



Genetic Improvement of Rice for Aroma, Nutrition and Grain Quality

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SUMMARY

Rice is the staple food for more than half of the world population satisfying the variable quality preferences of consumers across the globe. Therefore, there is no universal quality for rice. Besides, quantitative nature of their inheritance, effect of environment in their expression, destructive methods of estimation and high error rates in analysis, complicates breeding for quality traits. Moreover, the known traits are often unable to explain the end use quality variations. Non destructive trait descriptors that explain quality variations therefore need to be identified. These parameters can then be used to formulate regression models to predict end-use quality. Large number of QTLs and genes controlling quality has been reported. However, their functionality across genotypes needs validation for their effective utilization in breeding programmes. An in-depth understanding of the underlying mechanisms controlling grain quality will enable breeders to precisely connect the missing links with greater efficiency. The present chapter will attempt to provide an overview of the scientific and technological advances in rice grain quality research, with special emphasis to genetic studies and breeding efforts in India and abroad.

1. INTRODUCTION

Quality in rice refers to its physical and physico-chemical properties before and after cooking. Physical properties include the physical appearance of grain, its size, shape, color, uniformity in overall appearance, luster etc. Specific density of grain, hulling and milling recovery, head rice recovery etc. represents Millers' qualities. Physicochemical properties include the biochemical characters of the grain especially amylose, amylopectin, soluble starch, resistant starch, protein and micronutrient content besides gel consistency, gelatinization temperature, pasting properties, water uptake, volume expansion ratio, amylographic properties, etc. A new dimension to rice quality has been added in the form of its sensory qualities which include taste, flavor, uniformity of particles & mouth feel after chewing of the cooked whole grain etc. The sensory qualities are highly subjective in nature and vary widely from person to person. Among all other sensory qualities, aroma needs special mention due to its high market demand. Basmati rice is one such example where the premium cooking and eating quality along with pleasant aroma created a multi-billion dollar export industry to India. However, preference for aroma is highly location specific. Besides basmati there are other categories of aromatic rice, which fetch better prices in the



market. Looking into the wide acceptability of rice as staple, in cuisines and in desserts, the quality of this miracle grain can be divided under four broad categories namely its physical appearance, milling properties, cooking & eating characteristics and nutritional & nutraceutical quality. Nutritional quality includes grain protein, vitamins and micronutrient content while nutraceutical properties deal with the healing compounds. Individually each component plays significant role in maintaining good health.

Quality preferences in rice are highly variable. Different countries have different requirements for quality. Even within countries, an array of preferences can be observed. Therefore specific quality profiles need to be developed through extensive survey among consumers, millers and farmers in the target region of breeding. A preliminary survey on quality preferences of urban populations of eastern and southern India have been undertaken by Social scientists at IRRI. They concluded that medium slender grain type is preferred over long slender among both the populations. Aroma was priority for 37% respondents of eastern India. The preference for aroma was lower among the respondents of South India. However, a huge variation in grain type preference was observed among different cities of Eastern India revealing highly variable consumer preference for quality traits in this region. Appearance of rice grain, its cooking and eating qualities are highly significant in deciding the market value of produce. No rice is consumed if its cooking and eating quality does not match the preference of consumers. Besides the cooking quality of whole grain, rice processing quality i.e. ability of rice to be processed to different end products like puffed rice, popped rice, flattened rice etc., is gaining attention among millers and consumers. All these dimensions of rice quality should be understood well before initiating breeding efforts to improve it.

Due to quantitative nature of inheritance, quality traits in rice are highly influenced by environment. The existence of multiple genes and epistatic interactions among them complicates their breeding procedure. The situation is further complicated by the variable reports on genetic basis of quality traits in different populations and destructive nature of phenotyping for these traits. However, with the advancements in laboratory methods and instrumentation, more techniques for precise phenotyping are becoming available. Current advancements in the science of plant genomics will help the breeders to relate the phenotypes with specific genes or genomic regions which are expected to transform the breeding outlook for quality improvement in near future.

In the present chapter, we therefore have attempted to depict the shift of focus on rice quality in the time frame of last seventy years. How the research dimensions changed with respect to quality making it integral criteria in rice genetic improvement in today's world? Multidimensional aspects of rice grain quality have been discussed especially with respect to their genetic and molecular mechanisms. The underlying genes for various quality traits, their interactions and the resources available for precise breeding for these traits have been discussed in details, besides the significant achievements of classical breeding approaches. In Fig. 1, an overview of all the components of rice quality and their importance in varietal improvement has been presented graphically.

