Inter-relationship among Quality Traits in Fine Grain Scented Rice Mutants Derived through Recurrent Mutagenesis

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Abstract

Seventy five fixed mutant lines derived from recurrent mutagenesis (EMS, NG and their combinations) of PB 1, Pusa Sugandha 2 and a popular local scented rice variety Ketakijoha were assessed for seed yield and sixteen physical and cooking quality traits. Grain yield was unique to exhibit significant negative correlation with Kernel L/B ratio, but positive significant correlation with gel consistency among all 16 physico-chemical quality features. Volume expansion ratio had no significant correlation with yield and quality features except hulling percentage having significant negative correlation. Grain length had significant positive correlation with kernel length before and after cooking. So, it may serve as an ideal parameter to the consumers for better cooking quality. Hulling percentage was found to have significant positive correlation with gelatinization temperature but correlated negatively with gel consistency and alkali spreading value. This envisaged that the genotypes with high hulling percentage would separate upon cooking and develop less splitting of kernels which are desirable. Alkali Spreading Value (ASV) had perfect negative relationship with gelatinization temperature and significant positive correlation with gel consistency. Aroma had significant negative correlation with gel consistency indicating that the varieties which do not split upon cooking would have high aroma. The above information would be of immense value for breeding of basmati type genotypes for export value.

Key words: Fine grain scented rice mutants, inter-relationship, grain yield, physical and cooking quality traits.

India is an exporter of world class aromatic rice. Basmati rice is a resident of India by

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birth. Indian basmati - the soft cooking aromatic rice is acclaimed as the best quality rice in the world market. It has potential to contribute sizeable foreign exchange in the national economy. India is the largest producer and exporter of basmati rice in the world. The annual production in the country hovers at around 10-15 lakh tones a year, of which around two-third is exported. Self-sufficiency in rice production and enhanced purchasing power have resulted changed life style and awareness among consumers to improve and diversify diets including quality of rice consumed. Aroma and cooked kernel elongation are the most important quality traits, which differentiate the highly valued aromatic rice from the other rice types. Although genes/ QTLs of these two traits are linked and present on chromosome 8 (Jain *et al.,* 2006), in reality, there seems to have inconsistent correspondence between these two important traits (Golam *et al.,* 2010) owing to environmental influence particularly temperature for expression of the characters. The temperature of 25°C at day time and 21°C at night during the ripening stage has been found to have favorable effect in aroma as well as kernel elongation.

Rice being a major staple food, pleasant flavour in cooked rice becomes a highly valued quality attribute. More than 100 volatile aromatic compounds have been detected in fragrant rice among which biochemical basis of aroma is mainly attributed to 2-acetyl-1-pyroline (2AP) (Tanchotikul and Hsieh 1991) and the fragrance gene (*frg*) was successfully identified by Bradbury *et al.,* (2005) in the aromatic rice. Betaine aldehyde dehydrogenase (BADH-2) is the key enzyme in the biochemical path way for synthesis of 2AP. The *frg* gene has eight non-functional alleles and a few functional alleles among which a single recessive allele *badh 2.1* is predominant in virtually all fragrant rice varieties today including Basmati and Jasmine types. More recently, three SNPs and an eight base pair-deletion in exon 7 of the gene encoding betaine aldehyde dehydrogenase 2 (BAD2) on chromosome 8 of rice were identified as the probable cause of aroma enzyme in aromatic rice (Bradbury *et al.,* 2005). Kibria *et al.,* (2008) reported three SSR markers RM 223, RM 342A and RM 515 linked to fragrance gene (*fgr*) locus on chromosome 8.

Lengthwise elongation of cooked kernel without or minimum increase in girth is the characteristic feature of high quality basmati rice (Khush *et al.,* 1979). The inheritance pattern of rice kernel elongation is controlled by a single gene and is influenced by some modifier genes (Golam *et al.,* 2004). However, Sood *et al.,* (1983) reported the involvement of both non-additive and additive types of gene affects with the former playing a predominant role in kernel elongation.

