Bangladesh J. Bot. **51**(2): 343-351, 2022 (June) DOI: https://doi.org/10.3329/bjb.v51i2.60432

# MARKER-BASED ANALYSIS OF CMS LINES FOR GRAIN AND KERNEL TRAITS IN RICE HYBRIDS

**ASMA MAJID*, SHOWKAT A WAZA[1], ASIF B SHIKARI[2], NR SOFI[2] AND GA PARRAY[2]**

*Division of Genetics and Plant Breeding, FoA Wadura, SKUAST-K, J&K, India*

*Keywords:* Rice, Hybrids, Grain dimensions, Grain yield, Molecular markers

**Abstract**

The grain size is considered to be one of the complex traits affecting rice yield as it is directed by multifaceted gene combinations. In the present experiment, 34 pollen parents were crossed with four female lines in line × tester matting design and 136 hybrids were generated. Wide phenotypic differences were observed across the hybrids for grain length, grain width and grain length-width ratio. The correlation analysis depicted a strong association among two grain size regulating traits *viz.*, kernel length and kernel length-width ratio. However, kernel width shows negative correlation with these traits and influences the final grain size. The hybrids were also investigated at GS3 and GW8 loci. Gene based markers SF28 and DRR-GL showed strong association with the grain size related traits. The population structure analysis divided the entire material into four subgroups *viz.*, I, II, III and IV with demarcation of long, homozygous and short grain types. The analysis of molecular variance (AMOVA) as well as a pairwise $F_{ST}$ analysis indicated significant differentiation of population II as compared to rest of the populations, suggesting that only one among the four subgroups structured significantly different. Marker based evaluation in the present study provides the understanding for selection of suitable candidate markers for obtaining preferred grain shape/size and improving the grain yield through marker-assisted breeding.

## Introduction

Rice yields have witnessed a dramatic increase during the last few decades, thereby contributing towards the sustainability in food security (Priyanka *et al.* 2014, Dar *et al.* 2021). In addition to yield, the quality considerations also assume enhanced importance in the contemporary era and most of the rice producing countries are looking for ways to improve the quality of their produce. The improvement in human standard of living has also increased the demand for high quality rice. This necessitates the integration of preferred grain quality features in rice as the most important objective next only to yield enhancement. It is being anticipated that the grain quality will be even more important as more and more poor people are becoming economically better off and laying demands for higher quality rice (Welch and Graham, 2002, Majid *et al.* 2020). The rice is mainly consumed as whole kernels; therefore, the grain quality features are important considerations for plant breeders, farmers and the consumers (Sravan *et al.* 2016, Majid *et al.* 2019).

Grain size is one of the most important quality parameters determining market price of the produce (Jaiswal *et al.* 2015, Waza *et al.* 2016). Continuous efforts are being made by rice researchers towards the development of new varieties with desirable grain dimensions based on the understanding gained through inheritance studies (Waza *et al.* 2014) along with the mapping and cloning of genes/QTLs (Aluko *et al.* 2004). The complete whole genome sequence in rice has helped in identification of several QTLs and around 8500 QTLs governing different agronomically important traits including grain size have been mapped using various segregating populations

*Author for correspondence: <asmamajid173@gmail.com>. [1]Mountain Crop Research Station (MCRS) Sagam, SKUAST-K, J&K, India. [2]Mountain Research Centre for Field Crops (MRCFC) Khudwani, SKUAST-K, J&K, India.

generated from diverse parents (Doi *et al.* 2008). In recent years, number of QTLs affecting the rice grain size have been mapped and cloned across different genetic backgrounds. The QTL named GS3, is located in the pericentromeric region of chromosome 3 and is responsible for 80-90 % of the variation in kernel length (Aluko *et al.* 2004). Further, this locus has also been determined as a minor QTL for grain width and thickness (Fan *et al.* 2006). Another gene GW8 has been reported as one of the major regulators for grain shape (Wang *et al.* 2012, Zuo and Li 2014). The present study was undertaken to identify marker trait associations for grain size traits in rice hybrids. The gene specific markers can be utilised in marker assisted selection for improving grain size.

