plant^{-1})	RDW (g)	SDW (g)	LDW (g)	Adventitious roots (no.)	Length of the longest root (cm)
Cultivars								
IR64	5.0^{a}	5.6^{a}	1.82^{d}	0.76^{c}	4.06^{c}	2.74^{b}	194^{d}	17.6^{b}
IR64 *Sub1*	2.6^{b}	3.1^{b}	2.91^{b}	1.83^{b}	13.49^{b}	8.73^{a}	302^{b}	23.1^{a}
Swarna	4.8^{a}	5.7^{a}	2.45^{c}	0.93^{c}	5.66^{c}	3.57^{b}	231^{c}	19.3^{b}
Swarna *Sub1*	1.9^{b}	2.3^{b}	3.67^{a}	2.09^{a}	19.73^{a}	9.57^{a}	342^{a}	24.5^{a}
LSD$_{P=0.05}$	1.23	1.35	0.451	0.114	2.782	3.095	13.7	3.12
Seedling age (days)								
15	5.0^{a}	6.0^{a}	1.19^{f}	0.37^{c}	5.57^{e}	2.76^{d}	176^{f}	14.9^{f}
20	4.9^{a}	5.8^{a}	1.67^{e}	0.51^{c}	5.99^{e}	3.16^{d}	193^{e}	16.4^{e}
25	4.3^{a}	5.2^{a}	2.38^{d}	1.22^{b}	7.62^{d}	4.82^{c}	243^{d}	20.5^{d}
30	3.1^{b}	3.5^{b}	3.27^{c}	1.63^{b}	11.76^{c}	7.36^{b}	291^{c}	22.7^{c}
35	2.5^{bc}	2.7^{b}	3.76^{b}	2.11^{a}	15.85^{b}	9.19^{a}	342^{b}	25.4^{b}
40	1.8^{c}	2.1^{b}	3.96^{a}	2.26^{a}	17.17^{a}	9.82^{a}	358^{a}	26.8^{a}
LSD$_{P=0.05}$	1.12	1.76	0.183	0.447	1.491	1.532	16.8	1.61
Nitrogen application								
No N	3.1^{b}	3.6^{b}	1.02^{d}	0.62^{c}	4.82^{d}	3.53^{d}	157^{d}	15.3^{c}
NB	4.3^{a}	4.7^{a}	2.73^{c}	1.29^{b}	8.91^{c}	4.77^{c}	244^{c}	21.0^{b}
PF-N	2.9^{b}	3.4^{b}	3.13^{b}	1.81^{a}	12.47^{b}	6.84^{b}	299^{b}	22.7^{b}
NB+PF-N	4.1^{a}	4.5^{a}	3.81^{a}	2.02^{a}	16.47^{a}	9.71^{a}	370^{a}	25.9^{a}
LSD$_{P=0.05}$	1.01	1.28	0.394	0.592	3.334	1.258	15.2	2.27

LA-leaf area; RDW-Root dry weight; SDW-Shoot dry weight; LDW-Leaf dry weight; Values are means of measurements made after 10 days of complete submergence ± standard deviation; values having common letters are not significantly different at P=0.0; No-N: no nitrogen application, NB: application of basal nitrogen, PF-N: post-flood nitrogen application and; NB+PF-N: application of both basal and post-flood-nitrogen

Table 21 Yield attributes of rice cultivars as influenced by seedling age and nitrogen application

	No. of panicles plant^{-1}	Panicle length (cm)	Seed setting rate	Grains panicle^{-1}	Spikelets panicle^{-1}	Panicle wt. (g)	1000-SW (g)
Cultivars							
IR64	5.4^{c}	19.3^{a}	56.5^{b}	77.1^{d}	60.6^{c}	1.51^{c}	22.8^{a}
IR64 *Sub1*	12.3^{a}	20.5^{a}	81.9^{a}	119.3^{b}	79.9^{b}	1.92^{ab}	23.4^{a}
Swarna	7.6^{b}	20.3^{a}	61.7^{b}	92.4^{c}	64.5^{c}	1.77^{b}	19.1^{b}
Swarna *Sub1*	16.2^{a}	21.6^{a}	83.5^{a}	139.7^{a}	84.6^{a}	2.12^{a}	19.6^{b}
$\text{LSD}_{P=0.05}$	4.28	ns	10.75	9.16	4.11	0.223	2.46
Seedling age (days)							
15	1.8^{f}	18.9^{d}	44.5^{e}	72.2^{f}	49.8^{e}	1.24^{e}	20.5^{d}
20	3.5^{e}	19.3^{cd}	51.9^{d}	82.3^{e}	57.2^{d}	1.51^{d}	20.6^{d}
25	7.4^{d}	19.8^{bc}	69.4^{c}	98.5^{d}	68.7^{c}	1.79^{c}	20.9^{cd}
30	13.5^{c}	20.6^{b}	81.9^{b}	112.9^{c}	75.8^{b}	1.97^{b}	21.3^{bc}
35	16.9^{b}	21.7^{a}	87.8^{a}	131.3^{b}	86.9^{a}	2.14^{ab}	21.8^{ab}
40	18.5^{a}	21.9^{a}	89.9^{a}	145.9^{a}	89.5^{a}	2.23^{a}	22.4^{a}
$\text{LSD}_{P=0.05}$	2.14	0.88	7.06	9.57	7.49	0.131	0.72
Nitrogen application							
No N	4.1^{d}	19.1^{c}	51.4^{d}	77.2^{d}	53.3^{d}	1.47^{c}	20.4^{d}
NB	8.9^{c}	20.2^{bc}	69.8^{c}	101.9^{c}	66.7^{c}	1.79^{b}	20.9^{cd}
PF-N	12.5^{b}	20.8^{a}	77.8^{b}	114.8^{b}	75.8^{b}	1.91^{a}	21.6^{bc}
NB+PF-N	15.6^{a}	21.3^{a}	84.6^{a}	134.8^{a}	89.3^{a}	2.07^{a}	22.2^{a}
$\text{LSD}_{P=0.05}$	3.83	0.62	6.94	11.35	7.74	0.165	0.68

Values having common letters are not significantly different at *P=0.05*; No-N: no nitrogen application, NB: application of basal nitrogen, PF-N: post-flood nitrogen application and; NB+PF-N: application of both basal and post-flood-nitrogen

Seedling age also had marked variations among yield attributes, that is, number of panicles plant^{-1}, seed setting, and grains per panicles were around 9, 1.1, and 1.9 times higher in 40 d old seedlings compared to younger seedlings, that is, 15 d old. The role of N was similar as in survival of rice seedlings, that is, post-flood N contributed substantially to the higher yield attributing characters (Table 21).