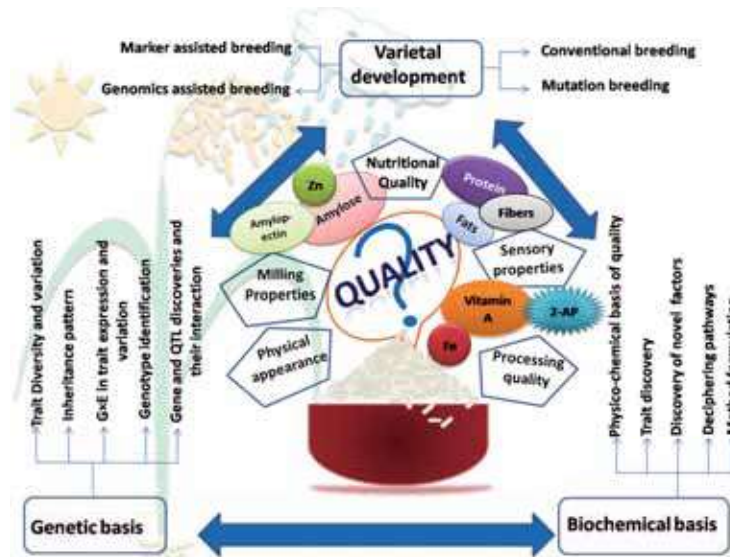


Fig. 1. Graphical representation of the chapter

2. STATUS OF RESEARCH IN RICE QUALITY

Several studies have been undertaken to understand the genetic basis of quality, and ways to improve it through established principles of genetics. Before moving into the detailed discussion on genetic improvement of quality traits, it is very essential to remember that genetic studies on any trait can be undertaken accurately only when precise methods of trait phenotyping do exist. Since grain quality traits are mostly controlled by biochemical components present in the grain, knowledge of biochemistry is very essential for understanding the variation in quality traits. Recognizing the importance of biochemistry in the study of quality traits, initial studies focused on investigating the physico-chemical basis of different quality traits and formulation of methods to quantify them. Work of Juliano at IRRI, Philippines and Bhattacharya at CFTRI, Mysore, India needs special mention. These two researchers contributed immensely to the understanding of physicochemical basis of rice quality during the early days of 1960's and 70's and throughout. Geneticists have utilized these advancements in that phenotyping for quality traits in classical genetic analysis as well as detailed molecular studies. Significant amount of information is now available to breeders for quality improvement with higher precision.

2.1. Phenotyping for quality

Among the various grain quality factors, aroma is one of the most easily recognizable sensory qualities which have been strongly selected by cultivators over generations. Chewing test has long been used for selection of aroma with the subsequent development of KOH test followed by development of more sophisticated



and sensitive instruments like gas chromatography mass spectrometry (GCMS) to precisely measure aroma and detect the volatile compounds responsible for it. Despite of the unavailability of standard measures for the sensory attributes including aroma, taste and flavor of cooked rice; the traits have been selected by the farmers over generations which is well revealed by their presence in local varieties and landraces.

There are several parameters to estimate the cooking and eating quality of rice. Amylose content, gel consistency, gelatinization temperature and pasting properties are objective parameters deciding the cooking and eating quality and have long been being recognized and worked upon by researchers. Sensory qualities like aroma, flavor and mouth feel are subjective parameters and are more recently recognized as important in deciding the market value of the produce. The traits have been preserved by farmers in form of landraces and local varieties at small scales for their own consumption. Sensory qualities largely depend on human perception and are therefore very complex to measure. Hence, the support of social scientists, psychologists and neurologists is also envisaged in formulating methods for precise measurement of these traits. Research efforts have been undertaken for identification of component traits which together decide the ultimate consumers' preference. The component traits are then subjected to scoring by trained panelists. However, such studies were realized to be loaded with human errors, as perception varies with health, environment, social and psychological status of the scorer. In order to overcome the variable human errors in those studies, instruments such as the texture analyzer, colorimeter, luster analyzer, taste meter, electronic tongue and electric nose have been designed to imitate human perception. Multiple regression equations based on the original data from human perceived scores and instrument perception were developed to provide indirect methods of measuring the sensory traits. The science of trait identification for better explanation of sensory qualities is in its nascent stage of evolution and needs further refinements. Methods to measure the nutritional quality of rice grain viz. protein, iron, zinc and other micronutrients evolved later with the advancements in instrumentation during twentieth century. Grain protein content is measured by the basic method of Kjeldahl while micro-nutrients are estimated by their basic property of emitting a particular wavelength of light when moved back to their ground state after ionization. Nondestructive methods of trait estimation have been formulated utilizing the principle of spectrophotometry and calibrating the same using multiple regression equations.

Milling qualities are comparatively easy to measure as they are highly correlated with the physical attributes of grain and are very important for the acceptance of variety in the market. Chalkiness has been found to significantly affect the milling qualities by disturbing the packing of starch granules in the grain. In the chalky area, the starch granules are loosely bound reducing its strength to endure the processing during hulling and milling. The grain breaks in the chalky area reducing the head rice recovery. Studies under Scanning electron microscope could correlate the orientation of starch molecules with chalkiness and milling recoveries. Table 1 represents the quality traits and tests available for their estimation.