Keeping in view the consumer's perspective and export value, high grain yield along with genetic amelioration of cooking quality of rice assumes an important objective in current breeding programmme. However, the crosses so far made involving Pusa Basmati-1, Taraori Basmati and Basmati 370 have not rendered any remarkable breakthrough in genetic enhancement for productivity in basmati type rice. Besides, many often the delicate genetic background conforming to the basmati standard is disturbed in the segregating population derived from recombination breeding. Better understanding of inter- relationship among quality features and seed yield can be helpful to breed superior aromatic rice genotypes. Therefore, an attempt was taken to investigate the nature of inter-relationship among physico-chemical quality features and grain yield in a set of fixed mutant lines derived from a few well adapted fine grain Indian scented rice varieties.

Materials and Methods

The experimental materials comprised 75 fixed mutant lines (M_8 generation) derived through recurrent mutagenesis with EMS (0.2, 0.4 and 0.6%), NG (0.01, 0.015 and 0.02% and a combination of 0.4% EMS and 0.015% NG (following a pre-soaking period of 10.5 h in distilled water), their parents (Pusa Basmati-1, Pusa Sugandha -2 and a popular land race of Odisha "Ketakijoha local") and two standard check varieties (Geetanjali and Pusa Sugandha-4). The experiment was laid out in randomized block design with three replications. Freshly harvested grains of the test genotypes were assessed for grain yield and 16 quality parameters. The quality tests e.g., gel consistency (GC) was based on the method described by Cagampang *et al.,* (1973), The varieties with gel consistency > 60 mm, between 40-60 mm, and < 40 mm were considered soft, medium and hard gel genotypes, respectively. Alkali spreading value (ASV) and Gelatinization temperature (GT) scores ranging from 1- 7 were assessed as per Little *et al.* (1958). GT is defined as the temperature at which 90% of the starch granules would swollen irreversibly in hot water and it is scored indirectly as an inverse measure of ASV. In the present investigation, the cooking qualities e.g., cooked kernel length, cooked kernel length/breadth ratio, elongation ratio upon cooking were calculated as per Verghese (1950), Volume Expansion Ratio (VER) were determined as per method of Juliano and Perez (1984). Presence or absence of aroma was scored (0-3 scale) after 10 min. (Sood and Siddiq 1978) during alkali digestion of milled kernels soaked in 1.7 % KOH for 23 hrs for determination of ASV at 30^{o}C. Assessment of all these quality features was repeated thrice to minimize experimental error. Routine statistical procedures were followed for analysis of variance and covariance as per Singh and Choudhary (1976). The correlation coefficient for each pair of characters were computed following Al-Jibouri *et al.* (1958) and the significance of correlation coefficients was tested by't'- test at n -2 degrees of freedom.

Results and Discussion

Genetic relationship of different quality traits is very important for selection of genotype for export value. Kernel length of more than 7 mm., L/B ratio more than 3.5, intermediate ASV, moderate GT, intermediate AC, intermediate GC, moderately high volume expansion after cooking and pleasant aroma are the minimum quality features of basmati types.

In the present investigation, efforts have been taken to study and implicate the relationship of seed yield with some easily observable quality traits that determine cooking qualities. Grain yield was unique to exhibit significant negative correlation (-0.50) with Kernel L/B ratio but had significant positive correlation (0.47) with gel consistency only (to be discussed later) (Table 1). Grain yield was also observed to have moderate positive correlation with kernel breadth but negative relationship with most of the quality features including grain length, kernel length and kernel L/B. Long fine grain and kernel types attract consumer's preference and have export value. *GS3*- an evolutionarily important gene controls grain length in rice. An association study revealed that a C to A mutation in the second exon of *GS3* (A allele) was associated with enhanced grain length in *Oryza sativa*, but was absent in other species of *Oryza* (Takano-kai *et al.,* 2009). Pusa 1121(Pusa Sugandha-4), an Indian basmati type rice is acclaimed as world's longest grain rice variety with exceptionally high cooked kernel elongation (Singh and Singh 2002). Besides, ORM 250-3 and ORM 228-3: two mutants of PS-4 included in this pursuit, had more longer kernel (9.12 mm) than even PS-4 (9.04 mm) (Tripathy *et al.,* 2012). Grain length exhibited significant positive correlation with grain L/B ratio, kernel length, kernel L/B ratio and kernel length after cooking as similar to the findings of Vivekanandan and Giridharan (1998). Besides, positive significant association of kernel length with kernel L/B ratio and cooked kernel length observed in this pursuit was in agreement with Lin (1978) and Somrith (1979). Hence, length of grain and kernel become ideal parameters to the consumers for better cooking quality. However, Chouhan (1996) reported significant positive association of kernel length with kernel L/B ratio but negative association with kernel breadth in some crosses of aromatic x non-aromatic varieties.