In the susceptible cultivars, 15 and 20 d old seedlings could not contribute to yield and the yield of older seedlings was also reduced due to submergence. Older seedlings (40 d old) of tolerant cultivars produced the higher

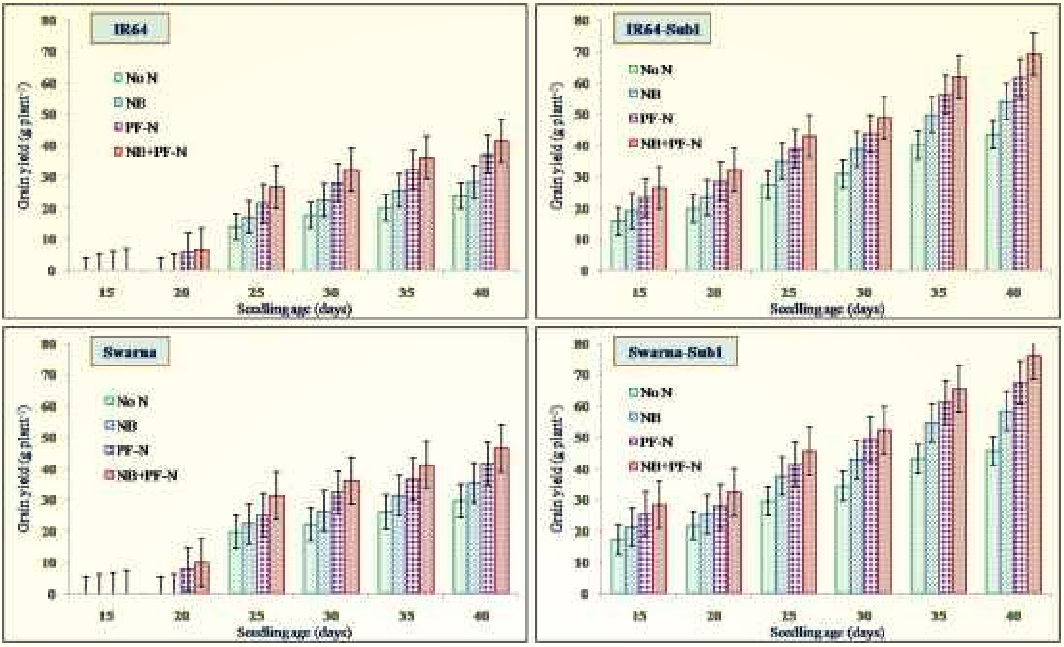


Fig. 35 Grain yield (g plant^{-1}) of IR64, IR64 *Sub1*, Swarna and Swarna *Sub1* as influenced by seedling age and nitrogen application (vertical bars in each line represents standard error).

yield which was around 169 and 119% higher in IR64 *Sub1*, 167 and 129% higher in Swarna *Sub1* over 15 and 20 d old seedlings. Nitrogen as a primary nutrient significantly contributed to the higher grain yield, especially when applied post-flood. As an interaction effect, it can be said that 40 d old seedlings of Swarna *Sub1* resulted in the highest grain yield with the application of post-flood and basal N (Fig. 35).

Older seedlings were taller, sturdier, and healthier; as a result, they could cope better with deeper water prevalent in coastal rainfed, low-lying areas. Older seedlings had higher dry biomass and carbohydrate storage at transplanting. Consequently, they were more tolerant of submergence and waterlogging in the field (Bhowmick *et al*, 2014). Seedlings of 15–25 ds did not perform well under submergence shallow water, whereas older seedlings (35 and 40 d) performed acceptably well in submerged conditions; post-flood N application further improved the tolerance of all the cultivars with a more prominent effect in *Sub1s*. It is, therefore, concluded that damage from submergence is most likely when the rice plant is small, and the damage seems higher if proper nutrient application was not followed. Survival, all the growth variables in shoots and roots, enzymatic activities, and carbohydrate status were reduced with submergence, but the magnitude of reduction was greater in younger seedlings.

10. On-farm validation of developed technology

The on-farm trial was conducted during 2014-15 to validate the results of our study (Gautam *et al*, 2014, 2015) i.e. basal P and post-flood N improves submergence tolerance and yield of rice, which was conducted under controlled conditions. For the experiment farmers' field were selected at Salipur block of Cuttack district (Table 22), where in the month of July-August, flash flood occurs completely submerging the plants for 10-15 d, because the field is inundated with Mahanadi river water, which adversely affect crop establishment and productivity. The improved variety i.e. Swarna *Sub1* recently developed for coastal lowland areas were released in 2009, were used in the experiment along with its recurrent parent Swarna. The flash flood experienced during seedling stage for 11 days (main field). Each on-farm experiment was laid as a randomized block design (RBD) with three treatments—T$_1$: farmer's field practice (FFP); T$_2$: basal P and K + urea broadcasting; and T$_3$: Basal P and K + urea foliar spray (Table 23). Field was prepared with a tractor drawn plough followed by puddling and laddering for transplanting of 30 d old rice seedlings. Seedlings were transplanted at a spacing of 20 x 15 cm and nutrient was applied as per the treatments.

10.1 Shoot elongation and plant survival

In the on-farm trial, nutrient management practices reduced the elongation and improved the plant survival of both the cultivars and locations with greater effects in Swarna *Sub1* (Table 24). Application of basal P and K+ post-flood N improved the survival by 35.5 and 40.1% in Swarna and Swarna *Sub1* over farmers' field practices (FFP), respectively.

10.2 Yield attributes and yield

Nutrient management options of rice seedlings had a significant impact on yield attributes except 1000-grain weight. Number of tillers, panicles, filled grains and panicle weight were higher in Swarna *Sub1* as

Table 22 Details of locations, varieties, and plot sizes of on-station and on-farm trials conducted during 2014-15 in the Cuttack, Odisha, India

Location	Longitude	Latitude	Variety	Plot size	Initial soil status			
					pH	N	P	K
						Kg ha^{-1}		
On-station trial								
ICAR-NRRI, Cuttack	85° 56$^{/}$ 4.10° E	20° 27$^{/}$ 13.46°N	IR64, Swarna, IR64 *Sub1*, Swarna *Sub1*	Pot experiment	6.4	212.5	10.8	118.7
On-Farm trial								
F1: Atoda, Salipur	86° 7$^{/}$ 9.11° E	20° 29$^{/}$ 3.66°N	Swarna, Swarna *Sub1*	300m^2	5.9	96	11.2	106.9
F2: Bahugram, Salipur	86° 7$^{/}$ 9.12° E	20° 29$^{/}$ 3.68°N	Swarna, Swarna *Sub1*	300m^2	5.6	105	11.5	109.8

Table 23 Details of the nutrient treatments executed in on-farm trial conducted during 2014-15 in the Cuttack, Odisha, India

Symbols	Treatments	Details
T_1	Farmers' Field practices	Dose: 40:20:20, NPK, P and K applied as basal and N applied at PI stage only; it varies from field to field and farmer to farmer
T_2	Basal P and K+ Urea broadcasting	P and K was applied @ 40 kg ha^{-1} as basal at the time of transplanting and N (100 kg ha^{-1}) in four equal splits, 25% as basal and remaining N in three equal splits (25%), one split as post-flood, whenever flood occurs; and at maximum tillering and panicle initiation stage. Urea broadcasting after complete receding of flood water.
T_3	Basal P and K + Urea foliar spray	P and K was applied @ 40 kg ha^{-1} as basal at the time of transplanting and N (85 kg ha^{-1}), 25 kg as basal and remaining N one as post-flood (Spray of urea (2% solution with knapsack sprayer) after desubmergence whenever flood occurs; and 25 kg each at maximum tillering and panicle initiation stage. Urea foliar spray if flood water not completely recedes from field.

Typical flash flood situation experienced in flood prone areas

Slow recession of flood water, plants come out from water, yellow leaves appears

Flood water receded and this is the time to apply N as foliar spray of urea of broadcasting for quick regeneration of plants

Post-flood N application led to better survival, quick recovery and higher yield of rice after facing submergence.

Recovered rice crop after application of post-flood nitrogen

Post-flood N application as foliar spray of urea led to better survival and quick recovery of rice

Healthy crop at maturity stage from regenerated seedlings

Adjustment in time and method of N application seems to be very effective low cost technology within easy reach of farmers for harvesting rich profits from the otherwise low productive floodprone areas

Fig. 36 Post-flood N and basal P application leads to higher recovery and productivity of rice

compared to Swarna. Apart from that, application of basal P and K^{+} post-flood N resulted in 38.1, 44.0, 10.2 and 6.6% higher number of tillers, panicles, filled grains and panicle weight, respectively as compared to FFP (Table 25). Similar to yield attributes and plant survival, grain yield was also affected by cultivars grown and nutrient management options (Fig. 37). Regardless of the nutrient management options, Swarna *Sub1* recorded 50.3 and 51.5 % higher grain yield over Swarna at F1 and F2 locations, respectively.