Table 1. Quality traits and their methods of evaluation.

Trait	Method
Length, breadth, width, etc	Vernier Callipers, Digital Annadarpan Image Analyzer
Moisture	Moisture meter
Cooking quality	Laboratory based methods viz. Alkali spreading value, KOH test
Pasting properties	Rheovisco analyzer (RVA)
Protein content	Kjeldhal method, Near Infra Red Spectrophotometer
Antioxidants	Spectrophotometry, HPLC
Micronutrient content	Atomic Absorption spectrophotometer (AAS), XRF
Appearance	Gloss meter, “Mido” meter, “Hunter Lab” Colorimeter
Aroma	KOH test, GC, GCMS, Electronic nose
Taste	HPLC, Enzyme kit, Taste sensor, Taste analyzer, Electronic tongue
Texture	Texturometer, Texture analyzer, Tensipresser

2.2. Major biochemical components of rice quality

For a comprehensive understanding of quality aspects of rice grains, it is very important to know about their biochemical bases. The major constituent of rice endosperm is starch which comprises about 90% of the total dry weight of polished grains. This is followed by protein, fats, fibers, vitamins and micronutrients. Comprising the bulk of the grain, starch quantity and quality play leading role in deciding almost all quality traits. Amylose and amylopectin together constitute the starch molecule and their relative abundance decides the starch quality. Amylose is long chained polysaccharide with less branching while amylopectin is highly branched. Higher content of amylose makes the rice fluffier, dry and non-sticky with better grain separation after cooking. However, beyond a certain limit (when amylose is >25%) it makes the rice hard when kept for longer time after cooking. On the other hand, lower values of amylose (<15%) or its absence makes the rice sticky and glutinous (waxy rice). However, deviations to this correlation do exist. These deviations have been explained on the basis of amylose-amylopectin ratio or degree of branching of these two chains. Besides cooking quality, starch is vital in deciding the eating and product processing quality in rice. Nature of binding of starch molecules during grain filling decides the overall appearance of raw grain.

The second major component of rice endosperm is protein (vary from 5–12% of grain weight) which is the lowest among cereals. However rice protein is best in digestibility among cereal protein due to the higher content of (~80%) glutelins. Rice protein is also most balanced among cereal proteins due to higher content of lysine and tryptophan, the limiting essential amino acids in cereals. In addition to the traditional quality features, the focus of developing rice as nutriceal has gained momentum in last two decades. Considering rice as staple food for billions, this shift in focus from food security to nutritional security bears great significance. Fats are important in the bran layer of rice as they are abundant there and are used to extract



high quality γ -oryzanol (rice bran oil) containing ω -3- fatty acids. Due to the low smoking point of rice bran oil it is mixed with other vegetable oils to improve its cooking quality. Fibres are important as roughage and decide the glyciemic index of rice. However, higher content of fibres, reduces palatability.

Half of the world's population is suffering from one or more vitamin and/or mineral deficiency. Vitamin A, iron and zinc seem to be most prominent limiting components among them. Hence, varieties rich in micronutrients and vitamins will be the only sustainable solution to the problem of hidden hunger. In general vitamin-A is absent in rice grains and micronutrients like iron and zinc are present in lower concentrations. Antioxidants are another important class of nutri-compounds that protect the cells from harmful effects of free radicals and reactive oxygen species released during various metabolic pathways of cells. Pigmented rice is rich in such compounds. The main phenolic compounds in pigmented rice are anthocyanins which are the major active components for antioxidation. Moreover, this rice is also rich in Vitamin B and E. Beside all these features, it also needs special mention that hundreds of end products are developed from rice in India and throughout the world. Each of these end products have their specific quality requirements.

