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Phenotyping and correlation studies of recombinant inbred lines derived from interspecific cross of *Oryza sativa* and *Oryza nivara*

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Abstract

Recombinant Inbred Lines (RILs) derived from crosses between elite cultivars and wild introgression lines are important sources of new genetic variability. In this study 177 RILs derived from a cross of Swarna / 166s (Swarna /*Oryza nivara*), were characterized for yield and grain shape related traits for two seasons *Rabi* 2018-19 and *Kharif* 2019. Phenotypic correlation analysis showed that, single plant yield had a positive association with productive tiller number, total tiller number, panicle weight, biomass and harvest index in both the seasons. Significant and positive correlation was observed between grain length and grain perimeter followed by productive tiller number and total tiller number. A perfect negative linear relationship was observed between filled grain percentage and unfilled grain percentage. Across the seasons, biomass weight consistently showed significant positive correlations with the majority of yield contributing traits, followed by days to 50 percent flowering. Grain shape traits (grain length, grain width, length/width ratio, grain area and grain perimeter) were interrelated with each other significantly. Trait association study in this mapping population showed that though the yield performance has always been the main focus of breeding programmes, the complex interaction of the yield attributes is also equally significant to understand the genetic mechanisms for yield improvement. Selected RILs from this study are being used in rice improvement programmes to detect favorable genes from *O. nivara* for yield improvement.

Key Words: Rice, yield, grain shape, correlation coefficient, recombinant inbred lines

Introduction

Rice (*Oryza sativa* L) is one of the most important staple food crops of the world providing nutritional and economic benefits to more than half of the world population (Roy and Shil, 2020). Rice yield needs to be continuously improved to fulfill the future demands of increasing population and to enhance living standards. However, it is the most challenging part in breeding programmes because yield is the most variable and complex trait influenced by several environmental and genetic factors (Pimsaen *et al*., 2010; Sreedhar et *al*., 2011; Christopher and Sangodoyin, 2011). The current practice of crossing between elite lines of *Oryza sativa* is expected to reduce genetic variability in the working germplasm and slow down the discovery of novel traits to improve yield. Yield level in the released varieties has plateaued due to the narrow genetic base in parental lines used in crossing procedure (Tanksley and McCouch, 1997). One of the alternatives for increasing agriculture production is to identify the genes lost or weakened during the domestication process and use them in subsequent targeted breeding programmes (Shakiba and Eizenga, 2014). The wild species of the genus *Oryza* serve as a nearly untapped reservoir of genetic diversity that can be used to improve the world's most important food crop, rice. The high diversity existing in wild rice species as they have not undergone rigorous human selection, makes them potential sources of important agronomic traits for exploitation by plant breeders for varietal improvement (Brar and Khush 1997; Gur and Zamir, 2004; Xie *et al*., 2008; Yadav *et al*., 2013; Sharma *et al*., 2013).

The genus *Oryza* has 22 wild species, with tremendous variability and contains numerous valuable genes for improvement of several traits. This was demonstrated by Khush *et al., (*1977), by introgressing the gene for resistance to grassy stunt virus from *O. nivara* to cultivated rice. Since then, wild species have been widely used for introgression of several agronomically important traits such as tolerance to biotic stresses like blast (Jeung *et al.,* 2007), brown planthopper (Rahman *et al*., 2009; Jena and Kim, 2010; Sarao *et al*., 2016), and bacterial leaf blight (Natrajkumar *et al*., 2011). *O. nivara*, a cross compatible wild species of rice with AA genome is known as a source of important genes like cytoplasmic male sterility (Li *et al*, 2005), resistance to grassy stunt virus (Brar and Khush, 1997), bacterial leaf blight (Cheema *et al*., 2008) and BPH (brown planthopper) (Madurangi *et al*., 2013; Sarao *et al*., 2016). Introgression from non-AA genome species *Oryza minuta* (CCDD) was used in the successful transfer of blast resistance genes and *O. australiensis* (EE) for blast and BPH resistance genes into *O. sativa* (Jena and Khush,2000; Fu *et al*., 2008; Suh *et al*., 2014).