Farmers' field practice obtained the lowest grain yield at both the locations, which was 65.7 and 37.9% lower in Swarna and Swarna *Sub1* over basal P, K and post-flood N management. Further, among post-flood N application, urea foliar spray proved its superiority in terms of higher grain yield (around 2%) over N supply through urea broadcasting.

10.3 Economics

Both productivity and profitability in terms

Table 24 Shoot elongation and plant survival of Swarna and Swarna *Sub1* influenced by nutrient management practices at farmers' field experiment

Treatments	Shoot elongation (%)				Plant survival (%)			
	Swarna		Swarna *Sub1*		Swarna		Swarna *Sub1*	
	F1	F2	F1	F2	F1	F2	F1	F2
Farmers' Field practices	79.2	74.6	48.8	44.6	38.5	39.6	62.8	65.3
Basal P and K+ Urea broadcasting	60.4	57.3	31.2	30.5	51.3	52.1	87.2	87.9
Basal P and K + Urea foliar spray	60.2	57.6	30.9	29.7	53.4	54.9	91.1	92.6

Table 25 Yield attributes of Swarna and Swarna *Sub1* influenced by nutrient management practices at farmers' field experiment

			Effective tillers m^{-2}	Panicles per hill	Filled grains per panicle	Spikelet fertility (%)	Panicle weight (g)	1000 grain weight (g)
F1	Swarna	T_1	215	6.2	61.4	67.2	1.58	19.1
		T_2	299	8.9	69.8	70.6	1.71	19.2
		T_3	317	9.1	72.6	71.5	1.75	19.2
	Swarna *Sub1*	T_1	288	8.5	119.2	85.5	2.01	19.4
		T_2	389	12.1	126.9	88.1	2.08	19.5
		T_3	407	12.4	128.1	88.2	2.11	19.5
F2	Swarna	T_1	229	6.5	62.5	69.1	1.61	19.2
		T_2	305	9.1	71.7	72.6	1.73	19.3
		T_3	326	9.6	74.2	73.3	1.78	19.3
	Swarna *Sub1*	T_1	302	8.8	121.1	86.3	2.04	19.6
		T_2	401	12.5	128.5	89.5	2.12	19.7
		T_3	412	12.9	130.3	89.6	2.14	19.7

of annual net return and B: C ratio must be considered for choosing suitable cultivars and their management options especially under environment prone to abiotic stress. The cost of production was lower when FFP was followed but the net returns and B: C ratio were higher when basal P, K and post-flood N management options were adopted because of higher grain yield under the treatment (Table 26). Net returns were around 355 USD higher in basal P, K and post-flood N management over farmers' field practices (FFP), irrespective of the cultivar and locations. Further, net returns were increased by 4.4% and 2.9% in Swarna and Swarna *Sub1* when urea foliar spray was applied over N supply through urea broadcasting as post-flood.

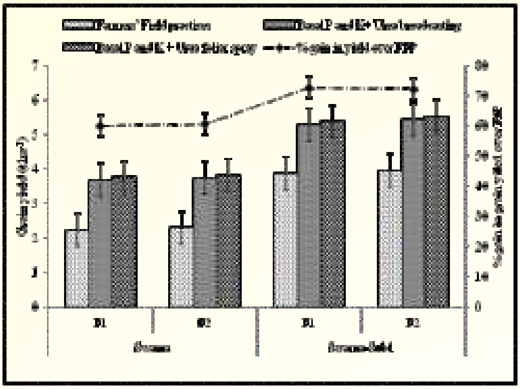


Fig. 37 Effect of fertilizer application on grain yield (t ha^{-1}) of Swarna and Swarna *Sub1* in on-farm trial conducted at farmers' field (vertical bars in each column and line represents standard error). Column bars on primary axis represents grain yield and lines on secondary axis represents % gain in grain yield over farmers' field practices.

11. Anaerobic germination (AG)

Rice is unique in being capable of growing well in waterlogged and submerged soils because of its well-developed aerenchyma system that facilitates aeration of the roots and the rhizosphere, thus alleviating most of the stresses experienced under low oxygen (Setter *et al*, 1997; Jackson and Ram, 2003). But it is extremely intolerant to anaerobic conditions during germination and early growth of the embryo (Yamauchi *et al*, 1993; Ismail *et al*, 2009; Angaji *et al*, 2010). Rice seeds can germinate and, to some degree extend their coleoptiles under hypoxic and even anoxic conditions, but fail to develop roots and leaves (Taylor, 1942; Ella and Setter, 1999), because of its limited ability to

Table 26 Economics of Swarna and Swarna *Sub1* influenced by nutrient management practices at farmers' field experiment

			Gross returns (USD ha^{-1})	Net returns (USD ha^{-1})	B:C ratio
F1	Swarna	T_1	599.9	171.2	0.40
		T_2	989.9	516.0	1.09
		T_3	1008.7	538.9	1.15
	Swarna *Sub1*	T_1	1041.0	612.4	1.43
		T_2	1420.3	946.4	2.00
		T_3	1444.5	974.7	2.07
F2	Swarna	T_1	616.0	187.4	0.44
		T_2	1006.0	532.2	1.12
		T_3	1024.9	555.0	1.18
	Swarna *Sub1*	T_1	1065.2	636.6	1.49
		T_2	1460.6	986.8	2.08
		T_3	1484.8	1015.0	2.16

mobilize and use energy reserves when oxygen is limiting (Ismail *et al*, 2009). Cell division is active during the first 48 hours of submergence, and that is the period when oxygen is mostly required (Atwell et al, 1982). Since cellular expansion consumes less energy than cell division, the latter is the main process governing elongation of the coleoptiles under anoxia.

Genotypes that are more tolerant of flooding during germination seem to have better capabilities for breaking starch into simple sugars, as demonstrated by faster depletion of starch in their germinating seeds compared with intolerant genotypes (Ismail *et al*, 2009). When flooding occurs just after direct seeding, tolerant genotypes germinate faster and their coleoptiles grow at a relatively faster rate to emerge from flooded soils. These genotypes are also capable of forming roots and leaves in shallow water depths (Ismail *et al*, 2009; Angaji *et al*, 2010).

Several major quantitative trait loci (QTLs) for tolerance of AG were identified from different landraces, and one of them, qAG-9-2 (AG1) was derived from Khao Hlan On, a landrace from Myanmar (Angaji *et al*, 2010). Near isogenic lines (NILs) containing AG1 in the background of IR64 were developed, two of them were IR93312-30-101-20-3-66-6 (IR64-AG131) and IR93312-30-101-20-13-64-21 (IR64-AG132). Subsequent studies confirmed that there was no yield penalty due to the introgression of this QTL in several genetic backgrounds, including IR64 (Toledo *et al*, 2015). Furthermore, the gene underlying AG1, OsTPP7, was identified through map-based cloning. This gene increases the sink strength in proliferating heterotrophic tissues by maintaining signaling for low sugar availability, thus enhancing starch mobilization to drive growth kinetics of the germinating embryo and elongating coleoptile, which consequently enhance AG tolerance (Kretzschmar *et al*, 2015).

The study was conducted using IR64-AG NILs (IR64-AG131 and IR64-AG132; tolerant to AG but intolerant to submergence), IR64 *Sub1* (tolerant to submergence; intolerant to AG), and IR64 (intolerant to submergence and AG) to evaluate the impact of AG tolerance conferred by the AG1 QTL in the IR64 background, and to compare the responses of these lines to selected agronomic practices desired to further augment expression of tolerance in famers fields. The study compared (i) germination and growth attributes of IR64-AG NILs with IR64 and IR64 Sub1 under controlled submergence; (ii) the use of different establishment methods across both tolerant and sensitive genotypes; and (iii) evaluated the effects of selected agronomic practices on the performance of AG tolerant and sensitive genotypes under flooded field condition.