2.3. Genetic studies on quality traits

Following the advent of standard methods to measure different quality traits, studies on exploring their genetic basis were undertaken. Most of the quality traits were perceived as quantitative, except for aroma and grain pigmentation which were perceived as present-absent type and were studied in form of discrete ratios. Segregation ratio of aroma varied among different studies. However, the studies could unanimously conclude that aroma gene is recessive in nature which was later tagged by Ahn et al. (1992) as the *fgr* gene on long arm of chromosome 8. Bradbury et al. (2005) cloned the gene and characterized it further. In aromatic genotypes, a deletion in exon 7 of the gene coding for betaine aldehyde dehydrogenase (*BADH2*) generates a premature stop codon which results into loss of function of the gene (Shi et al. 2008) leading to accumulation of 2- Acetyl-1- Pyrroline (2AP). This is the main volatile compound (out of nearly hundred reported volatiles) imparting aroma to rice grain. Despite of being the most elaborately studied trait among all other quality traits, the quest for aroma is still on. The search for new variants of aroma gene, other minor genes and elucidation of its pathway are going on. The major focus of aromatic rice breeding in India was development of basmati rice varieties in which role of ICAR-IARI, New Delhi; CCSHAU, Hissar; PAU, Ludhiana and GBPUAT, Pantnagar were very crucial. However comparatively lesser focus was given in case of non-basmati aromatic rice both for basic and applied research. In a compendium prepared by the Indian Agricultural Research Institute (IARI) and APEDA, varieties such as Kalanamak, Tilak Chandan and Jeerabati (Uttar Pradesh), Kalajeera (Orissa), Katrani (Bihar), Ambemohar (Maharashtra), Gobindbhog and Badshahbhog (West Bengal), Dubraj, Badshahbhog and Jawaphool (Chhattisgarh) and Kalajoha (Assam) have been identified which could be harnessed and developed for their export potential. Many traditional aromatic rice genotypes viz. Bindli, Dubraj, Durgabhog, Makarkanda,



Badshahog, etc. were reported to surpass basmati in one or more characteristics like aroma, texture, elongation on cooking and taste etc. and they mostly possess small to medium grain. Kalanamak and Dubraj were reported to command a premium price in the market. Breeding efforts for developing high yielding modern varieties of aromatic non-basmati rice have been quite limited when compared with intensive efforts for basmati. Mostly pureline selection and mutation approaches have been followed. However the semi-dwarf version of the Kalanamak landrace developed by researchers at IARI, New Delhi needs special mention. Chakraborty et al. (2016) explored the possible existence of any locus other than *BADH2* controlling aroma among 84 landraces of *indica* rice using functional marker (8-bp deletion) for *badh2* gene. Nearly 80 percent of the landraces carried the well-known *BADH2* deletion. However, eleven aromatic genotypes including wild ancestors lacked that particular functional allele. This indicated the existence of an alternate gene or allele controlling aroma in rice. Singh et al. (2010) however, have also established the role of *BADH1* gene and its haplotypes with aroma in rice.

Like aroma, anthocyanin pigmentation in rice grain was also studied as a qualitative trait due to presence or absence type of phenotypes. Unlike aroma, this trait shows dominant gene control and two complementarily acting genes, *Rc* and *Rd* control the pericarp pigmentation. The *Rd* locus codes for dihydro flavonol reductase (DFR) enzyme and the *Rc* gene codes for Basic Helix-Loop-Helix (HLH) Protein. The *Rc* locus has been cloned and its three allelic variants have been well characterized. Its null allele (*rc*) with 14-bp deletion creates frame shift mutation and a premature stop codon leading to white pericarp phenotype (Brooks et al. 2008). Several pigmented rice genotypes have been identified and characterized in India, including the Chakhao rice of Manipur, Kalbahat rice of Maharashtra, Njavara of Kerala, etc. Njavara is well known for its medicinal significance in Ayurvedic medicines. However, the identified pigmented genotypes are low yielding and susceptible to lodging. Breeding interventions to improve their plant type and yield shall be highly beneficial in their popularization.

The quality traits other than aroma and grain pigmentation have been studied considering them as quantitative traits. Gene action studies on these quality traits lead to variable conclusions by different researchers. Advent of molecular markers has opened a new era of QTL and gene discoveries. More than 600 QTLs have been reported for different quality traits like chalkiness, cooking and eating quality, grain dimensions, pasting properties, grain protein content, iron and zinc (<http://www.gramene.org>). Table 2 summarizes the identified loci information of major quality traits and availability of their corresponding functional markers.

Detection of such large number of QTLs poses a great challenge for the plant breeder to efficiently and intelligently use this information for the genetic improvement of the traits. However, a comprehensive analysis suggests that the QTLs and genes involved in amylose biosynthesis pathway play a significant role in deciding the physical attributes (head rice recovery, chalkiness and grain dimensions) of grain along with its cooking and eating quality. *Waxy* (*Wx*) locus was first identified as the



Table 2: Genes and functional markers for quality traits in rice.