**Materials and Methods**

The experiment was carried out at Mountain Research Centre for Field Crops (MRCFC) Khudwani, SKUAST-K during the *Kharif* season of 2019. Four Cytoplasmic Male Sterile (CMS) lines possessing WA (Wild Abortive) cytoplasm as the source of their male sterility were used as female parents in the present study. These lines were crossed with 34 male parents to generate 136 $F_1$ hybrids. Genetically diverse genotypes were used as male parents in the crossing programme. The parents and $F_1$s were raised under field conditions in randomized complete block design (RCBD) with 3 replications. The planting geometry of 20×15 cm was followed with recommended agronomic package of practices. After harvesting and proper milling, kernel length (KL) and kernel width (KW) was recorded (mm) as average of 10 unbroken kernels using scale and graph paper. The kernel length: width ratio (KLWR) was estimated by dividing mean kernel length with mean kernel width.

DNA was isolated from young leaves using the cetyl trimethyl ammonium bromide (CTAB) method as proposed by Murray and Thompson (1980). The purified genomic DNA was evaluated for quantity and quality parameters using 0.8% agarose gel electrophoresis and the plant DNA samples were diluted with TE buffer.

The rice genotypes were evaluated for the presence of two grain size related genes (GS3 and GW8) using functional/linked markers. The complete description of markers used in this study has been presented in Table 1. The PCR amplification of DNA was carried out in Thermal Cycler (TaKara, Japan). For the amplification, 10µl of reaction mixture was prepared with the ingredients as: 1µl of 1x PCR buffer [10mM Tris, pH 8.4, 50mM KCl, 1.8Mm $MgCl_2$], 1µl of 2.5mM dNTP mix (Fermentas, Lithuania, U.S.A), 0.5 µl of 10µM each of Forward and Reverse Primer, 0.6U Taq DNA Polymerase and Milli-Q water. The PCR thermal regimes set for the reaction comprised of: Initial denaturation at $94^0$C for 5 min followed by 35 cycles of 1 minute denaturation at $94^0$C, 1 min of annealing at varied temperatures and 2 min of primer extension at $72^0$C. Final extension was allowed for 7 min at $72^0$C. The PCR amplicons were resolved through electrophoresis using 3% agarose gel prepared in TAE buffer [242 g Tris-base, 57.1 ml glacial acetic acid and 100 ml 0.5 M EDTA (pH 8.0) dissolved in distilled water, and final volume made to 1000 ml]. Ethidium bromide was added at the rate of 5µl per 100µl of agarose solution. The gels were loaded along with the 3µl of a 100 bp DNA ladder (Puregene, Indore, India). The electrophoresis was carried out at a constant voltage of 65V for about 3-4 hrs and the gels were visualized under a UV light source in a Gel documentation system (Gel DocTM XR+, Bio-rad, Hercules, California).

The PCR products using CAPS primer (SF28-PstI) were cleaved with PstI restriction enzymes (New England Biolabs, Inc., USA), following the manufacturer's protocol. The digestion mixture was set up by adding 10µl of PCR product, 2µl of restriction enzyme (3U), and 2µl of 10 X buffer with addition of 18µl nuclease free water to make the final volume to 32µl. The reaction mixture was incubated at $37^0$C as per enzyme's requirement for 16 hrs. The digested product was separated

in 3% agarose gel and observed in UV Transilluminator. The markers were scored as present (1) or absent (0) to generate a binary matrix and used for further analysis.

**Table 1. Details of the molecular markers for grain size regulating genes used in the present studied.**

| Gene Locus | Primer | Marker sequence | Reference |
|---|---|---|---|
| GS3 (last intron) | RGS1 | F:5'TCCACCTGCAGATTTCTTCC3'<br>R:5'GCTGGTCTTGCACATCTCTCT3' | Ngangkham *et al*. (2018) |
| GS3 (final exon) | RGS2 | F:5'AGCGACACGGACTCTTCGT3'<br>R:5'GTGCATGATGCTTTCACCAC3' | Wang *et al*.(2010) |
| GS3 | DRR-GL<br>EFP<br>ERP<br>IFLP<br>IRSP | F:5'AGGCTAAACACATGCCCATCTC3'<br>R:5'CCCAACGTTCAGAAATTAAATGTGCTG3'<br>F:5'ACGCTGCCTCCAGATGCTGA3'<br>R:5'AACAGCAGGCTGGCTTACTCTCTG3' | Ramkumar *et al*. (2010) |
| GS3 (second exon) | SF28-Pst1 | F:5'TGCCCATCTCCCTCGTTTAC3'<br>R:5'GAAACAGCAGGCTGGCTTAC3' | Wang *et al*. (2010) |
| GW8 | GW8InDel | F:5'TTGTGATGGCAATTAGTAAGCAG3'<br>R:5'GTTCTCCAGCTCGTCGGCTA3' | Ngangkham *et al*. (2018) |