Three experiments were conducted to evaluate the performance of IR64, IR64 *Sub1*, and two AG NILs [IR64-AG131 (IR 93312-30-101-20-3-66-6) and IR64-AG132 (IR93312-30-101-20-13-64-21)] under varying flooding stress; and the impact of crop and nutrient management practices on their yield and yield attributes.

11.1 Germination and growth of IR64-AG NILs under controlled flooding (Experiment I)

First experiment was conducted during the *kharif* (wet) season of 2013 under controlled conditions, with the objective to evaluate the germination and growth attributes of IR64-AG NILs, IR64 and IR64 Sub1. Hundred pre-soaked seeds of each cultivar were directly sown in plastic trays (37 x 35 x 25 cm) containing 10 kg of farm soil (Sandy clay loam, pH 6.3, EC-0.071 dS m^{-1}, available N, P and K were 52.5, 4.2 and 61.4 mg kg^{-1} soil, respectively). Fertilizers at 0.49 g urea, 1.14 g SSP, and 0.31 g MoP were applied to each tray as N, P, and K sources, respectively. The trays were watered immediately after seeding and a water head of 5 cm was maintained above soil surface for 0 (saturated condition), 5, 9, 13, 17 and 21 d based on treatments.

11.1.1. Germination and growth of shoots and roots

Germination started after 2 d in the saturated condition and intolerant genotypes under flooding, but after 3–4 d in the sensitive genotypes when soils were flooded. Maximum germination was reached in all genotypes after 5 d in the saturated condition and 8 d in all flooding

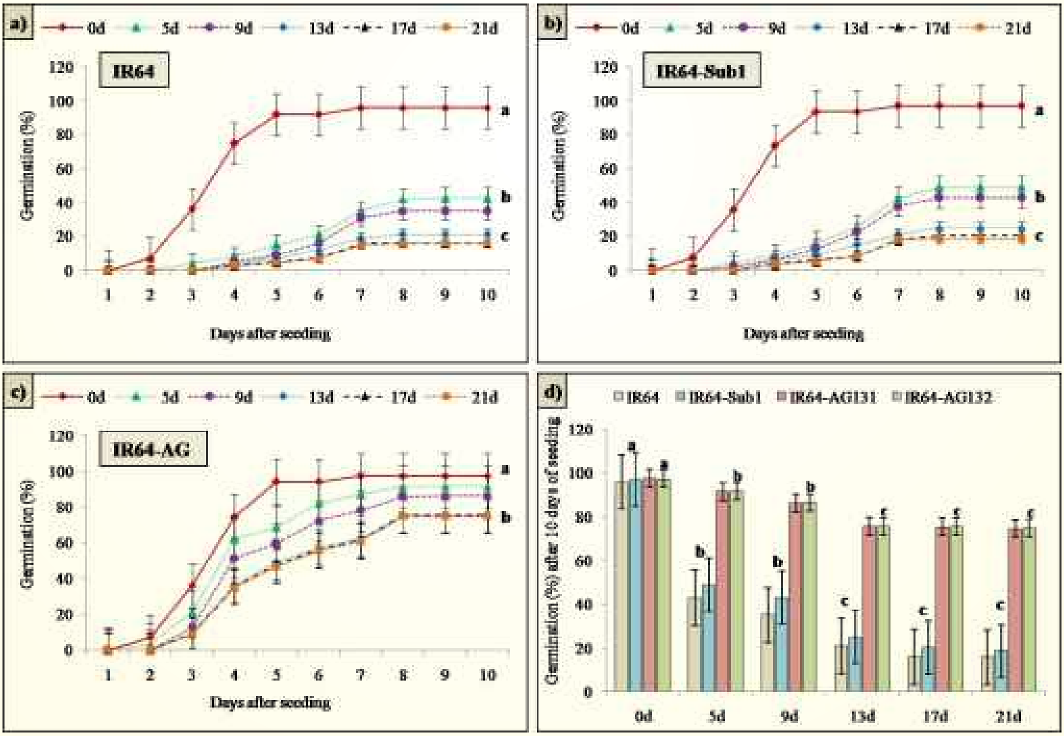


Fig. 38 Germination percentage during 10 d following seeding of a) IR64, b) IR64 *Sub1*, c) IR64-AG (mean of IR64-AG131 and IR64-132, as values were similar), after sowing and flooding for 0 to 21 d;, and d) germination % after 10 d of seeding in each treatment. Data are from experiment I conducted during the *kharif* (wet) season of 2013. Vertical bars in each line and column represent ±SE.

treatments (Fig. 38a–c), and was highest in the saturated condition and lowest following 21 d of flooding (Fig. 38d). The anaerobic germination tolerant lines showed higher germination during all flooding durations, which was 92% after 5 d flooding and 75% after flooding for more than10 d.

The lowest germination was observed in IR64 (17%) after 10 d of flooding. The duration of flooding also significantly influenced the germination percentage; in intolerant genotypes, 5 d flooding reduced germination by 50%, while 9 d flooding further reduced it to 18% and flooding for over 10 d caused complete suppression. Irrespective of the flooding duration, IR64–AG NILs (IR64-AG131 and IR64-AG132) had 121% and 98% higher germination than IR64 and IR64 *Sub1*, respectively. Lengths of shoots and roots were significantly influenced by flooding duration, with more effect on IR64 (Fig. 39).

In IR64-AG131 and IR64-AG132, shoot and root lengths were slightly reduced at 5 d, and moderately affected after 10 d of flooding. Shoot length of these tolerant genotypes decreased by 22.6, 31.8 and 42.8%; and root length by 21.2, 35.5 and 53.2%, respectively, following 5 d, 9 d and over 10 d flooding, compared with the saturated condition. IR64-AG132 had relatively longer root and shoot length than IR64-AG131. On average, shoots of IR64 and IR64 *Sub1* were respectively, 289% and 114% shorter than that of the saturated condition following flooding for over 13 d.

11.1.2. Starch degradation and soluble sugar utilization

Starch concentrations in germinating seeds of all cultivars followed similar trends in saturated condition conditions in seeds of all genotypes; with high concentrations in seeds before germination, then progressive and fast reduction during germination, until depleted at about 13 d after sowing.

Under flooded conditions however, starch depletion was relatively slower in all genotypes compared with the saturated condition; and it remained much faster in the tolerant IR64-AG NILs (Fig. 40). When flooded for 5 and 9 d,

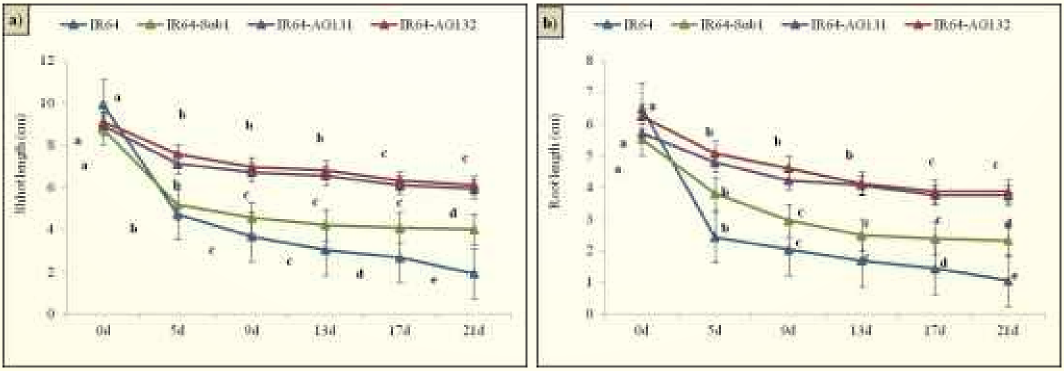


Fig. 39 Length (cm) of a) shoots and b) roots of IR64, IR64-*Sub1*, IR64-AG131 and IR64-AG132, following sowing and flooding for 0 to 21 d. Data are from experiment I conducted during the *kharif* season of 2013 at ICAR-NRRI Cuttack, Odisha. Vertical bars represents ± SE.