Previous studies showed that introgression from wild rice species also enhances tolerance to abiotic stresses like drought, salinity and cold (Koseki *et al*.,2010; Ndjiondjop *et al*., 2010; Yang *et al*., 2012), diversification of cytoplasmic male sterility sources (Li *et al*., 2005), adaptability and yield-enhancing components for improving rice yield (Swamy and Sarla, 2008; Brar and Singh, 2011; Shakiba and Eizenga, 2014; Furuta *et al*., 2014; Bhatia *et al.*, 2017; Haritha *et al.*, 2018; Kaur *et al,* 2018; Zhu *et al,* 2018) and grain quality (Yun *et al*., 2016;Gaikwad*et al*., 2018). It was reported that transferring yield-enhancing segments/ QTLs into the genetic background of elite varieties (Kaladhar *et al*., 2008; Fu *et al*, 2010; Bai *et al*., 2012; Thalapati *et al.,* 2012; Gaikwad *et al*., 2014; Swamy *et al.*, 2014; Surapaneni *et al*., 2017) helped in the identification of improved cultivars with wild introgressions (Sarla *et al*., 2009). In India, Dhanarasi and Jarava are two rice varieties released for increased yield and they were derived from crosses with *O. rufipogon* (Ram *et al.,* 2007, 2010). DRR Dhan 40, a medium duration rice variety (BIL derived from Swarna × *O. nivara* IRGC81848) was released for Maharashtra, West Bengal and Tamil Nadu states of India (Sarla, 2014; Haritha *et al*., 2016). Improving mega varieties which are well adapted, farmer accepted, popular and with good grain quality for any specific trait, is an important strategy in crop breeding programmes. In the present study, RILs derived from a cross between cultivar Swarna and BIL166s were used to understand the correlations among the yield and grain shape related traits.

Materials and Methods

Plant material

Swarna [(MTU 7029/ IET5656 (Vasistha/Mahsuri)] is a low-land high yielding *indica* type mega rice variety released in 1982 in India by RARS, Maruteru and it is a semi-dwarf variety maturing within 135-140 days with an average yield of 6.5 t/ha. 166s (male parent), a stable advanced backcross introgression line derived from a cross between Swarna and *Oryza nivara* IRGC81848 from IIRR, Hyderabad, is a late duration genotype, with strong culm strength and panicle weight. 166s has higher grain yield and yield stability than Swarna but with similar grain type to Swarna (Balakrishnan *et al*., 2016). Both were used in crossing programmes (Kavitha *et al.*, 2020). In the present study, the F_6 and F_7 generations of the cross were raised during *Rabi* 2018/19 (dry) and *Kharif* 2019 (wet) seasons respectively, at research farm, ICAR-Indian Institute of Rice Research, Hyderabad ($17^0$19’ N, $78^0$29’ E) at an altitude of 549 m above sea level in alkaline vertisol soil under irrigated field condition.

Phenotypic evaluation of RILs

The 177 RILs obtained from ICAR-IIRR were planted in the field as three-row families in both seasons. One row consisted of ten plants and each replication had thirty plants. A randomized complete block design was followed with two replications. Parental lines Swarna and 166s were used as check varieties. The spacing between rows was 20 cm and 15 cm between plants. The crop was grown under irrigated condition and phenotypic observations were recorded for yield and grain shape-related traits from the randomly selected three plants in the middle row of each family. Twelve yield related traits *viz*, days to 50% flowering (DFF), plant height (PH), number of productive tillers per plant (PTN), number of total tillers per plant (TTN), panicle length (PL), panicle weight (PW), 1000 grain weight (TGW), filled grain percentage (FG), unfilled grain percentage (UFG), single plant yield (SPY), biomass weight (BM) and harvest index (HI) were measured following Standard Evaluation System (IRRI, 2013). The days to 50 per cent flowering was taken as the number of days taken for 50 per cent heading in the family considering the 30 plants. Grain shape-related five traits were taken *viz*, grain length (GL), grain width (GW), grain length/width ratio (LW), grain area (GA) and grain perimeter (GP). All the parameters were measured using Image J (image analysis software) and the mean of 10 seeds was taken in each replication.

Statistical analysis

The mean values from each replication were subjected to statistical analysis. Correlation analysis was performed with Statistical Tool for Agricultural Research STAR (STAR 2.0.1, http://bbi.irri.org) and significant values were determined using Pearson coefficients at the 0.05 and 0.01 levels.