The correlations between different grain size traits namely kernel length (KL), kernel width (KW) and kernel length width ratio (KLWR) were carried out using software WindoStat version 9.1. The association between markers and kernel length was evaluated in TASSEL5 software. GenAlEx 6.5 Software was used to carry out the analysis of molecular variance (AMOVA) for separation of the total molecular variance between and within groups, and to estimate the significance of $F_{ST}$ (Peakall and Smouse 2006).

## Results and Discussion

The rice seed constitutes various types of tissues and its size is determined on the basis of control of growth of the embryo, the triploid endosperm and the seed coat (Li and Li 2015). The yield and nutritional value of rice grain is usually determined by the synthesis and storage of carbohydrates, proteins and minerals during grain filling. The quality is affected by the interaction of various enzymes to produce the final structure of the starch at molecular and granule levels (Zhu *et al*. 2003).

The phenotypic variation in grain size was determined in 136 cross combinations obtained from crossing between 4 CMS lines and 34 pollen parents. A substantial variation in grain size suggested a quantitative inheritance governed by multiple genes. Estimates for range, mean, standard deviation (SD), standard error (SE) and coefficient of variation (CV %) evaluated in the present study are being presented in Table 2. The mean of kernel length was found to be 6.19 ± 0.03 mm. The lines showed a range of 2.25 mm (5.40 to 7.65 mm) for kernel length, suggesting that apart from one or two major genes there might be some interactions between the genes and the environment. Similarly, the mean kernel width was recorded to be 2.06 ± 0.01. As equated with length, lower variation was seen in kernel width having range of 0.60 mm as expected and extended from 1.83 to 2.43 mm. However, in case of kernel length: width ratio, the mean was found to be 3.02 ± 0.02 mm with a range of 1.58 from 2.33 to 3.91. The coefficient of variation (CV %) for kernel length, kernel width and kernel length: width ratio was 6.30, 5.48 and 8.93%,

respectively (Table 2). The kernel length showed more phenotypic variation as compared to kernel length: width ratio, which in turn showed larger variation than kernel width. Since the availability of genetic variability for yield related components could be a valuable selection priorities of breeders for need based breeding in rice yield improvement, it is possible to effectively utilize the studied material of the varied grain length and grain length : width ratio effectively in rice yield enhancement. The genotypic correlation coefficients (r) were calculated among cooking quality traits (Table 3). Kernel length showed significant negative correlation with kernel width. Such linear negative correlation has also been reported in other grain yield or yield related studies (Ngangkham *et al.* 2018). Kernel length: width ratio showed positive and significant correlation with kernel length. This result suggests that there is a strong association among the three grain traits which play an important role in determining the rice grain size. Such patterns are in accordance with the concept that kernel length: width ratio contributes the major effects in combining the length and width of the rice grain. Further, the whole material was divided into four groups based on grain length as per SES-IRRI (2018). It was observed that the highest number of genotypes belonged to medium grain type. Based on length, out of 136 hybrids one was extra-long (EL), 15 were long (L), 119 were medium (M) and one was short (S). On the basis of shape, 65 were slender (S) and 71 were medium (M), however no hybrid was of bold (B) or round (R) grain type (Fig. 1).

**Table 2. Descriptive statistics for grain size traits.**

| Trait | Range | Mean | Standard deviation (SD) | Standard error (SE) | Coefficient of variation (CV %) |
|---|---|---|---|---|---|
| Kernel length | 5.40-7.65 | 6.19 | 0.39 | 0.03 | 6.30 |
| Kernel Width | 1.83-2.43 | 2.06 | 0.11 | 0.01 | 5.48 |
| Length-width ratio | 2.33-3.91 | 3.02 | 0.27 | 0.02 | 8.93 |

**Table 3. Genotypic correlation coefficient for kernel length, kernel width and kernel length width ratio.**

| | Kernel width | Kernel length width ratio |
|---|---|---|
| Kernel length | -0.56** | 0.92** |
| Kernel width | | -0.83** |

