Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.7122

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **7 (3):** 67-73 (2019)

Research Article

Capturing the Genetic Diversity for Grain Quality Attributes in a Set of Temperate Rice (*Oryza sativa* L.) Germplasm by Cluster Analysis and the Assessment of *Wx* gene Polymorphism

Arpit Gaur*, Ghulam Ahmad Parray, Asif Bashir Shikari and Sofi Najeeb

Division of Genetics and Plant Breeding

Sher-e-Kashmir University of Agricultural Sciences & Technology - Kashmir

Srinagar (190025), Jammu & Kashmir, India

*Corresponding Author E-mail: drarpitgaur@gmail.com

Received: 6.12.2018 | Revised: 11.01.2019 | Accepted: 20.01.2019

ABSTRACT

This study analysed the genetic diversity in a set of ninety elite genotypes of temperate rice on the basis of grain quality attributes and polymorphism in Wx gene. The UPGMA cluster analysis amalgamated the genotypes in five major cluster each having 8, 21, 36, 12 and 13 genotypes, respectively. The majority of genotypes were characterized with low amylose, soft gel consistency and intermediate gelatinization temperature. Two genotypes namely Mushkbudji and Kamad were registered as aromatic rice. The principal component analysis explained the contribution of each component to total variance. First three components viz. KLBC, KLAC and KBBC were found to be contributing in 52.34% of variation. The assessment of the polymorphism in Wx gene was also done with the help tightly linked RM190 microsatellite. A total eight different types allele were produced in 76 genotypes by RM190 SSR marker. The RM190 was successful in explaining 40.23% of total phenotypic variations. The UPGMA dendrogram, drawn on the basis of genotypic data, further cluster the 76 genotypes in six major clusters.

Key words: *Genotypes, Rice, Yield, Food, Grain*

INTRODUCTION

Rice (*O. sativa* L.) is a primary source of carbs in many parts of the world especially in Asian countries. Being a major staple food for millions of people the emphasis has always been given to the development of yield performing rice varieties. However, in past few decades the importance of rice grain quality attributes has widely been recognised. Since, rice is a cereal consumed as a whole, the consumers' preference for rice grain widely depends on its cooking quality, taste, aroma, shape and size[1,2]. Moreover, with improvement in the living standards of people the demand on the rice grain quality have become more diverse[3]. Realization towards the importance of grain quality had made it one of the primary considerations of plant breeder.

Cite this article: Gaur, A., Parray, G. A., Shikari, A.B. and Najeeb, S., Capturing the Genetic Diversity for Grain Quality Attributes in a Set of Temperate Rice (*Oryza sativa* L.) Germplasm by Cluster Analysis and the Assessment of *Wx* gene Polymorphism, *Int. J. Pure App. Biosci.* **7(3):** 67-73 (2019). doi: http://dx.doi.org/10.18782/2320-7051.7122

Success of crop improvement programmes greatly depend on the magnitude genetic variability in the base population. However, limited studies have been conducted to evaluate the extant of genetic variability for the grain quality attributes of rice of different geographical origins. Kashmir valley of India is enriched with numerous landraces of temperate rice (*O. sativa* L.). Little or no information is available in reference to their grain quality attributes are available in these landraces. Since landraces are the morgue of valuable gene complexes which have a wider adaptability towards the varying environmental conditions the assessment of these landraces or traditional rice varieties may prove invaluable for the future breeding programmes aimed to grain quality.

Therefore, the present investigation was designed with two major objectives. First, to uncover underlaying genetic diversity with the help of Principal Component Analysis (PCA) and cluster analysis techniques as, these techniques have been found extensively useful in unveiling the genetic diversity in various crops[4]. PCA is robust tool to reduce the multiple dimensions of the observed variables to a small central dimensionality of independent variables and identify the highest contributor to the total variation at each axis of differentiation[5,6]. Cluster analysis defines study material under different groups on the basis of variables[4]. Second, to detect the polymorphisms in the starch synthesising gene *i.e. Wx* gene with the help of a tightly linked polymorphic SSR marker RM190[7].