Genetic variation is a common feature for kernel recovery after hulling in any set of test material. The recovery of whole kernel varies between genotypes mainly due to extent of compactness of the starch grains in the endosperm, air space between hull and kernel, and hull thickness. About 20-25 per cent of grain weight is reduced due to hull after hulling depending upon the genotype. Besides, the whole kernel in which endosperm with aleurone layer and embryo remain intact, reflects the real nutritional value of kernel in terms of starch, vitamins and protein content. Hence, relationship of hulling percentage with other quality traits and yield *per se* is of great importance. In the present investigation, hulling percentage is found to have significant positive correlation with GT but correlated negatively with GC and ASV. This envisaged that the genotypes with high hulling percentage would separate upon cooking and develop less kernel splitting which is highly desirable.

Volume expansion ratio is the measure of relative increase in volume of kernel after cooking and thus, the genotypes having high volume expansion are of great value to the consumers. But, results indicated that there is no significant correlation of Volume Expansion Ratio (VER) with any of the quality features except hulling percentage with significant negative correlation which is undesirable. In contrast, it had considerable degree of positive association with grain yield indicating recovery of a few high yielding genotypes with high volume expansion. Chouhan (1995) indicated significant positive association of volume expansion with raw kernel length and kernel L/B ratio. However, Nayak *et al.* (2003) reported that genotypes with high kernel elongation after cooking resulted less volume expansion.

Kernel elongation ratio was shown to have positive correlation with cooked kernel length (r=0.523) and cooked kernel L/B ratio (r=0.567) (Table 1). Sadhukhan and Chattopadhyay (2000) observed positive association of kernel elongation ratio with kernel length after cooking. In contrast, elongation after cooking was also reported to be negatively correlated with kernel length (Kumari and Padmavati1991) and positively with kernel breadth (Bocevska *et al.,* 2009).

Gel consistency (GC) separates high amylose rices into hard gel (26-40 mm) and low amylose rices into soft gel (>61mm). Rice varieties with medium gel consistency (41-60 mm) are preferred as the cooked rice remains separate (non-sticky) and soft (palatable). GC measures the tendency of cooked rice to harden when it cools down and therefore, it serves as a good index of cooked rice texture. The differences between hard and soft, hard and medium, and medium and soft gel consistency are under monogenic control and that modifiers affect the expression of the trait. Multiple alleles in the same locus designated as *geca* for medium gel consistency and *gecb* for soft gel consistency, were recessive to the wild type allele (GEC) for hard gel consistency; and *geca* was dominant over *gecb* (Tang *et al.,* 1991). In the present investigation, grain yield/plant was observed to have moderate positive significant correlation (0.47) with gel consistency only. High gel consistency (>61mm) is the indication of low AC which is undesirable leading to inferior cooking qualities; particularly, the kernels become soft, sticky and intermingled after cooking and thus, not suitable for table purpose. Hence, high yielding genotypes are qualitatively poor and these have limited scope for export purpose. Sadhukhan and Chattopadhyay (2000) reported positive association of grain length with AC. In contrast, Samal *et al*. (2014) observed negative correlation of AC with kernel length in a set of aromatic germplasm.

ASV, GT and GC had shown to have typical *inter se* correlation which are usually taken as indicators for AC of a genotype and hence the cooking quality. ASV had perfect negative relationship with GT and significant positive correlation with GC. GT refers to the range of temperature within which starch granules start swelling irreversibly in hot water. In other words, GT determines the time taken to cook rice. Rice genotypes with high gelatinization takes longer time to cook, expand more, elongate less and many often remain uncooked under standard cooking procedure (Singh *et al.,* 2002) and hence, least preferred. The GT of rice varieties are classified as low (55-69^{0}C), intermediate (70-74^{0}C) and high (75-79^{0}C).

