Chapter

Breeding for Grain Quality Improvement in Rice

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Abstract

Oryza sativa holds a unique position among domesticated crop species as it is one of the most important staple foods globally. Without rice, the day will not be fulfilled in most of the Asian countries. Requirement of rice for consumption is anticipated from 450 million tons in 2011 to about 490 million tons in 2020 and to around 650 million tons by 2050 globally. To meet the food demands, it has been estimated that 40 per cent more rice is needed to be produced by 2050 for the ever increasing population. Increasing incidences of both biotic and abiotic stresses under changing climate are the major constraints in rice production to meet the rapidly escalating population. Crop improvement in rice will not be completed lacking of grain quality analysis. Rice grain quality embraces storage, milling, market quality, cooking and eating quality and nutritive quality of grain. Demand for high quality rice has increased globally in recent years and continues to trend upward due to the taste preferences. Since, consumer demand in Asia and all over the world are diverse due to varied demographics and culture, defining uniform attributes to grain quality becomes more challenging. The Middle Eastern consumers highly prefer long grain, well milled rice with strong aroma while European consumers prefer long grain non aromatic rices. In Asia, Chinese consumers prefer semi-aromatic rice to pure aromatic rice. Cooked kernel elongation is the most important quality traits, which differentiate the highly valued basmati rice from other rice types. Kernel elongation after cooking is an important character of fine rice and the most rice consumers prefer lengthwise elongation.

Keywords: quality, rice, aroma, kernel elongation, basmati

1. Introduction

Rice (*Oryza sativa* L.) is ranked as first in human food crop for more than half of the world's population [1] and economically imperative food crop with nutritional diversification that helps in poverty alleviation. With the intensifications of diverse food demands and living standards of global populations, rice grain appearance and grain quality have become a primary concern for rice breeders [2]. Rice grain quality characters has been grouped into two classes: (i) grain appearance and (ii) cooking and eating qualities. The grain appearance includes kernel length, kernel breadth, kernel length-breadth ratio, endosperm translucency and cooking and eating qualities includes cooked kernel elongation, amylose content, gelatinization temperature (measured as alkali spreading value), aroma and volume expansion. In addition processing qualities, hulling percentage,

milling percentage and head rice recovery are important for market value. Most of the quality traits are complex trait governed by quantitative inheritance.

Generally, Japanese people prefer short and sticky rice, while Indians prefer aromatic basmati rice which elongates when cooked. Indian basmati rice and Thai jasmine rice are highly priced due to their distinctive aroma when cooked and some European countries have led consumers to prefer better quality rice. Due to consumer's demand for better rice quality, rice breeders are striving to develop rice varieties with improved qualities that meet local demand. Basmati varieties has superior quality traits *viz*.

Superfine slender grains, fine cooking quality, pleasant aroma, and lengthwise elongation during cooking and fetched with premium price in the local and global market [3]. The physicochemical and cooking characteristics are the good indicators of grain quality [4, 5]. Most of the consumers prefer rice with soft to medium gel consistency, intermediate amylose content and gelatinization temperature.

Kernel elongation after cooking is an important character of fine rice and most of the rice consumers prefer length-wise elongation. Rice kernels absorb water during cooking and increase in length through swelling of kernels. Breadth-wise increase is not desirable, whereas length-wise increase without increase in girth or a crack on the kernel is a desirable characteristic of high quality rice. In northeast India, cultivated varieties having high kernel elongation might also have originated from this region. The elongation genes might have moved towards both eastern and western parts from the periphery of the centre of origin. Some varieties showed marked elongation in eastern countries such as Burma and Thailand. However varieties in western region with Punjab showed good kernel elongation.

2. Measurement of kernel elongation ratio

Cooked kernel elongation can be measured by three ways *viz*., grain elongation ratio, proportionate change, grain elongation index. Grain elongation ratio is defined as the ratio of the length of cooked rice (L_2) to the length of milled rice (L_1). Kernel elongation ratio indicates the proportionate change of rice grain after cooking. Sood and Siddiq [6] followed following formula to measure kernel elongation

$$\text{Proportionate change} = \frac{L_F / B_F - L_0 / B_0}{L_0 - B_0} \quad (1)$$

Where, L_F – kernel length after cooking, B_F – kernel breadth after cooking, L_0 – kernel length before cooking, B_F – kernel breadth before cooking. Grain elongation ratio is a better index of cooking quality than grain elongation index (A) or proportionate change [7].

$$A = \frac{L_2 / W_2}{L_1 / W_1} \quad (2)$$

Where, L_2 – cooked kernel length, W_2 – cooked kernel breadth, L_1 - milled rice length, W_2 - milled rice breadth.