MATERIAL AND METHODS

Material and Variables: In the present investigation a total of ninety elite genotypes of temperate rice (*Oryza sativa* L.), procured from the germplasm repository of MRCFC, SKUAST-Kashmir, were studied to capture the underlaying genetic diversity on the basis of various grain quality parameters like kernel length before (KLBC) and after cooking (KLAC), kernel breadth before (KBBC) and after cooking (KABC), length to breadth ratio (LBR), kernel expansion ratio (KER), amylose content (AC), alkali spreading value (ASV) and gelatinization temperature (GT). In addition to this assessment of polymorphism in *Wx* gene was also done with the help of simple sequence repeat (SSR) marker RM190, a tightly linked polymorphic microsatellite[7].

Phenotyping and Genotyping: The phenotyping of the procured study material was done by the standard methods previously adopted by Gaur *et al.*[8]. Furthermore, for the assessment of *Wx* gene polymorphism total genomic DNA was extracted from the 21 days-old seedlings of all the genotypes using CTAB (Cetyl Trimethyl Ammonium Bromide) method of Murray and Thompson[9]. DNA amplification was carried out in PCR tubes containing 10µl reaction mixture. Reaction mixture contained 2µl (30ng/µl) of template DNA, 1µl (0.5µl Reverse + 0.5µl Forward) SSR Primer RM190 (F: *5'CTTTGTCTATCTCAAGACAC-3'* and R: *5' TTGCAGATGTTCTTCCTGATG-3'*)[7], 1.3µl 10x PCR Buffer with $MgCl_2$, 1µl of dNTPs (2.5mM) and 1U of *Taq* DNA polymerase. Amplifications were performed on a Takara PCR thermo-cycler with a PCR profile of initial denaturation of 95°C for 4 minutes, denaturation of 94°C for 45 seconds, annealing of 55°C for 30 seconds, extension of 72°C for 1 minutes for a total of 35 cycles repetition from denaturing and a final extension of 72°C for 10 minutes followed by storage cycle at 4^oC for ∞ time. The amplified products were resolved on the silver stained 12% PAGE {consisting of 4ml of Acrylamide: Bisacrylamide (29:1), 4ml of ddH_2O, 2 ml of 5X TBE buffer, 163µl of 10% APS (Ammonium Persulphate) and 8µl of TEMED (Tetramethylethylenediamene)}. The bands were visualized on white light illuminator. Bands were seen and scored on the basis of band size in base pair by comparing with a reference of 100 bp molecular DNA ruler. All the scorable bands were considered as single locus/allele.

Statistical Analysis: Suitable R-packages were used for statistical analysis.

RESULTS AND DISCUSSION

Cluster analysis: The UPGMA agglomerative clustering analysis amalgamated all ninety genotypes into five major clusters (Table 1), while covering 63 percent of total germplasm lines in Cluster III (*n = 36*) and II (*n = 21*). Whereas, rest 37 percent of the germplasm was unequally distributed in cluster V (*n = 13*), IV (*n = 12*) and I (*n = 8*). This supports the existence of substantial genetic diversity among the studied genotypes.

Table 1: Clustering of 90 temperate rice genotypes

Cluster	No. of Genotypes	Name of Members
I	**8**	Kosar, Mushkandi, Mehvan Green, Noor Miree, Mushkbudji, SK-402, Kamad, Kuch
II	**21**	Aziz Beoul, Katwar, Kuch, Never, Kalbrar, Mirzug, Kaw Kareer, Nikaloul Anzul, Prenev Wal, Qadir Beigh, IRBN, Shal Kew, Irbn 2008 V20, Safid Kuch, Tral Zagad, Zagar, Purple, SK/PBG/93, SK/PBG/95, SAW/GM2/224, SAW/GML/188, K-39, Reshim
III	**36**	Begum, Bala Baber, Baber, Baber Safed, China Baber, Gura, Keon, Gull Bara, Kew, Mehavan Purpule, Niver Zag, Nika Katwar, Pren Zagir, Ramhall, Rehman Balli, Peeneh Wal, Siga, Tilzag, Tumla Hall, Watezag, Wazul Krer, IRBN 2008 V8, Mazhale, SK/PBG/51, SK/PBG/64, SK/PBG/67, SK/PBG/75, SK/PBG/79, SK/PBG/84, SK/PBG/86, SK/PBG/90, SK/PBG/94, SAW/GML/280, SAW/GML/295, SAW/GML/317, K-332, SK-408
IV	**12**	Bala Koun, Gurah, Hapat China, Kaw Qudur, Larbeoul, Laul Anzul, Pren Niver, Poot Brar, Sig Safed, Zagia, SK/PBG/68 SK/PBG/91
V	**13**	Black Rice, Budgi, SK/PBG/98, SAW/GM2/214, SK 87041-TR-990-11-2-1, SAW/GML/252, SAW/GML/310, SAW/GML/327, SR-1, SR-2, SR-3, IRBN 2008 V III, SAW/GM/286