Trait	Gene	FM Availability
Aroma/Fragrance	<i>Badh2</i>	Available
Amylose content (AC)	<i>Wx</i>	Available
Grain size	<i>GS3</i>	Available
Gelatinization temperature	<i>SSIIa</i>	Available
Iron (Fe)	<i>OsYSL1, OsMTP1, OsFER1, OsFER2</i>	Yet to be developed
Zinc (Zn)	<i>OsARD2, OsIRT1, OsNAS1, OsNAS2</i>	
Fe and Zn	<i>OsNAS</i> gene family <i>OsNAS3, OsNRAMP1, Heavy metal ion transport, APRT</i>	

Source: Adapted from Lau et al. (2015)

major locus controlling majority of the variation in amylose content in grain which in turn was known as the sole major factor affecting the cooking and eating quality of rice. The *Wx* locus codes for an enzyme Granule Bound Starch Synthase I (GBSS I) and has a minor effect in controlling gel consistency, but has no effect on gelatinization temperature. Alternate splicing of the *Wx* transcript leads to several allelic variations altering the degrees of amylose content in grain. The locus *Alk* is the major locus in controlling Gelatinization temperature and codes for the enzyme Soluble Starch Synthase IIa (Umemoto et al. 2005). *Wx*, *SSIIa*, *SBE3* (Starch Branching Enzyme3), *PUL* (Pullulanase) have been reported to affect the different aspects of the pasting properties in rice. Existence of cross talks and pleiotropy among the loci has also been reported. Cloning and characterization of several genes controlling grain appearance traits led to better understanding of their regulation and can therefore be utilized more efficiently in breeding programs. Chalkiness in the endosperm was attributed to the loose binding of the starch. However, among many QTLs reported for chalkiness only *Chalk5* was isolated and characterized well (Li et al. 2014). Interestingly, some of the QTL clusters controlling grain dimensions (grain size and grain width) are reported to have pleiotropic effect with chalkiness.

Besides the above mentioned QTLs related to starch component of rice grains, a large number of QTLs have also been reported for the nutritional components like content of protein, iron, zinc etc. in grains. Expression of these traits is highly affected by growing environment and cultural practices followed to grow the crop. This makes their detection very difficult. Earlier, tight negative linkage was reported to exist between yield and grain protein content (GPC). In rice, many QTLs along with associated markers have been identified covering all 12 chromosomes for GPC among which chromosomes 1, 2 and 7 harbor most of the QTLs. The QTL *qPC1* present on long arm of chromosome 1 control GPC through its regulation of synthesis and accumulation of glutelins, prolamins, globulins, albumins and starch. It encodes a putative amino acid transporter (*OsAAP6*) and control GPC without affecting growth and grain yield suggesting that GPC and nutritional quality could be improved without reduction in grain yield (Peng et al. 2014). Several QTLs have been reported for higher



concentration of iron and zinc in rice grains some of which have been listed in Table 2. However, all these studies need support of bioavailability data for the micronutrients. In the last decade, Indian researchers have given major attention for improving the nutritional quality of rice grains with major emphasis on increasing grain protein, iron and zinc concentrations. Researchers from ICAR-IIRR, Hyderabad reported that wild relatives of rice such as *O. nivara*, *O. rufipogon*, *O. barthii*, and *O. longistaminata*, and African cultivated rice *O. glaberrima* have higher level of Zn. The QTLs for Zn content were detected in Ch 3, 7 and 12 by using RIL population derived from Madhukar x Swarna. They also developed high grain zinc containing rice variety DRR Dhan 45 through conventional breeding. It is the first rice variety with high zinc content to be notified at national level with an average zinc content of 22.6 ppm in polished rice.

Significant progress has been achieved in development of transgenic lines especially in terms of nutritional quality in rice. However, their large scale application in form of cultivars is yet to be established. The biggest success in this regard was achieved in the development of beta-carotene rich golden rice which was developed by transformation of genes from daffodil and a soil bacterium *Erwinia* into rice and expressing them in the grain. The concentration of beta carotene was further improved by transforming the *phytoene synthase* gene from maize (Paine et al. 2005). Scientists from IARI, New Delhi have transferred the genes for golden rice traits in popular Indian rice varieties through backcross breeding strategy. In order to improve the iron content in grain, *ferritin* gene has been transferred from soybean into rice. Efforts have been made to reduce the phytic acid content which is a major anti-nutritional factor in rice grain. It declines the bioavailability of iron in body by chelating the same at low pH in the stomach. Simultaneously transgenics have been developed to improve the micronutrient content in grain to combat the problem of hidden hunger. Table 3 compiles the successful transformation events undertaken to improve the nutritional quality of rice grain. However, there is need to thoroughly analyze the stability of the events and their utility in trait transfer into high yielding genetic back grounds through breeding interventions.

Table 3. Genes used for transformation of rice to improve vitamin and mineral content.