Grain elongation index is a more reliable estimate of kernel elongation during cooking [8].

$$\text{Elongation index} = \frac{BGS}{GS} \quad (3)$$

Grain elongation index values were less precise and less sensitive than elongation ratio. It needs the additional measurement of grain width, use of kernel L/B ratio instead of length offered no advantage of sensitivity since, grain expanded both length and width wise during cooking [9, 10].

$$\text{Cooked rice elongation} = \frac{\text{After cooking length} - \text{Before cooking length}}{\text{Before cooking length}} \times 100 \quad (4)$$

3. Assay of cooked rice elongation

Ten selected grains were soaked in distilled water for 30 min which were then placed between two pieces of wet filter paper in a petri dish filled with an appropriate amount of water. The dish was then placed in a covered container and the grains were cooked by steaming over boiling water for 10 min and simmering for 10 min (with power off). The cooked rice grain was transferred onto a piece of dry filter paper at the bottom of a fresh petri dish, which was placed in a desiccator with a constant temperature (19°C). Then the CRL was measured [11].

Ten randomly chosen intact polished grains were boiled in a water bath for 7 min after soaking in 7.5 ml of distilled water for 20 min. The cooked rice grains were then placed on absorbent paper for 15 min, and the boiled grain length (BGL, mm) and width (BGW, mm) were measured using the same scanner. The treatments were repeated twice, and the average grain length and width before and after cooking were calculated for estimation of elongation index.

The length of 10 whole rice kernels after cooking was measured by using the micro-scale, and the average kernel length determined. Kernel elongation ratio was calculated by dividing the average length of cooked kernel by the average length of the raw (uncooked) rice.

An inter-laboratory collaborative test was conducted for measurement of grain elongation of milled rice during cooking in 19 laboratories in 16 countries. Details of individual laboratory methods for measurement of grain elongation given below [9].

Collaborator	Methods
2	10–20 grains soaked for 30 min in 20 ml water in 25 × 100 mm test tubes and tubes placed directly in 100°C bath for 15 min
4	Aggregate length and width of 20 raw and cooked grains measured in graduated grooved board. Rice (2 g) placed in boiling water bath without pre-soaking and cooled until centre becomes translucent, determined by sampling every minute from 10 min onwards
6	Fifty grains (not presoaked) boiled for 15 min and sampled at 2 min intervals until 90% of the grains had translucent centers, after which 10grains are measured
7	Rice grains (2 g) boiled for 22 min without presoaking.
8	Milled rice (7 g) is washed, soaked for 30 min and a duplicate 7 g sample soaked for 60 min in 12·6 g (1·8 volumes) water in aluminum pudding cup and cooked in a rice cooker with water (200 ml) for 32 min (at least 20 min at >99°C). Cooked rice is allowed to stand 15 min before measuring 10grains in the middle layer with caliper gauge
11	No presoaking. Grains measured directly with microscope fitted with an eyepiece graticule
14	15 min cooking time in boiling water
15	Aggregate length and width of 10 random raw and cooked grains measured
16	28 min heating of rice (10 g) in water (50 ml) in 250 ml beaker using hot plate

4. Mechanism of kernel elongation

Although the mechanism of kernel elongation of rice is not clear. Water uptake might be the only trait showed positive and significant influence on kernel elongation. The anatomical study of the endosperm of elongating and non elongating varieties showed variation in the shape and arrangement of cells. Elongation pattern might be influenced by either size and arrangement of cells in endosperm or structural arrangement of starch molecules within cells and in non elongating rice, long belt of radially arranged cells might restrict linear expansion of cells while cells in the intermittent region may tend to expand towards the periphery leads to breadthwise elongation [12]. Aging also influences the kernel elongation [13].

Both genetic and environment factors influence the kernel elongation. The ambient temperature of about 25°C during day time and 21°C during night at the time of ripening is a favourable condition for maximum elongation [14]. Pre-soaking before cooking also play major role in kernel elongation [15].