The cluster mean values of each 11 traits are presented in Table 2. It is evident form the table that members from cluster I, II, III and IV possess short and bold type of gains. Whereas, cluster V is characterized with long and bold type of grains. Furthermore, it was reported that all the clusters were characterized with low amylose content [AAC] (10-19%), soft gel consistency [GC] (>60 mm) and intermediate gelatinization temperature [GT] (70-74 °C). The mean value of thousand kernel weight (TKW) ranged from 13.87 g to 12.83 g between five clusters. Members of cluster I had a distinguish trait called aroma. Among the members of cluster I a genotype namely *Mushkbudji* was characterized with strong 2AP aroma however, rest of the members were characterized with mild to low level of aroma. These results were in confirmation with previous studies Sandhyakishore *et al.*[10], Garg *et al.*[11].

Table2: Cluster mean of 90 temperate rice genotypes for elven traits:

Traits	Cluster				
	I (8)	**II (21)**	**III (36)**	**IV (12)**	**V (13)**
TKW	13.73	13.50	13.87	12.83	12.99
KLBC	5.27	5.92	5.77	5.63	6.83
KLAC	7.79	7.49	8.62	7.64	9.57
KBBC	2.83	2.93	2.83	2.92	2.58
KBAC	4.24	3.55	3.66	3.57	3.56
LBR	1.87	2.03	2.05	1.94	2.68
KER	1.49	1.27	1.50	1.35	1.42
GC	70.71	58.38	64.16	127.61	69.3
ASV	5.12	4.05	5.08	4.25	4.62
ACC	14.72	15.32	12.61	13.91	15.44
Aroma	0.88	0.00	0.00	0.00	0.08

*in parenthesis number of total members in the respective cluster

Principal component analysis: results from the PCA explained the degree of contribution of each component to total variance and existing genetic diversity in our study material. The principal components were selected by following the criterion of Clifford and Stephenson[12], according to which first three components accounted for about 52.34% of total variations with 23.97%, 15.96% and 12.4% individual proportion of total variance, respectively (Table 3). To determine the traits with larger effects the criterion of Raji[13], which states that traits with loading values > 0.3 have a large enough effect to be considered important while those traits having loading values < 0.3 do not have significant effects. As per this criterion, in our study, the PC1 was most contributed with negative loading values of KLBC, KLAC, and LBR and positive loading value of KBBC. As a result, the PC1 differentiated those germplasms which had low KLBC, KLAC, and LBR values and registered with high KBBC. The PC2 further differentiated germplasm lines registered with higher KLAC, KBAC and KER as these traits contributed most positively here. Furthermore, germplasm lines with higher values of KLBC, KBBC, KBAC, and AAC and low values of GC were differentiated by PC3.

Table 3: Coefficient of first three Eigen Vectors for eleven grain quality traits:

Variables	**PC1**	**PC2**	**PC3**
TKW	0.1146	-0.0231	-0.1151
KLBC	-0.4374	-0.2613	0.3157
KLAC	-0.4431	0.4252	0.1804
KBBC	0.4259	-0.068	0.3617
KBAC	0.2015	0.3379	0.5149
L.B	-0.5917	-0.1179	-0.0414
KER	-0.0849	0.7072	-0.0711
GC	0.102	0.0311	-0.5122
ASV	0.057	0.1895	0.1469
ACC	-0.0108	-0.2443	0.3932
Aroma	0.0781	0.1453	-0.1154
Standard deviation	1.6239	1.3249	1.1681
Proportion of Variance	0.2397	0.1596	0.124
Cumulative Proportion	0.2397	0.3993	0.5234
Eigen Values	2.637	1.7555	1.3645

A two-dimensional biplot was developed between PC1 (*x-axis*) and PC2 (*y-axis*) with the help of loading values (Figure 1). Our results from UPGMA clustering were superimposed with biplot representation. This validates and confirm the results from UPGMA clustering. These results are in agreement with Gaur *et al.*[8] and Chakravorty *et al.*[4].