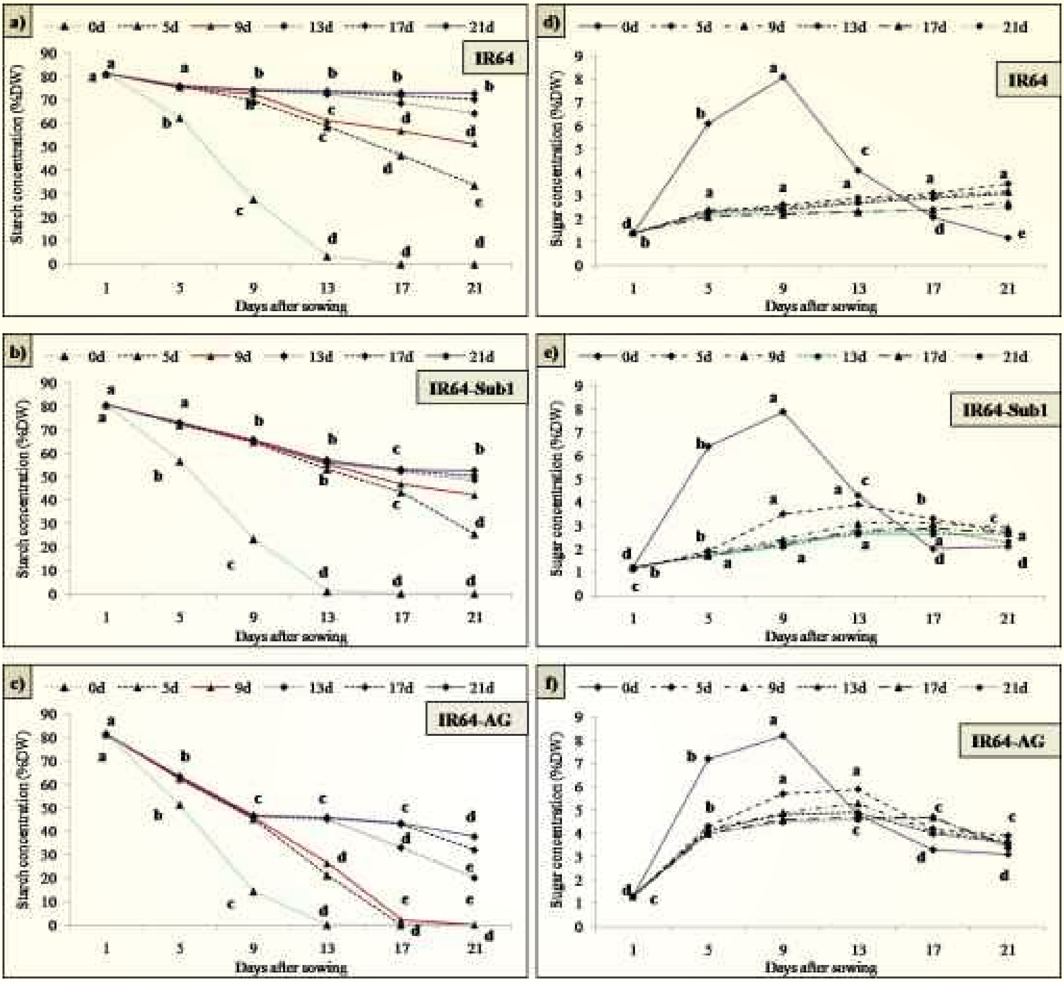


Fig. 40 Depletion of starch (%DW) from seeds of a) IR64, b) IR64 *Sub1*, c) IR64-AG (mean of IR64-AG131 and IR64-AG132) and soluble sugar concentration (%DW) in coleoptiles of d) IR64, e) IR64 *Sub1* and f) IR64-AG (mean of IR64-AG131 and IR64-AG132), from seeding to 21 d under different flooding treatments. Data are from experiment I conducted during the *kharif* season of 2013. Lower case letters *indicates* means are significantly across flooding treatments.

starch in seeds of these tolerant genotypes was completely depleted by 16–17 d, but when the duration of flooding was longer, the depletion became progressively slower. For the intolerant genotypes IR64 and IR64 *Sub1*, starch depletion was much slower, and was partial even after shorter flooding of 5 and 9 d. Under saturated conditions, soluble sugar concentrations followed similar pattern in all genotypes, where it increased faster in emerging coleoptiles until 8–9 d, then decreased thereafter. Overall, flooding considerably reduced soluble sugar concentrations in emerging coleoptiles during the first 9–23 d of flooding, however, the tolerant genotypes, IR64-AG NILs maintained higher soluble sugar concentrations than the other two intolerant genotypes in all flooding treatments (Fig. 40).

11.2. Responses of IR64-AG NILs to different crop establishment methods (Experiment II)

Second experiment was carried out to compare the performance of IR64-AG NILs (IR64-AG131 and IR64-AG132), IR64 and IR64 *Sub1* using different methods of crop establishment during *kharif* seasons of 2013 and 2014. Flooding was imposed for 21 d. The experiment was laid out in a split plot design with plot size of 35 m^2, with three replicates. Three crop establishment methods were used as main plots; (i) direct dry seeding in unpuddled soil, (ii) wet seeding in puddled soil using drum seeder (iii) wet seeding in puddled soil broad casting (farmers' practice in lowland condition). The genotypes constituted the subplots. For direct dry seeding, a fine seed bed was prepared with 2-3 cultivations and planking after one irrigation before sowing. Manual seeding was accomplished during the first week of July, in rows 20 cm apart and at a depth of less than 3 cm; the seed rate was 25 kg ha^{-1}. For direct wet seeding in puddled soil, the field was prepared by dry ploughing followed by irrigation and puddling. Seeds were soaked in water for 24 h then incubated for another 24 h before seeding. Sprouted seeds were then sown using a drum seeder or broadcasted. The fields were irrigated immediately after sowing and a water depth of 2-3 cm was maintained above the soil surface for 21 DAS. Fertilizers at rates of 80-40-40-25 kg N-P_2O_5-K_2O and $ZnSO_4.7H_2O$ per ha were applied, with half of N and all of P_2O_5, K_2O and $ZnSO_4.7H_2O$ applied as basal, the remaining nitrogen was applied in two equal splits at tillering (35 DAS) and panicle initiation stages (60 DAS). At 21 DAS, the water was drained and seedlings were allowed to recover for 10 d. In the IR64-AG NILs, the plant population was maintained and thinned in some cases, because of high germination percentage, whereas for IR64 and IR64 *Sub1*, germination was poor with gaps. The plant population was evenly distributed in half of the plot using clonal tillers and the remaining half was kept as it is was. This replanting was done to assess the impact of gap filling on grain yield, as practice by some farmers when crop establishment is poor.