Results and Discussion

As a complex quantitative character, estimation of grain yield depends on contribution from other traits which ultimately affects the overall yield potential of the genotype. The trait association which is either in positive or negative direction helps the breeder to select traits to improve grain yield in breeding programmes. Hence, association analysis was undertaken to determine the direction of selection and to get an understanding of the characters to be considered in improving grain yield. The phenotypic correlation coefficient among seventeen characters was assessed related to yield and grain shape-related traits using RILs derived from Swarna / 166s, in *Rabi* 2018-19 and *Kharif* 2019 with two replications (Table 1).

The r-value for Karl Pearson's correlation coefficient helps in identification of an association between two distinct traits. Though it does not measure the magnitude of association it does give an idea of the relationship (Yusuff *et al*., 2018). For the correlation coefficient interpretations, Ratner, (2009) gave a standard accepted guideline. The r-value of 0, +1, and -1 indicates no linear relationship, a perfect positive linear relationship, and negative linear relationship, respectively. The values that range from 0 to 0.3, 0.3 to 0.7, and 0.7 to 1 indicate a low, moderate, and strong positive linear relationships, respectively, while the values that range from 0 to -0.3, -0.3 to -0.7, and -0.7 to -1 indicate a low, moderate, and strong negative linear relationships, respectively.

In the present study, single plant yield showed association with one of the main yield components, panicle weight and a moderate positive association with tiller number in *Rabi* 2018/19 and *Kharif* 2019 seasons, respectively. Further single plant yield showed significant and positive association with panicle weight, biomass and harvest index. Panicle length had a positive association with days to fifty percent flowering, plant height, biomass and panicle weight. Panicle weight showed significant and positive association with days to fifty percent flowering, plant height, panicle length, single plant yield and thousand grain weight, while thousand grain weight showed significant and positive association with harvest index and panicle weight. As expected, percentage of filled and unfilled grain had a perfect negative linear relationship. Filled grain had significant and positive

Table 1: Correlation coefficients among yield and grain shape-related traits in Swarna x 166s RILs in *Rabi* 2018/19 and *Kharif* 2019

	DFF	PH	PTN	TTN	PL	PW	TGW	FG	UFG	SPY	BM	HI	GL	GW	LW	GA	GP
DFF	**1**	0.407**	0.135*	0.084	0.357**	0.412**	-0.097	-0.112*	0.112*	0.318**	0.432**	-0.236**	-0.055	0.009	-0.046	-0.016	-0.068
PH	0.392**	**1**	0.052	-0.019	0.443**	0.519**	0.057	-0.052	0.052	0.393**	0.528**	-0.302**	-0.077	-0.016	-0.008	-0.079	-0.078
PTN	0.072	-0.166**	**1**	0.952**	0.146**	0.074	-0.084	0.018	-0.018	0.561**	0.565**	-0.134*	-0.129*	-0.048	-0.031	-0.099	-0.134*
TTN	0.098	-0.177**	0.968**	**1**	0.097	-0.001	-0.09	0.042	-0.042	0.478**	0.533**	-0.188**	-0.155**	-0.024	-0.065	-0.094	-0.158**
PL	0.294**	0.512**	-0.063	-0.068	**1**	0.677**	-0.079	-0.054	0.054	0.392**	0.389**	-0.099	0.002	0.0293	-0.027	0.025	0.005
PW	0.315**	0.455**	-0.137**	-0.146**	0.601**	**1**	0.109*	0.054	-0.054	0.579**	0.387**	0.076	0.014	-0.07	0.064	-0.047	0.008
TGW	-0.221**	0.081	-0.229**	-0.247**	-0.061	0.134*	**1**	0.034	-0.034	0.097	-0.067	0.173**	0.011	0.061	-0.033	0.037	-0.006
FG	-0.233**	-0.115*	0.071	0.054	-0.049	0.266**	0.095	**1**	-1.00**	0.036	-0.118*	0.184**	-0.119*	-0.039	-0.019	-0.078	-0.122*
UFG	0.233**	0.115*	-0.071	-0.054	0.049	-0.266**	-0.095	-1**	**1**	-0.036	0.118*	-0.184**	0.119*	0.039	0.019	0.078	0.122*
SPY	0.065	0.088	0.509**	0.517**	0.102	0.217**	-0.059	0.254**	-0.254**	**1**	0.689**	0.154**	-0.018	-0.057	0.042	-0.054	-0.035
BM	0.226**	0.175**	0.523**	0.536**	0.110*	0.053	-0.178**	-0.066	0.066	0.602**	**1**	-0.574**	-0.088	0.044	-0.087	-0.016	-0.091
HI	-0.153**	-0.045	0.081	0.067	0.032	0.267**	0.131*	0.393**	-0.393**	0.608**	-0.195**	**1**	0.095	-0.136**	0.171**	-0.051	0.074
GL	-0.021	0.043	0.098	0.082	0.019	-0.048	0.001	-0.091	0.091	-0.091	-0.061	-0.063	**1**	0.064	0.483**	0.529**	0.953**
GW	-0.026	-0.036	-0.061	-0.044	0.043	-0.029	0.006	-0.062	0.061	-0.08	-0.037	-0.095	-0.026	**1**	-0.821**	0.846**	0.198**
LW	-0.022	0.047	0.068	0.055	-0.052	-0.028	-0.055	-0.006	0.006	-0.014	-0.054	0.025	0.477**	-0.118*	**1**	-0.458**	0.349**
GA	-0.02	0.004	0.0004	-0.002	0.082	-0.032	0.071	-0.1	0.101	-0.111*	-0.035	-0.101	0.497**	0.08	-0.503**	**1**	0.648**
GP	-0.021	0.042	0.069	0.057	0.049	-0.055	0.013	-0.109*	0.109*	-0.127*	-0.081	-0.088	0.955**	0.013	0.331**	0.627**	**1**