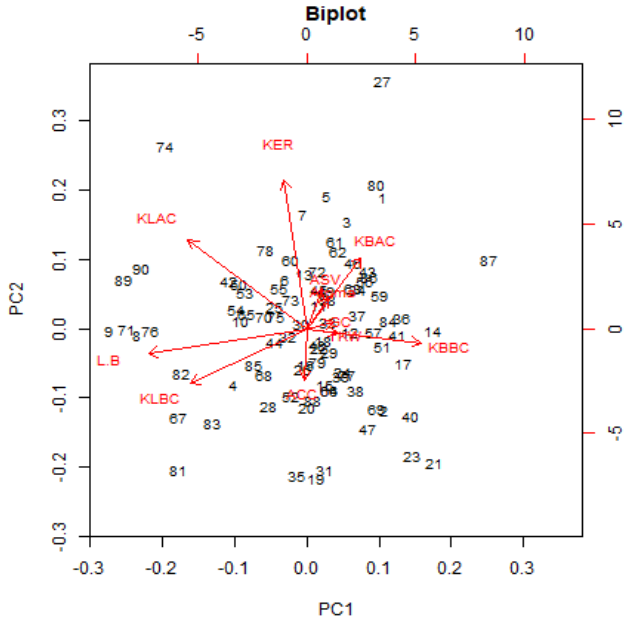

Fig. 1:

Polymorphism in Wx gene: Genotypic results produced from RM190 microsatellite was found useful in identifying substantial allelic variations in the studied germplasm (Figure 2). Out of ninety genotypes clear and ambiguous bands were produced in total 76 genotypes (Table 4). A total of eight different alleles explained at least 40.23% of phenotypic variations for amylose content among our germplasm lines. Using the results produced by RM190 microsatellite a dendrogram was developed which clustered all the 76 genotypes in to six major clusters based on the similarity distance between them (Figure 3). Phenotypic differences in the amylose content among genotypes with similar alleles might be due to the environmental effects and/ or due to the genetic background of those genotypes[14]. Previous studies have supported environmental factors may cause up to six percent variation in amylose content in a given genotype. Furthermore, calorimetry method used in the quantification of amylose content also detect various amount of long chain amylopectin. These results are in confirmation with Mahalingam *et al.*[15], Sharma *et al.*[16] and Vohara *et al.*[17].

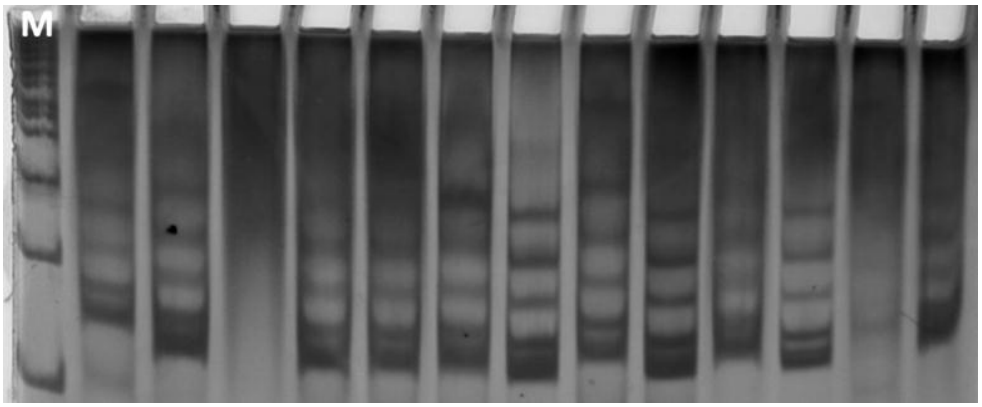

Fig. 2: A representative photograph of allelic variations in the ninety germplasm lines for amylose content by the microsatellite RM190 on PAGE