In the present investigation, GT was numerically scored 1-7 for low to high in relation to high or low ASV value. In general, varieties with low alkali spreading value and gel consistency are expected to harbor high AC. But, a genotype should have an intermediate AC of 20-25% to fulfill the minimum standard of basmati types. Hence, selection of a genotype with delicate balance of ASV, GT and GC at intermediate or moderate level is indispensable for their suitability for export purpose.

Aroma had significant negative correlation with GC indicating that, in the present set of material, the varieties which do not split upon cooking would have high aroma. This association is desirable in basmati types and a priority consideration for breeding of genotypes for export value. In contrast, the local types, barring a few do not bear such favorable association. For instance, Leelavati - a short bold grain local land race gives high scent upon chewing and cooking, but its kernels become sticky and split upon cooking due to high gel consistency making it not suitable for table rice. The present study on analysis of physical and quality traits of aromatic rice will be of value in breeding high yielding Basmati type rice.

References

Al-Jibouri H. A., Mulle, P. A. and Robinson, H. F. 1958. Genotypic and environmental variances and covariances in a upland cotton crosses of interspecific origin. *Agronomy Journal 50:633-636.*

Bocevska M., Aldabas I, Reevska D. and Ilieva V. 2009. Gelatinization behavior of grains and flour in relation to physico-chemical properties of milled rice (Oryza sativa L). *Journal of Food Quality* 32(1): 108–124.

Bradbury L. M., Fitzgerald T. L., Henry R. J., Jin Q. and Waters D. L. 2005. The gene for fragrance in rice. *Plant Biotechnology Journal.,* 3: 363-370.

Cagampang G. B., Perez C. M. and Juliano B. 1973. A gel consistency test for eating quality of rice. *Journal of the Science of Food and Agriculture* 24; 1589-1594.

Chouhan J. S., Chouhan V. S. and Lodh S. B. 1995. Cooking quality components and their inter-relationship with some physico-chemical characters of rainfed upland rice grain. *Oryza,* 32:79-82.

Chouhan J. S. 1996. Segregation analysis and estimation of genetic parameters for some quality traits in F2 generation in rice. *Oryza* 33: 168-173.

Golam Faruq, Mohamad. O., Hadzim K. and Craig. A. M. 2004. Inheritance of kernel elongation in rice. *In: Proceedings 4th International Crop Science Congress(Poster session), September Brisbane, Australia.p*-1-4.

Golam Faruq., NorZulaani K., Jennifer A.H., Subha B., Zulqarnain M., Osman M., Nazia A. M., Zulqarnian M. and Mohammad. O. 2010. Evaluation of kernel elongation ratio and aroma association in global popular aromatic rice cultivars in tropical environment.

African Journal of Agricultural Research, 5(12): 1515-1522.

Jain N., Jain S., Saini N. and Jain Rajinder K. 2006. SSR analysis of chromosome 8 regions associated with aroma and cooked kernel elongation in Basmati rice. *Euphytica* 152: 259-273.

Juliano B. O., and Perez C. M., 1984. Results of a collaborative test on the measurement of elongation of milled rice during cooking. *Journal of Cereal Sciences* 2: 281-292.

Kibria K., Islam M. M., and Begum S.N., 2008. Screening of Aromatic rice Lines by phenotypic and molecular markers. *Bangladesh Journal of Botany* 37(2): 141-147.

Little. R.R., Hilder G.B., and Dawson E.H., 1958. Different effect of dilute alkali on 25 varieties of milled white rice. *Cereal Chemistry* 35: 111-126.

Nayak. A.R., Chaudhury. D. and Reddy. J.N. 2003. Inter-relationship among quality characters in rice. *Indian Journal of Agricultural Research* 37(2): 124-127.

Samal, K.C., Rout, G.R. and Das, S.R. (2014). Study of genetic divergence of Indigenous Aromatic Rice (*Oryza Sativa* L.): Potentials and consequences of on-farm management in traditional farming. *International Journal of Agricultural Sciences*, 4(4): 176-189.

Singh. R.K. and Choudhary. B.D. 1976. Biometrical techniques in genetics and plant beeding. *(Varghese TM ed.), International Bioscience Publishers, Hissar, India* p. 56-71.

Singh. L., Singh. J.D., and Sachan. N.S., 2002. Inter-character association and path analysis in paddy. *Annals of Biology* 18(2): 125-128.