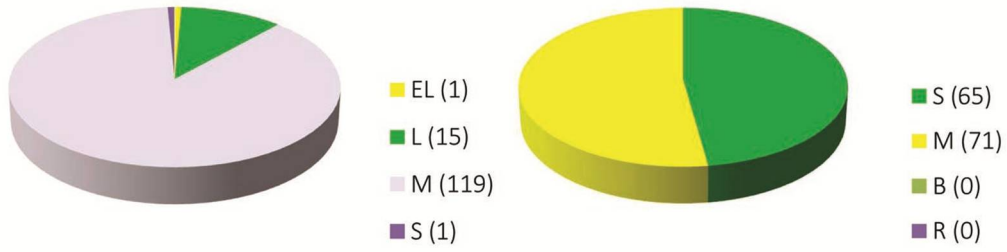


Fig 1. Classification of hybrids on the basis of kernel length and length width ratio.

The allelic distribution of genes related to grain size was examined in 136 rice hybrids using genic/linked markers, and accordingly genotypes were divided into different classes. Further, phenotypic allelic frequencies were also estimated. In this study, four genic/ functional markers (SF28-PstI, RGS1, RGS2 and DRR-GL) were used to assess the allelic pattern in GS3 locus in 136 hybrids. These markers revealed polymorphism between long grain and short grain types except for RGS2. The SF28-PstI is a functional CAPS marker using PstI endonuclease enzyme differentiating `C/A' SNP mutation in second exon of GS3 gene which produces a truncated protein without functional domain that is associated with an enhanced rice grain length (Yan *et al.* 2011). Using marker SF28-PstI primers, all lines generated PCR products of approximately 140 bp in size. Since CTGCAG sequence (restriction site of PstI enzyme) is present in the PCR products, the 140 bp PCR products was digested by PstI endonuclease enzyme into 110 bp and 30 bp smaller fragments (Wang *et al.* 2010). Since SF28-PstI requires an additional step of digestion, another SNP marker combination namely DRR-GL (EFP/ERP, EFR/IRSP and IFLP/ERP) was used. The external primers EFP/ERP amplify a region of 365 bp as a common allele in both dominant and recessive alleles. While the allele-specific primer pairs, EFP/IRSP and ERP/IFLP, amplify regions of 147 bp and 262 bp, respectively. Thus, the long grain genotypes possess 365 bp and 262 bp, while 365 bp in combination with 147 bp indicates that genotypes are of short grain types, while in heterozygotes all the three (365 bp, 147 bp as well as 262 bp) bands are present. To check the accuracy and reliability of this marker based genotyping, it was cross checked with marker SF28-PstI, and the results depicted that this marker was accurate and reliable. The RGS1 (SSR marker for GS3) gene produces 200 bp and 180 bp fragments which corresponded to long and short grain types, respectively (Fig. 2). The GW8 gene synonymous with OsSPL16, encodes a protein of an SBP-domain transcription factor that regulates grain size by positively regulating the cell proliferation of grain (Wang *et al.* 2012). The GW8-indel marker having 10 bp deletion is responsible for grain size variation. On the occurrence of 10 bp deletion, this marker generates two alleles, A and B corresponded to Basmati and indica-type alleles, respectively (Wang *et al.* 2012, Lee *et al.* 2015, Ngangkham *et al.* 2018). In the current study, the grain quality SSR primers RGS1, CAPs marker SF28-Pst1 and DRR-GL marker combination (EFP/ERP, EFP/IRSP and IFLP/ERP), revealed a distinguishing banding pattern. The short grain type was seen to be dominant over the long grain type.

**Table 4. L.D/$R^2$ value depicting association of grain length markers (RGS1, SF28, DRR-GL) with its corresponding trait.**

| | RGS1 | SF28 | DRR-GL | Kernel length |
|---|---|---|---|---|
| RGS1 | - | 0.32 | 0.06 | 0.06 |
| SF28 | | - | 0.09 | 0.18 |
| DRR-GL | | | - | 0.20 |
| Kernel length | | | | - |

Gene based markers DRR-GL and SF28, and SSR marker RGS1 were validated on the test cross population. Among these, DRR-GL and SF28 showed strong association with grain length, whereas RGS1 revealed weak association with the trait. Analysis of variance carried out using TASSEL5 software has been presented in Table 4.