Fig. 41 Enumeration of population at 21 DAS and performance of AG NILs in NRRI field

Fig. 42 Germination of IR64-AG under a) broadcasting, b) drum seeding and c) dry direct seeding

11.2.1. Effect on plant population

Plant population was slightly higher during 2014 as compared 2013 but not significantly different (Fig. 43). Among the establishment methods, the plant population was highest in broadcasting followed by drum seeding. IR64-AG NILs had 81.1 and 217.5% higher plant populations over IR64 Sub1 and IR64, respectively, irrespective of the establishment methods. The anaerobic condition soon after sowing restricted the emergence of rice seedlings in IR64 and IR64 *Sub1*. After 10 d of receding the floodwater, the gap filling in half of the plots of each treatment was done with clonal tillers in IR64 and IR64 Sub1; thinning was done in IR64-AG NILs to maintain the plant population evenly in the whole plot. After gap filling, the plant population in IR64-AG NILs was normal, whereas, in IR64 and IR64 *Sub1* plant population was distributed upto some extent and it was nearly normal in case of IR64 *Sub1*. After the gap filling and redistribution, the plant population per m^2 difference in IR64 *Sub1* and IR64 was 20.1 and 45.4% lower as compared to IR64-AGNILs over non-gap filling plots.

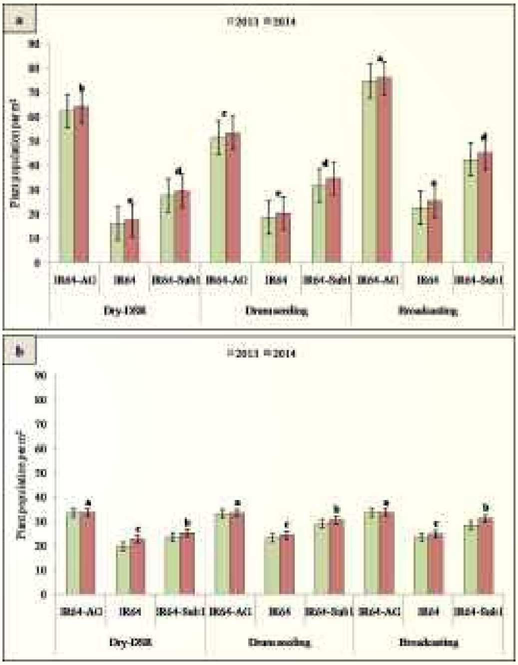


Fig. 43 Plant population per m^2 of IR64, IR64 *Sub1* and IR64-AG (mean of IR64-AG131 and IR64-AG132), a) without gap filling and b) after gap filling, following sowing and flooding for 0 to 21 d. Data are from experiment II conducted during the kharif seasons of 2013 and 2014 at ICAR-NRRI Cuttack. Vertical bars represents ± SE.

11.2.2. Effect on yield attributes and grain yield

The numbers of effective tillers per m^2, panicles per plant, grains per panicle and spikelet fertility were significantly influenced by crop establishment method and genotype, but 1000-grain weight and panicle length were not (Table 27). IR64-AG NILs significantly outperformed IR64 and IR64 Sub1 in the number of effective tillers m^{-2}, panicles plant^{-2}, grains panicle^{-1} and spikelet fertility.

Table 27 Effect of crop establishment method on yield attributes of IR64-AG NILs, IR64 and IR64 *Sub1* in experiment II conducted during the *kharif* seasons of 2013 and 2014 at ICAR-NRRI, Cuttack, Odisha.

Establishment method	Genotypes	No. of effective tillers m^{-2}	Panicles $plant^{-1}$	Panicle length (cm)	Grains $panicle^{-1}$	Spikelet fertility (%)	1000-grain weight (g)
2013							
Dry-DSR	IR64-AG[a]	324	10.3	24.1	124	82.4	24.0
	IR64	204	6.3	23.7	109	69.1	22.5
	IR64 *Sub1*	260	8.8	24.0	116	76.5	23.1
Drum seeding	IR64-AG	379	12.2	24.4	132	85.7	24.2
	IR64	234	7.5	24.0	115	72.1	22.8
	IR64 *Sub1*	326	10.1	24.2	121	79.4	23.5
Broadcasting	IR64-AG	357	11.5	24.4	131	84.7	24.0
	IR64	220	7.1	23.7	113	70.2	22.7
	IR64 *Sub1*	302	9.7	24.1	120	78.9	23.2
$LSD_{0.05}$		28.6	2.11	ns	12.8	9.62	ns
2014							
Dry-DSR	IR64-AG	338	11.2	24.2	129	85.1	24.1
	IR64	211	7.1	23.6	112	72.5	22.4
	IR64 *Sub1*	271	9.3	24.0	120	80.1	23.2
Drum seeding	IR64-AG	388	12.9	24.5	135	88.7	24.2
	IR64	243	8.1	23.8	119	75.4	22.9
	IR64 *Sub1*	340	11.1	23.9	126	83.2	23.3
Broadcasting	IR64-AG	369	12.3	24.4	134	87.5	24.0
	IR64	228	7.8	23.8	117	73.9	22.8
	IR64 *Sub1*	316	10.4	24.2	125	82.7	23.1
$LSD_{0.05}$		31.4	2.16	ns	13.7	10.12	ns

Among the crop establishment methods, the highest yield attribute values were observed when drum seeding was used and the lowest values were observed under dry–DSR (Table 27). The fewer tillers m^{-2} and panicles plant^{-1} in 2013 resulted in lower grain yield. Drum seeding resulted in significantly higher grain yield during both years followed by broadcasting (Table 28). Grain yield in drum seeding was respectively, 11.3% and 5.5% higher during 2013 and 12.1% and 4.8% higher during 2014 over dry–DSR and broadcasting (Table 28). Grain yield of IR64-AG NILs was respectively, 36% and 15% higher in 2013 and 52% and 22% higher in 2014 than IR64 and IR64 *Sub1*, irrespective of the establishment method. Harvest index of IR64 was lowest followed by IR64 *Sub1*. In the half of the plots where plant population was evenly distributed by gap filling and thinning, the difference in grain yield was reduced. After gap filling, the grain yield in IR64 and IR64 *Sub1* was increased upto 9.1 (2.5 q ha^{-1}) and 11.2% (3.6 q ha^{-1}), as compared to non-gap filled plots, respectively (Table 28).

Table 28 Effect of crop establishment methods on grain and straw yields and harvest index of IR64-AG NILs, IR64 and IR64 Sub1 in experiment II conducted during the kharif seasons of 2013 and 2014 at ICAR-NRRI, Cuttack, Odisha

Establishment methods	**Varieties**	**Grain yield (t ha^{-1})**				**Harvest index**	
		Without gap filling		**After gap filling**			
		2013	**2014**	**2013**	**2014**	**2013**	**2014**
Dry-DSR	IR64-AG[a]	3.38	4.12	3.55	4.23	0.40	0.41
	IR64	2.35	2.78	2.66	3.02	0.35	0.36
	IR64 *Sub1*	2.92	3.27	3.32	3.77	0.38	0.39
Drum seeding	IR64-AG	3.73	4.68	3.89	4.87	0.43	0.45
	IR64	2.83	3.04	3.08	3.28	0.37	0.39
	IR64 *Sub1*	3.21	3.88	3.64	4.19	0.39	0.40
Broadcasting	IR64-AG	3.59	4.44	3.78	4.68	0.42	0.44
	IR64	2.67	2.91	2.84	3.16	0.37	0.38
	IR64 *Sub1*	3.17	3.69	3.47	3.93	0.40	0.42
$LSD_{0.05}$		0.564	0.623	0.578	0.634	0.024	0.028

[a] mean of both IR64-AG131 and IR64-AG132 was presented as values were similar

11.3. Response of IR64-AG NILs to varying seed rates and nutrient supply (Experiment III)

Third experiment was conducted during the kharif season of 2015 to evaluate the performance of the same genotypes used in the first and second experiments, under different seed rates and nutrient management. Three different seed rates, 40, 50 and 60 kg ha^{-1}; and three nutrient management treatments (i) control (No fertilizer), (ii) recommended dose (80-40-40-N-P_2O_5-K_2O per ha), and (iii) recommended N-P_2O_5-K_2O + 20% additional P (basal) were used. The experiment was laid out in a factorial RCBD with three replications. Cultural practices were the same as in experiment II, except that fertilizers were applied based on the treatment. Wet seeding was used with seeds directly sown in puddled soil, followed by flooding from 0 to 21 DAS. Half of the N and all of P and K were applied as basal fertilizer at the time of sowing in all of the treatments except the control, and the remaining N was applied in two equal splits at tillering (35 DAS) and panicle initiation (60 DAS).