Grain width and length, endosperm cell size and starch granule structure and arrangement are influenced the cooked kernel elongation. No specific mechanism to determine kernel elongation, but it might be due to physical and chemical phenomenon occurred after cooking [16].

Soaking of grains upto 30 min increases kernel elongation during cooking and leads to grain cracking. Elongating and non-elongating rices showed no difference in the composition of endosperm cell walls.

In basmati rice, equidistant pentagonal or hexagonal cells arranged in honeycomb fashion in the elongating variety and in breadth wise swelling types, long, rectangular and arranged radially in columns extending from the centre to the periphery. Microscopic examination of endosperm sections of the experimental materials revealed differences between elongating and non elongating groups of rice in respect of shape and arrangement of cells as represented by Sood et al. in **Figure 1**

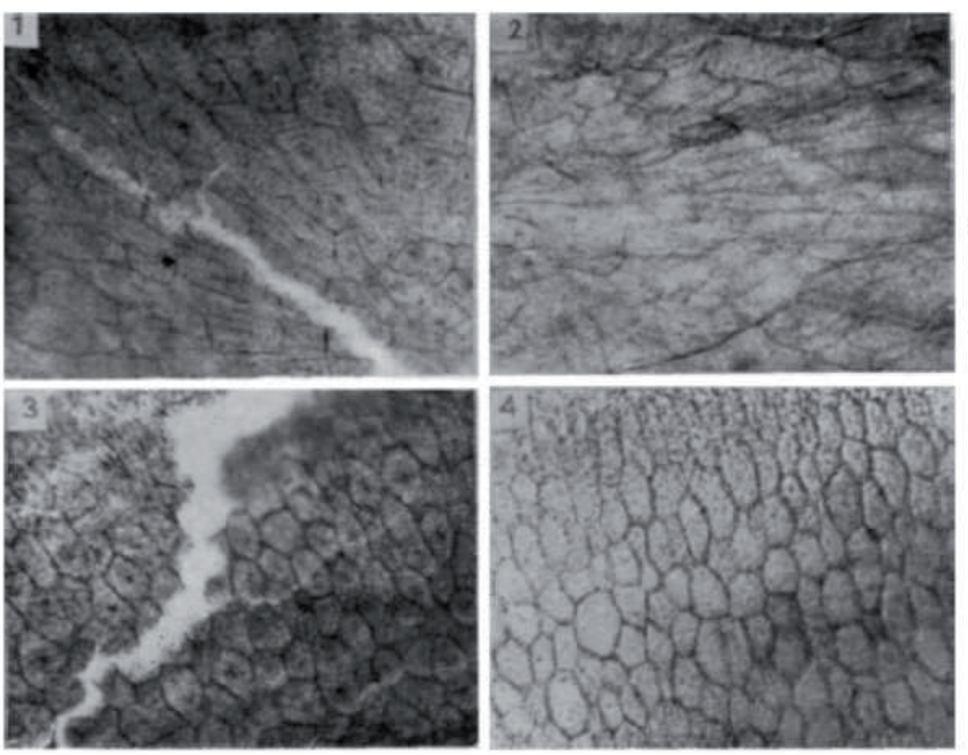

Figure 1.
Anatomical study of elongating and non elongating rice kernels. (1, 2) Transverse sections of elongating and non elongating types (3, 4) Longitudinal sections of elongating and non elongating types. Source: Sood et al.

Internal kernel cracks were higher in aged rice kernels than the non-aged rice kernels in three popular Malaysian rice cultivars (Mahsuri, Mahsuri Mutant and Puteri). The number of cracks in kernel during cooking increased at higher temperature (110°C) compared to lower temperature (90°C). Kernel aging and temperature positively associated with internal physical structure of the rice kernel. From the above study, it is concluded that ageing and internal kernel cracks might influence the cooked kernel elongation [17].

5. Genetics of kernel elongation

Kernel lengthwise increase without increase in girth is a desirable characteristic in high quality premium rice. Bao *et al.,* [18] reported that in *indica* rice variety, cooked kernel elongation was governed mainly by major gene with environmental interactions. Genetic improvement of any characters depends on efficiency of selection among the progenies which has different genetic value. Gene actions are reported to be associated with breeding value. To estimate the breeding value of progeny by studying the additive and dominance effects and their interactions which would benefit breeders to select the most appropriate breeding approaches to develop the superior variety.