The 4 populations from CMS lines (SKUA-7A, SKUA-11A, SKUA-19A and SKUA-21A) namely I, II, III and IV were screened using molecular markers. Analysis of molecular variance estimated through GenAlEx (Graphical Analysis in Excel) Software 6.5 revealed that 8% of

variance existed among populations and 92% within populations (Fig. 3, Table 5). Thus, the maximum variance (92%) was contributed by the male parents with respect to the CMS lines within same population. However, only moderate variance (8%) existed due to different CMS lines, indicating somewhat similarity between the CMS lines. Further the pair-wise fixation indices (FST) indicated that the 8% (moderate) difference among the populations was due to difference of population substructure (Table 6).

**Table 5. Analysis of molecular variance (AMOVA).**

| Source | DF | SS | MS |
|---|---|---|---|
| Among populations (crosses) | 3 | 12.750 | 4.250 |
| Within populations | 136 | 93.000 | 0.684 |
| Total | 139 | 105.75 | 4.934 |

**Table 6. Pair-wise $F_{ST}$ estimates among four populations.**

| | SKUA-7A | SKUA-11A | SKUA-19A | SKUA-21A |
|---|---|---|---|---|
| SKUA-7A | 0 | | | |
| SKUA-11A | 0.188 | 0 | | |
| SKUA-19A | 0 | 0.188 | 0 | |
| SKUA-21A | 0 | 0.188 | 0 | 0 |

$F_{ST}$ value below diagonal.

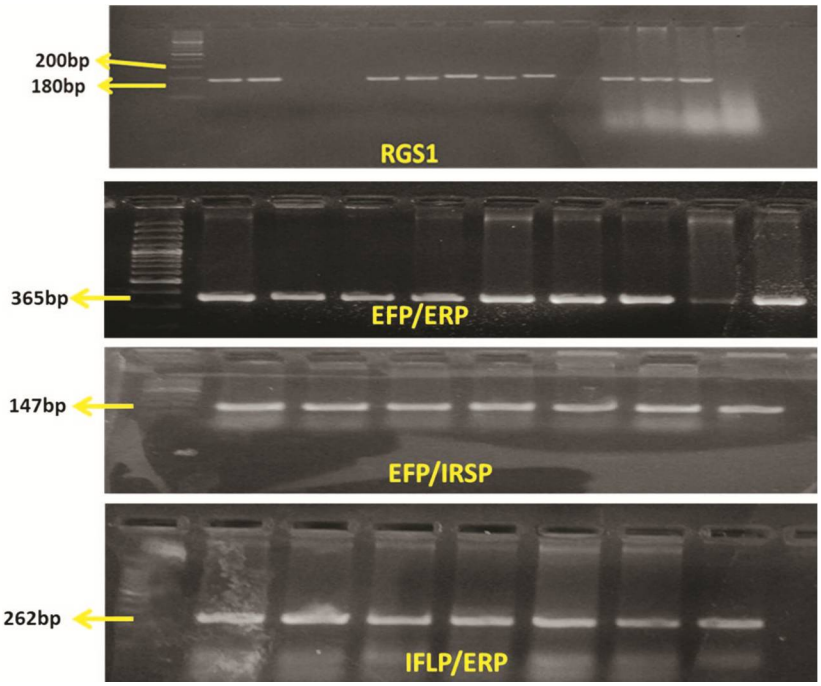


Fig 2. Representatives of PCR amplified fragments of linked/functional markers.

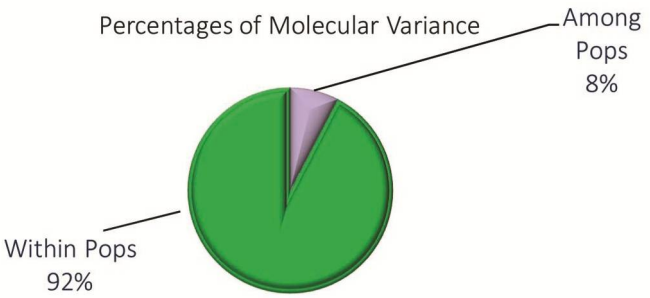


Fig 3. Percentage of Molecular Variance Where, pops indicate populations.