S. No	Nutrient	Genes used
1	Vit A	<i>Nppsy1</i> , <i>Eucrt1</i> ,
	β-carotene content	Daffodil <i>Phytoene Synthase</i> and <i>Erwinia Phytoenedesaturase</i>
2	Fe	<i>Osnas2</i> , <i>Aphytase</i> , and <i>Osnas1</i> , <i>Osnas3</i> , <i>OsYSL2</i> , <i>Ferritin</i> genes: <i>SoyferH1</i> , <i>PyFerritin</i> , <i>rgMT</i> , <i>Gm ferritin</i> ,
3	Zn	<i>Osnas2</i> , <i>Gm ferritin</i> , <i>Aphytase</i> and <i>Osnas1</i>
5	Fe and Zn	<i>Nicotianamine synthase (NAS) genes</i> [<i>OsNAS1</i> , <i>OsNAS2</i> , and <i>OsNAS3</i> , <i>OsNAS3-D1</i> , <i>HvNAS1</i> , <i>AtNAS1</i> and <i>HvNAS1</i>], <i>HvNAAT</i> , <i>HvNAAT-A</i> , <i>HvNAAT-B</i> , <i>Osfer2</i> , <i>SoyFerH1</i> , <i>SoyFerH2</i> , <i>Pvferritin</i> , <i>OsIRO2</i> , <i>OsYSL2</i> , <i>OsYSL15</i> , <i>HvNAS1</i> , <i>Aphytase</i> ,
23	Zn, Cu, and Ni	<i>OsNAS3</i>

Source: Adapted from Mahender et al. (2016)



Due to increased incidences of diabetes in world population, development of rice genotypes with low glycemic index is receiving global attention. However the research in this particular area is still in nascent stage and remained limited to development of effective protocols and evaluation of limited set of germplasm. Any significant breakthrough in terms of detection of genes/QTLs for this trait is still not achieved. Although systematic breeding efforts for developing low glycemic index rice is in its nascent stage in India, through screening of existing varieties, some varieties like Swarna (low GI reported by IRRRI), Improved SambhaMahsuri (ICAR-IIRR, Hyderabad) and Madhuraj-55 (IGKV, Raipur) have been identified as low-GI-rice.

Besides all the traits discussed here, the ‘Komal-Saul’ (soft rice) of Assam with soak-and-eat property needs special mention. However, the unique property of this rice is highly location specific and expressed in just a few districts of north eastern India. Assam Agriculture University, Jorhat has released a few varieties of Komal-Dhan namely Aghonibora, Bhogalibora, etc. However, the underlying biochemical and molecular mechanism of this unique property is yet to be deciphered.

Indian rice breeding programmes are traditionally directed towards yield improvement. Targeted improvement for grain quality was carried out only in few cases. With a few exceptions, only advanced generations of high yielding materials are evaluated in nationwide trials for grain quality under All India Coordinated Rice Improvement Project (AICRIP). However, the huge amount of data generated from these studies have created a very useful database for the rice researchers in the country especially for study of Genotype x Environment interaction for grain quality along estimating the heritability, genetic gains and selection of genotypes for generating mapping population.

Many of the reported QTLs for different quality traits are either population specific, or control a minor portion of variation, or their expression is highly dependent on environment. As a result such QTLs become difficult for use by breeders. Sometimes the QTL region is so large that it becomes difficult to transfer the region intact. Therefore studies shall be carried out to identify and customize the QTLs and more preferably the gene combinations that can be effectively utilized under target specific breeding programs. These QTLs and gene combinations will largely vary depending on the target environment and market, and therefore needs validation through multi environmental trials.

2.4. Rice quality research at NRRI

The Institute was established in the backdrop of ‘The great Bengal famine’ of 1942 caused due to huge yield loss by *Helminthosporium*, as a mitigation strategy to avoid such disasters further. Since its inception, the institution is incurred with the responsibility to feed millions of hungry bellies of an infant nation. Therefore the main focus from the beginning was to enhance yield. *Indica-japonica* hybridization program of FAO as well as trials of other introductions provided a very strong initial support for generation of valuable variations and development of high yielding promising genotypes. Rice cytology and genetic analysis of important traits



contributed significantly in our present understanding of Rice science. Concern for rice quality along with yield got attention of visionary researchers even in days of immense pressure to feed the nation.

Breeding for fine grain began in 1953. Crosses were made to improve the grain characters of otherwise high yielding, highly adapted early maturing, coarse and red kernelled variety *Ptb10* using pedigree method of breeding. In 1961, cultures of X-ray irradiated *Ptb10* were received which segregated for kernel color and were subjected to further testing. Several mutation breeding programs have been undertaken to improve grain quality of otherwise popular varieties like TN1, Taichung65, and CR2001. Neelabati was identified as a high yielding fine grained genotype recommended for cultivation in coastal/saline areas. Investigations on cooking quality of genotypes using the KOH test were initiated during 1963-64 and genotypes with superior cooking qualities were identified. As soon as a quick and reliable method to estimate the cooking quality was adapted, inheritance study was undertaken by crossing of contrasting genotypes. The inheritance study revealed that there are 2-3 genes that control cooking quality in rice and the trait can be subjected to genetic improvement. The study also indicated the possible role of minor genes for small modifications in trait expression. However varietal development program specifically focusing on the cooking and eating quality have not been undertaken yet.