Singh.V.P., and Singh. A.K., 2002. Pusa 1121: a rice line with exceptionally high cooked kernel elongation and basmati quality. *International Rice Research Newsletter* 27(1):25-26.

Sood. B.C., Siddiq. E.A. and Zaman. F.U, 1983. *Genetic analysis of kernel elongation in rice.* Indian Journal of Genetics *43: 40-43.*

Sood. B.C. and Siddiq. E.A.1978. *A rapid technique for scent determination in rice.* Indian Journal of Genetics & Plant Breeding *38: 268-271.*

Takano-Kai Noriko. T., Jiang. H., Kubo Takahiko, Sweeney. M., Matsumoto. T., Kanamori. H., Padhukasahasram. B., Bustamante. C., Oshimura Atsushi Y., *Doi Kazuyuki., and McCouch Sushan 2009. Evolutionary History of GS3, a Gene Conferring Grain Length in Rice*. Genetics 182*(4): 1323-1334.*

Tanchotikul. U. and Hsieh. T.C.Y. 1991. *An improved method for quantification of 2-acetyl-1-pyroline: a popcorn-like aroma, in aromatic rice by high-resolution gas chromato-graphy/ mass spectrometry/selected ion monitoring.* Journal of Agriculture & Food Chemistry *39: 944-947.*

Tang. S.X., Khush. G.S. and Juliano. B.O. 1991. Genetics of gel consistency in rice (*Oryza sativa* L.). *Journal of Genetics* 70(2): 69-78.

Tripathy Swapan K., Mishra Dibya R,, Rajesh Ranjan., Bastia D.N., and Das. S.R. 2012. Identification of some useful scented rice mutants for grain yield and quality traits. *Oryza*, 49(3): 227-230.

Verghese E.J. 1950. A standard procedure for cooking rice for experimental purposes. *The Madras agriculture Journal* 37: 217-221.

Vivekanandan P, and Giridharan S. 1998. Genetic variability and character association for kernel and cooking quality traits in rice. *Oryza* 35(3): 242-245.

Table 1: Correlation among grain yield/ plant and sixteen quality characters in 20 selected rice genotypes

Chara-cters	G.B	G.L/B	K.L	K.B	K.L/B	Hull. %	C.L	C.B	C.L/B	E.R	V.E.R	A.S.V	G.T	G.C	Aroma	GY/P
G.L	0.32	0.74**	0.85**	-0.25	0.70**	-0.08	0.61**	0.26	0.32	-0.18	-0.06	0.09	-0.09	0.11	0.37	-0.41
G.B		-0.39	0.20	0.36	-0.08	-0.31	0.16	0.05	0.04	-0.05	-0.17	0.12	-0.12	0.05	0.09	-0.20
G.L/B			0.69**	-0.49*	0.74**	0.13	0.46*	0.23	0.26	-0.17	0.06	0.03	-0.03	0.10	0.29	-0.23
K.L				-0.19	0.76**	0.17	0.63**	0.22	0.38	-0.33	-0.27	0.01	-0.01	0.01	0.22	-0.35
K.B					-0.78**	-0.25	-0.22	-0.20	-0.05	-0.05	-0.09	0.11	-0.11	0.12	-0.33	0.40
K.L/B						0.27	0.55*	0.24	0.29	-0.17	-0.13	-0.04	0.042	-0.06	0.38	-0.50*
Hull. %							0.17	0.02	0.14	0.03	-0.36	-0.53*	0.53*	-0.52*	0.39	-0.07
C.L								-0.05	0.81**	0.52*	-0.19	-0.08	0.08	-0.11	0.25	-0.42
C.B									-0.60**	-0.30	0.06	-0.13	0.13	0.35	-0.18	0.03
C.L/B										0.56**	-0.09	0.01	-0.01	-0.25	0.28	-0.27
E.R											0.07	-0.10	0.10	-0.14	0.05	-0.13
V.E.R												0.10	-0.10	0.28	-0.23	0.37
A.S.V													-1.0**	0.51*	-0.34	-0.01
G.T														-0.51*	0.34	0.01
G.C															-0.47*	0.47*
Aroma																-0.34

***- significant at $P_{0.05}$, **-significant at $P_{0.01}$**