Relationship of grain shape markers *viz.*, RGS1, SF28-Pst1 and DRR-GL (EFP, ERP, IFLP and IRSP) for grain length and GW8InDel for grain width with yield was estimated. The hybrids and parents grouped into short, heterozygous and long grain types based on RGS1 recorded an average grain yield of 5.73 ± 0.08, 6.18 ± 0.09 and 5.2 ± 0.099 t/ha, respectively. Based on marker SF28-Pst1, these recorded grain yield of 5.66 ± 0.014, 5.76 ± 0.024 and 5.56 ± 0.022 t/ha, respectively. On the basis of DRR-GL (EFP, ERP, IFLP and IRSP) marker, these recorded the grain yield of 5.13 ± 0.034, 5.82 ± 0.038 and 5.50 ± 0.042 t/ha, respectively. GW8InDel for grain width depicted grain yield of 5.48 ± 0.022, 6.74 ± 0.027 and 6.72 ± 0.034 t/ha for bold, medium and slender grains, respectively. It was observed that $F_1$s exhibited more grain yield than their corresponding parents (Fig 4). GS3 localized on chromosome 3 has been reported as the most important gene for grain size (grain length), regardless of genetic background (Lu *et al.* 2013).

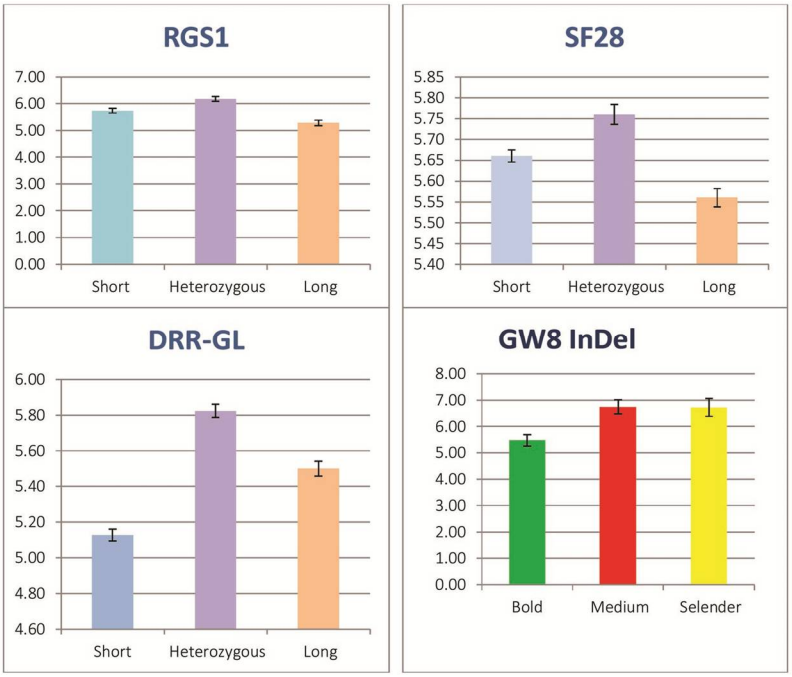


Fig 4. Grain shape markers depicting the relation with yield.

In the present study, different genes possessing different allelic combinations would be helpful in the depiction of final grain shape and size, thus providing means to understand the complex mechanism of rice grain dimensions. The alleles identified could be helpful in pyramiding the preferred grain size trait. The marker loci that are strongly associated with grain size trait would be highly informative and efficient in the selection of recombinants in new rice breeding populations. The availability of genetic variability for yield related components could be valuable selection priorities of breeders for need based breeding in rice yield improvement. Therefore, it will be helpful to utilize the studied material of varied grain length and grain length: width ratio effectively in rice yield enhancement programs.

## Conflicts of Interest

The authors declare that no conflict of interest is involved

## References

Aluko G, Martinez C, Tohme J, Castano C, Bergman C, Oard HJ 2004. QTL mapping of grain quality traits from the interspecific cross *Oryza sativa* and *O. glaberrima*. Theor. Appl. Genet. **109**: 630-639.

Dar MH, Bano DA, Waza SA, Zaidi NW, Majid A, Shikari AB, Ahangar MA, Hossain M, Kumar A, Singh US 2021. Abiotic Stress Tolerance-Progress and Pathways of Sustainable Rice Production. Sustainability **13**(4): 2078.

Doi K, Yasui H, Yoshimura A 2008. Genetic variation in rice. Curr. Opin. Plant. Biol. **11**(2): 144-8.

Fan C, Xing YZ, Mao HL, Lu TT, Han B, Xu CG, Li XH, Zhang QF 2006. GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor. Appl. Genet. **112**(6): 1164-1171.

Jaiswal HK, Waza SA, Sravan T 2015. HUBR-16: A high yielding early maturity rice line coupled with extra-long kernel and high kernel elongation. Indian J. Plant Genet. Resour. **28**(2): 260-262.