Head Rice Recovery (HRR) percentage after hulling was also investigated during 1960s which ranged from 23.8 to 74.5%. Nature and texture of endosperm along with shape and size of grain were reported to influence HRR. Presence of abdominal white was found not to affect HRR. Highest recovery percentage (79.2) was reported in *Changsan* variety from Manipur. HRR percentage of japonica rice was not significantly different from that of *indica* types. Thorough study on the effect of time of harvesting after flowering, moisture content during harvesting on head rice recovery after milling was undertaken. It was observed that some genotypes like GEB24, T141, T90, etc. were more resistant to breakage when harvested within 30 days after flowering. However, ill filled kernels are a problem associated with early harvesting. The study could recommend that field harvesting after 30-40 DAF at 20-23% grain moisture level and shade drying to 11-13% moisture, gives highest hulling recovery by reducing the development of sun cracks in the kernels during field drying.

Inheritance of red kernel colour was studied during 1960s in segregating generation of a cross between GEB24 and *Ptb10*. *Ptb10* being well recognized for its superior grain type. Association with other traits was studied and their linkages established. Sanghamitra et al. (2017) reported low amylose content in purple rice genotypes, Chakhao, Mamihunger and Manipuri Black. Lower yield along with the low grain amylose content in these genotypes needs improvement through breeding. Significantly higher antioxidation property in the grains of these genotypes has been recognized. Higher heritability of the trait suggested greater scope for genetic improvement of anthocyanin and antioxidant content in pigmented rice.

ICAR-NRRI took important initiatives for genetic improvement of short grained aromatic rice. The aromatic landrace collections of the institute were characterized

based on phenotypic descriptors and molecular markers (Roy et al. 2014 & 2016). Study of variation in nuclear and chloroplast DNA sequences also provided a greater insight into the population structure and origin of aromatic landraces (Roy et al. 2016). Integration of participatory plant breeding with marker assisted pureline selection helped in better genetic gain from selection in three landraces Kalajeera, Machhakanta, and Haladichudi (Roy et al. 2017). Several superior pureline varieties like NuaKalajeera, NuaDhusara and NuaChinikamini were developed and released (Patnaik et al. 2014). Marker assisted pedigree selection helped to develop high yielding aromatic genotype (CR Sugandh Dhan-907) by crossing Pusa 44 and Dubraj (Patnaik et al. 2015). CR Sugandh Dhan-907 was similar to Dubraj landrace in terms of its grain quality. Besides aromatic short grain varieties, long slender grained genotypes with aroma (Poombhog and Geetanjali) have been developed through mutation of basmati genotypes and released. The Geetanjali variety is popular among the farmers and has been used for establishment of rice value chain in Odisha.



Fig.2. Field photograph of two aromatic rice varieties developed at ICAR-NRRI along with their respective grain type.

Protein in rice has ever been a major concern of rice researchers in past as well as in present due to its lowest grain protein content among cereals. Positive correlation of bran and aleurone layers with protein and thiamine content was identified and high protein genotypes were found to have a cellular patch in spermoderm (CRRRI Annual Reports, 1949-50 and 1950-51). Studies on variation in protein content and aleurone layer thickness were undertaken in 450 representative genotypes of *japonica*, *indica* and *javanica* rice. Existence of positive correlation between aleurone layer thickness



and percent protein content in rice kernels was reported. Protein content in rice kernels varied from 6.1% to 10.1% while the aleurone layer thickness varied from 11.2 μ to 75.0 μ . Moreover, protein content in *japonica* rice was found to be higher than in white kernelled *indica* types. Inheritance of protein content was studied by the Rice Technology Section established during 1963 and they recorded frequency of occurrence of high protein genotypes among different duration classes of rice varieties in temperate and tropical zones. Due to 4-5% milling, a loss of 11-12% protein as compared to brown rice was recorded during 1966. Studies on inheritance of protein content suggested the role of polygenes in trait expression. Appearance of transgressive segregants in F₂ suggested the possibility to breed true for higher protein than in parents. Near infrared spectroscopy (NIR) was therefore calibrated and validated for large scale and high throughput phenotyping of GPC (Bagchi et al. 2015). Breeding for high protein rice was initiated involving two landraces from Assam. High protein trait was transferred to high yielding backgrounds of Naveen and Swarna through backcross breeding to release the first high protein variety in Naveen background as CR Dhan 310 (released by CVRC for Odisha, Uttar Pradesh and Madhya Pradesh) with 10.3% GPC in 2016. Another variety CR Dhan 311 has been released by State variety Release Committee of Odisha in the name of 'Mukul' with GPC of 10.1% and Zn content of 20 ppm. By using a backcross derived mapping population, a consistent QTL (*qGPC1.1*) over the seasons have also been identified (DARE/ICAR Annual Report 2015-16 and 2016-17 and NRRI Annual Report 2014-15).