11.3.1. Seedling emergence

Fig. 44 Experimental view after treatment imposition

Increase in the seed rate significantly increased emergence and seedling establishment of all genotypes. The highest emergence was recorded in the tolerant genotypes, IR64-AG131 and IR64-AG132 and the lowest in IR64, followed by IR64 *Sub1*. Using a seed rate of 60 kg ha^{-1} resulted in the highest emergence of rice seedlings, with relatively greater effects in the intolerant genotypes, although the difference was apparently much less than the contribution of genetic tolerance. The highest seed rate respectively, increased the populations of IR64 by 57% and 53%, and that of IR64 *Sub1* by 24% and 22.2%, over 40 and 50 kg seed rates (Fig. 46). The data suggest using higher seed rates even with tolerant genotypes when flooding is anticipated during crop establishment.

11.3.2. Crop growth parameters

Seeding rate significantly influenced days to emergence, maximum tillering (MT), panicle initiation (PI), heading, flowering and maturity (Table 29). Emergence of IR64-AG131 and IR64-AG132 occurred 4–5 DAS irrespective of the seed rate, while it ranged from 7.8 to 10 d for IR64 and 5.7–8 d for IR64 *Sub1*.

Fig. 45 Germination of a) IR64, b) IR64 *Sub1* and c) IR64-AG

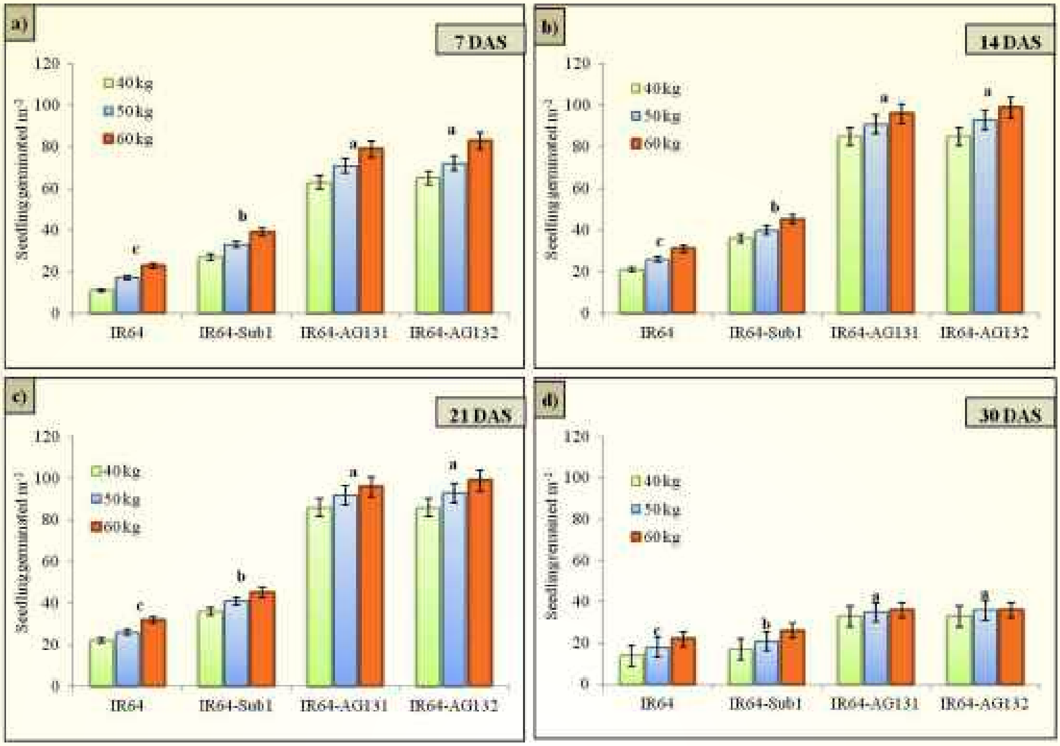


Fig. 46 Seedlings per m^2 of IR64, IR64 *Sub1*, IR64-AG131 and IR64-AG132 at a) 7 days after sowing (DAS), b) 14 DAS and c) 21 DAS and d) remained after 30 DAS as influenced by seed rate. Data are from experiment III conducted during the *kharif* season of 2015 at ICAR-NRRI Cuttack, Odisha . Vertical bars indicate ± SE.

The use of higher seed rate reduced the duration to reach different growth stages. The tolerant genotypes, IR64-AG131 and IR64-AG132 were least affected by variation in seed rate and reached different stages at similar durations. However, larger variation was observed in the sensitive genotypes to reach different stages based on seed rate, ranging from 3 to 8 d in IR64 and 2–5 d in IR64 *Sub1* (Table 29).

Application of nutrients and use of higher seed rates significantly improved plant height at all growth stages. Tiller numbers were highest at MT, but gradually decreased at PI and flowering stage. The highest number of tillers m^{-2} was observed in IR64-AG131 and IR64-AG132, which was, respectively, around 136% and 78% greater than that of IR64 and IR64 *Sub1*, irrespective of the growth stage (Table 30). Nutrient application also enhanced tillering; adding 20% Pin addition to the recommended N-P_2O_5-K_2O resulted in 64% more tillers over the control. Leaf area index gradually increased from MT to flowering in a trend similar to that of tillering; and was largest at higher seed rate. IR64 had the lowest LAI, which was 65% and 37% below that of IR64-AG (mean of both IR64-AG NILs) and IR64 *Sub1*, respectively. Like tillering, LAI was highest when 20% more P was added with the recommended N-P_2O_5-K_2O.

Table 29 Effect of seed rate on days to emergence and other stages of IR64, IR64 *Sub1*, IR64-AG131 and IR64-AG132. Data are from experiment III conducted during the *kharif* season of 2015 at ICAR-NRRI Cuttack, Odisha.

Seed rate	Varieties	Emergence	MT	PI	H	F	M
40 kg ha^{-1}	IR64	10.0	55	69	88	99	128
	IR64 *Sub1*	8.0	52	66	85	95	125
	IR64-AG131	5.0	45	60	80	91	120
	IR64-AG132	5.0	45	60	79	91	119
50 kg ha^{-1}	IR64	9.4	53	67	86	97	126
	IR64 *Sub1*	6.3	51	65	84	95	124
	IR64-AG131	4.5	44	59	79	89	119
	IR64-AG132	4.4	44	59	79	89	119
60 kg ha^{-1}	IR64	7.8	51	64	84	95	124
	IR64 *Sub1*	5.7	49	62	82	94	122
	IR64-AG131	4.2	45	60	80	89	120
	IR64-AG132	4.2	45	60	80	89	120
LSD$_{0.05}$		**0.28**	**3.54**	**4.12**	**4.88**	**4.92**	**4.96**

MT : maximum tillering, PI : panicle initiation, H : harvesting, F : flowering, M : maturity

11.3.3. Yield

The trend observed in yield attributes was reflected in grain yield (Table 31), with the highest yield when 60 kg ha^{-1} seed rate were used. This high seed rate increased grain yield of IR64 by 7.17% and 3.1% and that of IR64 *Sub1* by and 11.4% and 9.8% over that in seed rate of 40 and 50 kg ha^{-1}, respectively. Regardless of the seed rate and nutrient application, IR64-AG131 and IR64-AG132 had the highest grain yield; amounting to 92% over IR64 and 58% over IR64 *Sub1*. Addition of 20% P increased grain yield by about 20% over the control and by 10% over the recommended N-P_2O_5-K_2O treatment, irrespective of seed rate and genotype.