Inheritance pattern of cooked kernel elongation ratio is controlled by single gene and influenced by some modifiers [13]. Kernel elongation governed by additive [19] or non-additive [20–28] or both additive and non additive gene action [29]. Hence genetics of kernel elongation has not been elucidated due to its complex nature and inconsistent pattern [30].

Elongation ratio and elongation index are highly heritable [31, 32]. This trait under polygenic control, involving epistasis, additivity, and genotype × environment interactions [33]. Three crosses were made among Mahsuri mutant, Mahsuri and 9192 to study the segregation pattern of cooked kernel elongation. The frequency distribution of kernel elongation ration of the population showed bimodal curve. However, in crosses of Mahsuri mutant and mahsuri (and its reciprocal crosses) the bimodal was skewed towards lower kernel elongation. Hence this character might be influenced by one or two loci [13].

The parental lines did not showed significant differences in kernel elongation ratio, however the progeny exhibited much larger range of variation due to transgressive segregation. It is difficult to conclude about inheritance pattern of kernel elongation in rice since the segregation pattern is not stable in different crosses and very little information is available on about inheritance pattern of kernel elongation of rice [34].

Genetic models used to analyse the cooked rice elongation traits of indica rice in two environments and identified that rice grain quality was mainly controlled by genotype, major gene effects and environment interactions. Milled and cooked rice length exhibited higher narrow sense heritability's than cooked rice elongation which indicate that early selection would be possible for milled and cooked rice length but not for cooked rice elongation [33].

6. Association of kernel elongation with other quality traits

Waxy gene allele involved in determination of cooked rice elongation. The closely linked marker of waxy locus will facilitate the replacement of poor quality parent alleles [11] and waxy gene region control the cooked rice grain quality traits viz., elongation, length, width, width expansion ad water absorption [35].

Elongation index showed positive significant association with most of the grain quality traits which might be due to starch deformation caused by soaking or boiling.

Water absorption showed positive association with cooked rice elongation [36]. Aroma showed positive correlation with kernel elongation and both are highly influenced by environment. Elongation of rice can be influenced by both the l/b ratio and the amylose contents [37]. Kernel elongation is a physical phenomenon and influenced by gelatinization temperature [38]. Grain elongation has been associated with intermediate amylose and low gelatinization temperature.

Gene or quantitative trait loci for aroma and cooked kernel elongation were linked and present on chromosome 8.

7. QTL associated with cooked kernel elongation ratio

A QTL was identified on chromosome 8 for kernel elongation [39]. Rani [40] found that a functional marker targeting an SNP in the GS3 is associated with kernel elongation. Tian *et al*. [36] detected 3, 2, and 2 QTLs for water absorption, volume expansion and cooked rice elongation, respectively in a DH population.

Amarawathi *et al.* [41] identified a QTL (*elr11–1*) for linear elongation ratio in chromosome 11 in the marker interval of RM1812 – RM209. Mallikarjuna *et al.* [42] reported that 1 QTL on chromosome 3 in *Oryza nivara* × Swarna derived backcross populations in the marker interval RM55–RM520. Two QTLs were identified for KLAC and both were derived from *O. nivara*, and these were located on chromosomes 5 and 12. Dewei *et al.* [43] identified 12 QTLs for rice elongation traits were detected on chromosomes 3, 4, 6, 8, 9, 10, and 11, among which two QTLs for MRL were located on chromosome 3, one QTL for MRL on chromosome 8, four QTLs for CRL on chromosome 3, 6, 8, and 9, and five QTLs for CRE on chromosome 4, 6, 9, 10, and 11.