Fig.3. Field photograph of two high protein rice genotypes developed at ICAR-NRRI

3. KNOWLEDGE GAPS

Amylose is known to be the major biochemical factor influencing majority of endosperm traits in rice. However sufficient variation for physico-chemical properties has been observed among genotypes having same amylose content. Later, amylopectin and protein content were also designated to play role in deciding physico-chemical characteristics of grain. However, all these factors taken together, more often fail to predict the physico-chemical quality of the grain. Therefore there is need to discover some new factors that play significant role in deciding different aspects of grain quality along with those identified earlier.



Knowledge regarding biochemical and physiological mechanisms of development of superior grain quality needs to be generated for all quality traits. In case of aroma, the gene and the biochemical factors are well characterized. However; the biochemical pathway producing aroma is still not well understood. Similarly, knowledge regarding the mechanism of higher accumulation of grain nitrogen in high protein rice is still to be deciphered.

Quality traits are highly affected by environment but very few studies have been undertaken on the effect of environmental factors on different quality traits. Moreover, the quantification of the environmental component on different quality traits is required to formulate mathematical equations to predict the trait expression in any environment more accurately.

Regarding the grain protein content, the mechanism of higher accumulation of grain protein along with the genes/QTLs having greater effect on the trait need to be identified. Molecular and physiological basis of micronutrient accumulation in grain requires further understanding. Stable sources of low phytic acid and high antioxidant content in rice need to be identified so that the problem of mal-nutrition can be overcome in more economic way. Researchers also need to give more emphasis to the non-conventional quality traits especially low Glycemic Index, Soak and Eat property, product making quality etc. as demand for the varieties carrying such properties are expected to increase further in coming years.

4. RESEARCH AND DEVELOPMENT NEEDS

- There is need to develop methods to precisely quantify the quality traits in a small sample volume without destroying it.
- There is need for intervention of engineers to design instruments for precise estimation of quality traits with more automation and thereby reducing variable human errors.
- More information on surrogate traits for different aspects of quality like grain type, cooking and eating quality, nutritional value etc. needs to be generated.
- Information on differential expression of quality traits under variable environments need to be generated to identify the environments for best expression of different quality traits. The information will support the breeders to evaluate and select genotypes for their breeding program in these environments.
- More QTLs related to quality working across genotypes and environments should be identified, characterized and cloned. Functional markers for such QTLs shall be highly beneficial for the breeders to undertake marker assisted breeding of quality traits.
- There is need to develop quality preference map for the country depending on quality preferences under different rice ecologies of different regions. This will support breeders to decide upon the quality traits to target in their respective



breeding programs. A global database needs to be developed that integrates the phenotyping data, genetic information and marker based genotyping data against all genotypes being studied. Such database shall be easy to handle so that the breeders can select the parents based on the available information.

5. WAY FORWARD

Since quality traits are highly influenced by environment (season/location/year), multi environment testing shall be an integral part of any quality breeding program. Quality evaluation data should always be supported with the GPS, agro-meteorological and soil quality data to improve the precision in quality breeding and provide a readymade information to the breeders to decide upon 'when and where' to select. Moreover, integrating the participatory approach of selection during the later stages of breeding program will facilitate in accelerated popularization of the developed varieties after release.

Popular landraces known for different quality traits shall be improved through appropriate breeding strategies to remove their critical limiting features for which farmers are compelled to stop their cultivation. Wild species may also be evaluated for their quality traits which may serve as useful donors for specific quality features. An elite population comprising of large number of fixed and intermating breeding lines with different permutations and combinations of quality QTLs/ genes shall be developed in elite backgrounds. This will serve the need of elite genotypes for breeding in variable ecologies and provide a wide genetic base to avoid bottlenecks.

Discovery of new functional markers for QTLs and genes controlling quality in rice are very important so that the costly and destructive sampling methods for quality analysis may be avoided. However, exhaustive genotyping using the available marker information along with multi-location phenotyping must be undertaken among the genotypes selected for gene discovery. This will prevent unnecessary duplication of research (jumping into same QTLs/ genes) and provide strong support for identification of new genetic factors. Moreover, these studies shall not remain limited to demarcating the QTL region, but should also further characterize, clone and validate the genomic regions for their effective utilization in breeding programs. A global database for the genotypic and phenotypic data will be useful for selection of parents for breeding. Near isogenic lines (NILs) may be developed for these regions to study the effects of predicted genes individually and in combinations. Those single gene NILs and pyramided NILs will be very helpful to refine our understanding about the biochemical basis of quality. Rice quality breeding therefore, has a long way to go.

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