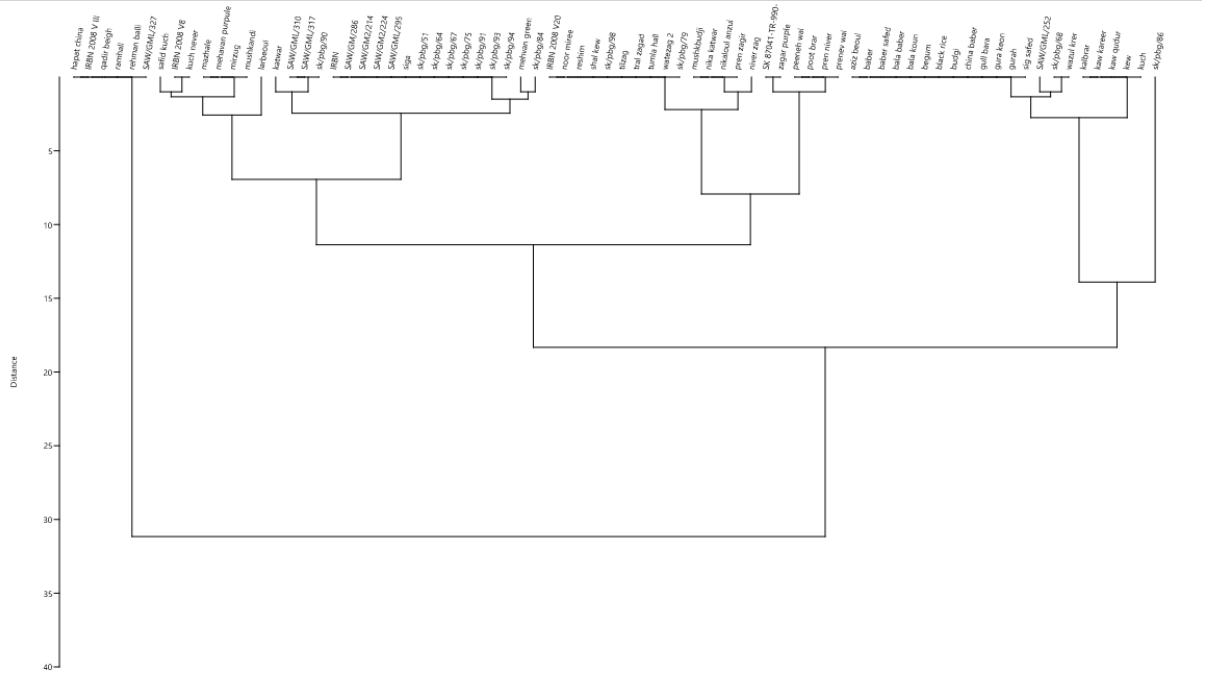

Fig. 3: UPGAM Dendrogram showing clustering of 76 genotypes based on the *Wx* gene polymorphism

SUMMARY AND CONCLUSION

The grain quality attributes of rice directly affect its market value. Therefore, in recent years rice grain quality breeding has become a major attraction. However, availability of genetic diversity for grain quality attributes is a perquisite for such breeding programmes. We evaluated a total of ninety germplasm lines of temperate rice for elven traits defining rice grain quality. Substantial genetic diversity was reported by us in the studied material for all those traits. These germplasm lines can be taken as breeding material in future breeding programmes especially focusing on the rice grain quality. Furthermore, two landraces namely, *Mushkbudji* and *Kamad* can be used as source of aroma gene(s). We also reported a considerable level of genetic divergence for the *Wx* gene using RM190 microsatellite marker. RM190 was found to be useful in explaining genetic diversity for amylose content at molecular level. Henceforth, it can be utilised in marker assisted breeding programmes.

Acknowledgment

Authors are highly thankful to Department of Biotechnology (DBT Project No. DBT/AGII//935/2014) and AICRP-Rice for financial supports. Authors are also thankful to Associate Director Research, MRCFC Khudwani for providing invaluable experimental plant material.

Note: Phonetic and linguistic barriers might have led to errors in designations of genotypes in typing.