Acga *et al.* [44] identified two QTLs for grain elongation on chromosome 2, designated *qGE-2-1* and *qGE-2-2*. The *qGE-2-1* mapped to the interval RM53-RM174, while *qGE-2-2* was mapped to the marker interval RM525-RM6, One QTL for grain elongation was previously reported on chromosome 2 in the marker interval R2510 - RM211 in the study conducted by Ge *et al*. [35]. Chen [45] reported that RM44 is associated with kernel elongation. Sathyasheela [46] reported that RM 209 was associated with LER. Liu *et al.* [47] detected three CRE QTLs on chromosome 4, 5, and 12, respectively, and the *qCRE-4* on chromosome 4 near *qER-4* was detected in this study. Li *et al.* [48] mapped a CRE QTL on chromosome 3, with the favorable allele obtained from the African rice *O. glaberrima*. Using a RIL population, Wang *et al.* [10] identified four CRE QTLs on chromosome 3, 6, 7, and 8, respectively.

8. Applications of CRISPR/Cas9 for rice grain quality improvement

Rice grain quality improvement through targeted genome editing is a fast, sustainable and cost effective approach. Conventional plant breeding methods depends on naturally existing germplasm variations. The introgression through backcrossing requires much time and screening of large population by marker assisted selection requires more energy. The reverse genetic approaches enhance the speed of plant breeding through targeted genome modifications [49] (**Figure 2**).

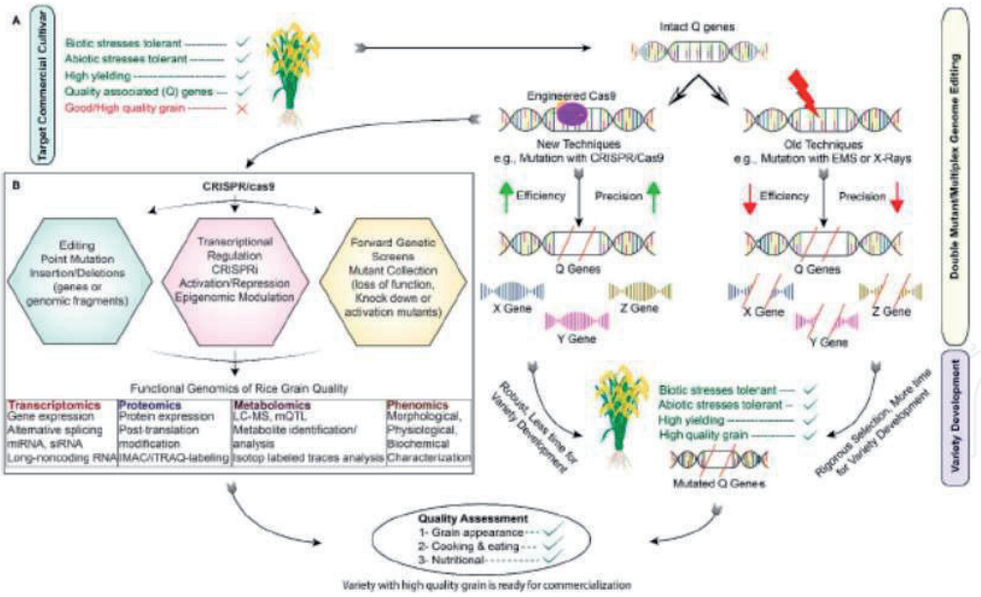

Figure 2.
An illustration of rice grain quality improvement through the CRISPR/Cas9 system.

9. Conclusions and future perspectives

Premium quality rice grain is the demand of a growing population with better living standards. Presently, the CRISPR/Cas9 system has all genome editing capabilities, e.g., knock-in, knockout, knockdown, and expression activation. This system has tremendous untapped potential, has formed an ever-expanding genetic toolbox for plant biologists to investigate functional genomics, and is a helping hand for breeders to integrate important genes into the genomes of important crops. The successful application of CRISPR/Cas9 for tissue engineering and human stem cell modification has led to further developments in the field of precise genome editing. The ability to target multiple genes via multiplexed genome editing strategies can facilitate pathway-level research to engineer complex multigenic rice grain quality attributes. Previously, few studies have been conducted that are related to targeted mutagenesis for rice grain quality improvement. The pathways of rice grain quality are not well understood, and they can be investigated for the genetic mechanisms controlling quality attributes. The development of novel regulatory components from naturally existing peripherals (genes, promoters, cis-regulatory elements, small RNAs, and epigenetic modifications) can facilitate the engineering of regulatory pathways for different elements of rice grain quality. The rapid shift of research toward the utilization of CRISPR/Cas9 systems for targeted mutagenesis could be a promising approach for overcome barriers to breeding improved quality rice.

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