12. Future directions

A wide knowledge gap still exists between researchers and farmers about the need and progress in rice technology development for flood-prone environments. Even the available technologies have not reached their target users because of the poor extension service networks in most of these areas. Poor characterization of the soil and hydrology of flood-prone environments also seems to limit technology development and

Table 30 Plant height, tiller number and leaf area index of IR64, IR64 *Sub1*, IR64-AG131 and IR64 AG132 at maximum tillering (MT), panicle initiation (PI) and flowering (F) stages as influenced by seed rate and nutrient application.

	Plant height (cm)			**No. of tillers m^{-2}**			**Leaf area index**		
	MT	**PI**	**F**	**MT**	**PI**	**F**	**MT**	**PI**	**F**
Seed rate (kg ha^{-1})									
40 kg	48	86	102	183	181	178	2.6	3.1	4.2
50 kg	57	91	106	239	233	224	3.6	4.4	5.1
60 kg	62	95	113	297	285	275	3.8	4.9	5.4
LSD$_{0.05}$	**4.5**	**4.8**	**6.3**	**22.5**	**20.4**	**19.7**	**0.24**	**0.32**	**0.38**
Varieties									
IR64	44	83	99	142	135	128	2.2	2.7	3.9
IR64 *Sub1*	52	89	105	181	176	168	3.1	4.1	4.9
IR64-AG131	64	94	111	317	306	301	3.9	4.9	5.5
IR64-AG132	65	96	112	321	314	308	4.0	5.1	5.6
LSD$_{0.05}$	**4.7**	**5.1**	**6.8**	**28.4**	**25.3**	**21.1**	**0.29**	**0.36**	**0.42**
Nutrient application									
Control	50	88	107	171	167	162	2.3	2.9	3.7
N-P_2O_5-K_2O	56	92	109	269	257	251	3.7	4.7	5.4
N-P_2O_5-K_2O +20%P	63	94	104	281	274	264	3.8	4.5	5.6
LSD$_{0.05}$	**4.5**	**4.8**	**6.3**	**22.5**	**20.4**	**19.7**	**0.24**	**0.32**	**0.38**

Data are from experiment III conducted during the kharif season of 2015 at ICAR-NRRI Cuttack, Odisha.

adoption on a wider scale. One of the major constraints to rice productivity enhancement across flood-prone environments is the lack of seeds of suitable improved and nutrient-efficient and responsive varieties. Post-flood nutrient management (combination of macro and micro-nutrient) for better recovery growth after recession of floods is also least studied and suitable technologies are still not available. Future research should therefore focus on

i) Developing new varieties with a high level of tolerance for the prevailing flood type but are responsive to inputs and crop management;

ii) Suitable management packages at different stages of crop growth and extensive testing and validation;

Table 31 Grain and straw yields (t ha^{-1}) of IR64, IR64 *Sub1*, IR64-AG131 and IR64-AG132 as influenced by seed rate and nutrient application.

Seed rate	Varieties	Grain yield (t ha^{-1})			Straw yield (t ha^{-1})		
		Control	N-P_2O_5-K_2O	N-P_2O_5-K_2O + 20% P	Control	N-P_2O_5-K_2O	N-P_2O_5-K_2O + 20% P
40 kg ha^{-1}	IR64	1.84	1.98	2.22	2.54	2.73	3.07
	IR64 *Sub1*	2.29	2.47	2.58	3.05	3.25	3.71
	IR64-AG131	3.59	3.93	4.28	4.47	4.86	5.29
	IR64-AG132	3.64	3.97	4.32	4.53	4.89	5.33
50 kg ha^{-1}	IR64	1.91	2.06	2.30	2.82	3.05	3.39
	IR64 *Sub1*	2.41	2.57	2.85	3.18	3.46	3.77
	IR64-AG131	3.68	4.07	4.47	4.43	4.81	5.28
	IR64-AG132	3.75	4.11	4.59	4.50	4.91	5.23
60 kg ha^{-1}	IR64	2.09	2.19	2.44	2.99	3.13	3.49
	IR64 *Sub1*	2.49	2.68	2.91	3.32	3.55	3.84
	IR64-AG131	3.71	4.12	4.53	4.43	4.77	5.25
	IR64-AG132	3.80	4.17	4.64	4.47	4.89	5.26
$LSD_{0.05}$		**1.75**	**1.82**	**2.12**	**2.33**	**3.21**	**3.46**

Data are from experiment III conducted during the *kharif* season of 2015 at ICAR-NRRI Cuttack, Odisha.

iii) Integrating research results into extension efforts for rapid transfer to and adoption of effective practices by farmers; and

iv) Developing strategies to incorporate effective management practices in seed delivery and validation in farmers' fields through farmer's communities, government and other institutional agencies and dissemination as a package with improved varieties to ensure wider adoption.

13. Publications

1. Gautam P, Nayak AK, Lal B, Bhattacharyya P, Tripathi R, Shahid M, Mohanty S, Raja R, Panda BB. 2014. Submergence tolerance in relation to application time of nitrogen and phosphorus in rice (*Oryza sativa* L.). *Environmental and Experimental Botany*. **99**: 159–166.

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3. Lal B, Gautam P, Rath L, Haldar D, Panda BB, Raja R, Shahid M, Tripathi R, Bhattacharyya P, Mohanty S, Nayak

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4. Gautam P, Lal B, Raja R, Baig MJ, Mohanty S, Tripathi R, Shahid M, Bhattacharyya P, Nayak AK. 2015. Effect of nutrient application and water turbidity on submergence tolerance of rice (*Oryza sativa* L.). *Annals of Applied Biology*. **166**: 90–104.

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8. Gautam P, Lal B, Raja R, Tripathi R, Shahid M, Baig MJ, Puree C, Mohanty S, Nayak AK. 2016. Effect of simulated flash flooding on rice and its recovery after flooding with nutrient management strategies. *Ecological Engineering*. **77**: 250-256.

9. Gautam P, Lal B, Tripathi R, Shahid M, Baig MJ, Raja R, Maharana S, Nayak AK. 2016. Role of silica and nitrogen interaction in submergence tolerance of rice. *Environmental and Experimental Botany*. **125**: 98–109.

10. Gautam P, Lal B, Tripathi R, Shahid M, Baig MJ, Raja R, Maharana S, Puree C, Nayak AK. 2016. Beneficial effects of potassium application in improving submergence tolerance of rice (*Oryza sativa* L.). *Environmental and Experimental Botany*. **128**: 18-30.

11. Lal B, Gautam P, Nayak AK, Raja R, Shahid M, Tripathi R, Singh S, Septiningsih EM, Ismail AM. 2016. Agronomic manipulations can enhance the productivity of anaerobic tolerant rice sown in flooded soils in rainfed areas. *Field Crops Research*. http://dx.doi.org/10.1016/j.fcr.2016.08.026.

12. Gautam P, Lal B, Tripathi R, Baig MJ, Shahid M, Maharana S, Bihari P, Nayak AK, 2017. Impact of seedling age and nitrogen application on submergence tolerance of Sub1 and non Sub1 cultivars of rice (*Oryza sativa* L.). *Journal of Plant Growth Regulation*. DOI 10.1007/s00344-016-9661-7.

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