REFERENCES

1. Juliano, Bienvenido, O., *Rice chemistry and quality*. Manila, Philippines: Philippine rice research institute (2003).
2. Gaur, Arpit, Shabir, H., Wani, Sah, S. K. and Asif, B., Shikari, Biochemical, Genetic and Genomic Perspectives of Flavor and Fragrance in Rice. **In**: *Science and Technology of Aroma, Flavor, and Fragrance in Rice*, pp. 289-312. Apple Academic Press (2018).
3. Balakrishnan, Divya, Robin, S. and John Joel, A., Waxy gene polymorphism and its association with grain quality traits in selected landraces of rice. *ORYZA-An International Journal on Rice*. **50(3):** 214-221 (2013).
4. Chakravorty, Ashim, Ghosh, P. D. and Sahu, P. K., Multivariate analysis of

phenotypic diversity of landraces of rice of West Bengal. *American Journal of Experimental Agriculture*. **3(1):** 110-123 (2013).

5. Kumari, T., Jindal, Y. and Satpal, Genetic diversity and variability analysis in oats (*Avena* sp) genotypes. *Electronic Journal of Plant Breeding*. **10(1):** 1-8 (2012).
6. Sharma, P. K., Gupta, P. K. and Balyan, H. S., Genetic diversity in a large collection of wheats (Triticum spp.). *The Indian Journal of Genetics and Plant Breeding*. **58(3):** 271-278 (1998).
7. Ayres, N. M., McClung, A. M., Larkin, P. D., Bligh, H. F. J., Jones, C. A. and Park, W. D., "Microsatellites and a single-nucleotide polymorphism differentiate apparent amylose classes in an extended pedigree of US rice germ plasm." *Theoretical and Applied Genetics*. **94(6-7):** 773-781 (1997).
8. Gaur, A., Parray, G. A., Shikari, A. B., Wani, S. H., Malav, A. K., Najeeb, S. and Bhat, M. A., An Assessment of Temperate Rice (*Oryza sativa* L.) Germplasm for Grain Quality Attributes. *Int. J. Curr. Microbiol. App. Sci*. **6(6):** 728-735 (2017).
9. Murray, M. G. and Thompson, W. F., Rapid isolation of high molecular weight plant DNA. *Nucleic acids research*. **8(19):** 4321-4326 (1980).
10. Sandhyakishore, N., Babu, V. R., Ansari, N. A. and Chandran, R., Genetic divergence analysis using yield and quality traits in rice (*Oryza sativa* L.). *Crop Improvement*. **34:** 12-15 (2007).
11. Garg, P., Pandey, D. P. and Kaushik, R. P., Genetic divergence for yield and quality traits in rice (*Oryza sativa* L.). *Journal of Rice Research*. **4(1):** 1-5 (2011).
12. Clifford, H. T. and Stephenson, W., An introduction to numerical classification. Academic Press (1975).
13. Raji, A. A., Assessment of genetic diversity and heterotic relationships in African improved and local cassava (Manihot esculenta Crantz) germplasm. Unpublished doctoral dissertation). University of Ibadan, Ibadan, Nigeria (2002).
14. Asaoka, M., Okuno, K. and Fuwa, H., Effect of environmental temperature at the milky stage on amylose content and fine structure of amylopectin of waxy and nonwaxy endosperm starches of rice (*Oryza sativa* L.). *Agricultural and Biological Chemistry*. **49(2):** 373-379 (1985).
15. Mahalingam, A., Saraswathi, R. and Ramalingam, J., Simple sequence repeat (SSR) markers for assessing genetic diversity among the parental lines of hybrid rice (*Oryza sativa* L.). *African Journal of Biotechnology*. **12(33):** 5105-5116 (2013).
16. Sharma, S., Singh, A. K., Kumar, B. P. and Salgotra, R. K., Microsatellite marker based genetic diversity analysis in Basamti rice. *The Ecosacan*. **9(1-2):** 389-392 (2015).
17. Vhora, Z., Trivedi, R., Chakraborty, S., Ravikiran, R. and Sasidharan, N., Molecular studies of aromatic and non-aromatic rice genotypes for quality traits using microsatellite markers. *The Bioscan*. **8(2):** 359-362 (2013).