Lee CM, Park J, Kim B, Seo J, Lee G, Jang S, Koh HJ 2015. Influence of multi-gene allele combinations on grain size of rice and development of a regression equation model to predict grain parameters. Rice **8**(1): 33.

Li N and Li Y 2015. Maternal control of seed size in plants. J. Exp. Bot. **66**(4): 1087-1097.

Lu L, Shao D, Qiu X, Sun L, Yan W, Zhou X, Lin Y, Yuqing H, Sibin Y, Zhong XY 2013. Natural variation and artificial selection in four genes determine grain shape in rice. New Phytol. **200**(4): 1269-1280.

Majid A, Parray GA, Sofi NR, Shikari AB, Waza SA, Maqbool S, Khan MA, Sheikh FA 2019. Evaluation of Grain Quality Traits in High Altitude Rice Hybrids (*Oryza sativa* L.). Ind. J. Pure App. Biosci. **7**(5): 525-530.

Majid A, Parray GA, Sofi NR, Shikari AB, Waza SA 2020. Combining Ability Effects for Various Agro-Morphological Traits in Rice under Temperate Conditions. Curr. J. Appl. Sci. Technol. **39**(38): 47-58.

Murray MG. and Thompson WF 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. **8**: (19).

Ngangkham U, Samantaray S, Yadav MK, Kumar A, Chidambaranathan P, Katara JL 2018. Effect of multiple allelic combinations of genes on regulating grain size in rice. PLoS ONE **13**: (1).

Peakall RO, Smouse PE 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes **6**(1): 288-295.

Priyanka K, Jaiswal HK, Waza SA, Sravan T 2014. Identification of superior hybrids among the crosses between various rice genotypes. Appl. Biol. Res. **16**(1): 31-35.

Ramkumar G, Sivaranjani AKP, Pandey MK, Sakthivel K, Rani NS, Sudarshan I, Prasad G SV, Neeraja CN, Sundaram RM, Viraktamath BC, Madhav MS 2010. Development of a PCR-based SNP marker system for effective selection of kernel length and kernel elongation in rice. Mol. Breeding **26**: 735-740.

SES-IRRI 2018. Standard Evaluation System for Rice. Los Banos. Philippines: International Rice Research Institute.

Sravan T, Jaiswal HK, Waza SA, Priyanka K 2016. Analysis of variability and character association in indigenous aromatic rice (*Oryza sativa* L.). Electron J. Plant. Breed. **7**(1): 159-164.

Wang S, Wu K, Yuan Q, Liu X, Liu Z, Lin X, Zeng R, Zhu H, Dong G, Qian Q, Zhang G, Fu X 2012. Control of grain size, shape and quality by OsSPL16 in rice. Nat Genet. **44**(8): 950.

Wang C, Chen S and Sibin Y 2010. Functional markers developed from multiple loci in GS3 for fine marker-assisted selection of grain length in rice. Theor. Appl. Genet. **118**: 107-115.

Waza SA, Jaiswal HK, Sravan T, Bano DA, Priyanka K, Umesh U 2016. Heterosis for yield and quality traits in rice (*Oryza sativa* L.). J. Appl. Nat. Sci. **8**(3): 1510-1522.

Waza SA, Rastogi NK, Verulkar SB, Sharma D, Bano DA 2014. Microsatellite based estimation of genetic diversity between parental lines and its relationship with yield potential of rice hybrids. Bioinfolet **11**(2): 591-593.

Welch RM, Graham RD 2002. Breeding Crops for Enhanced Micronutrient Content. Plant Soil **245**: 205-214.

Yan S, Zou G, Li S, Wang H, Liu H, Zhai G, Guo P, Song H, Yan C, Tao Y 2011. Seed size is determined by the combinations of the genes controlling different seed characteristics in rice. Theor. Appl. Genet. **123**(7): 1173.

Zhu T, Budworth P, Chen W, Provart N, Chang HS, Guimil S, Su W, Estes B, Zou G, Wang X 2003. Transcriptional control of nutrient partitioning during rice grain filling. Plant Biotechnol. J. **1**(1): 59-70.

Zuo J and Li J 2014. Molecular genetic dissection of quantitative trait loci regulating rice grain size. Annu. Rev. Genet. **48**: 99-118.

*(Manuscript received on 02 June, 2021; revised on 19 February, 2022)*