Significance levels: *P< 0.05, and **P< 0.01. Upper diagonal shows the phenotypic correlation of mean data in *Rabi* 2018/19 and lower diagonal shows phenotypic correlations of *Kharif* 2019

DFF-Days to 50% flowering, PH-plant height, PTN-number of productive tillers per plant, TTN-number of total tillers per plant, PL-panicle length, PW-panicle weight, TGW-1000 grain weight, FG-filled grain percentage, UFG-unfilled grain percentage, SPY-single plant yield, BM-biomass weight, HI- harvest index, GL-grain length, GW-grain width, LW-grain length/width ratio, GA-grain area and GP-grain perimeter association with harvest index while it showed significant and negative association with grain perimeter and days to fifty percent flowering. Unfilled grain percentage showed significant and negative association with harvest index and significant, positive association with grain perimeter and days to fifty percent flowering. Biomass showed significant positive correlations with the majority of other traits such as days to fifty percent flowering, plant height, productive tiller number, panicle length, panicle weight, unfilled grains and single plant yield in *Rabi* 2018/19 followed by days to fifty percent flowering with plant height, productive tiller number, panicle length, panicle weight, unfilled grains, single plant yield and biomass.

Rice grain yield is contributed by many component traits measured mainly by four indices: panicles per unit area, number of spikelets per panicle, the weight of spikelets (1000 grain weight) and spikelet fertility or filled grain percentage (Fageria, 2007; Xing and Zhang 2010; Ikeda *et al*., 2013). Also, shoot dry weight, grain harvest index, and nitrogen (N) harvest index are positively associated with grain yield (Fageria, 2007). Supportive to the idea of four main components of grain yield, Bagati *et al*., (2016) using a RIL population derived from a cross between Pusa Basmati and Basmati 370, reported that tillering ability, spikelet fertility percentage, number of grains per panicle and 1000-grain weight had a significantly positive correlation with grain yield. A study carried out by Kar *et al.*, (2018) with low land rice varieties indicated that grain yield was positively correlated with fertile grain number, fertility percentage, days to flowering and plant height indicating the importance of such traits for the realization of high yield in rice. Haritha *et al.,* (2017) showed that yield per plant was significantly correlated with above-ground biomass, total dry matter and harvest index using ILs of a cross between *Oryza sativa* (KMR3) × *Oryza rufipogon* (WR120). Yusuff, *et al.,* (2018) reported that, the yield per hectare showed strong, positive and highly significant association with tillers per hill and panicles per hill. Sudhakar *et al*., (2012) reported that grain yield per plant (single plant yield) showed a significant positive association with grain weight per panicle (PW) using ILs of KMR3 x *O. rufipogon*.

In *Kharif* 2019, the same pattern of correlation of biomass with days to fifty percent flowering, plant height, productive tiller number, panicle length and single plant yield was observed; followed by days to fifty percent flowering with plant height, panicle length, panicle weight, unfilled grains and biomass with significant and positive correlation for the majority of the yield contributing traits. Biomass showed significant and negative association with harvest index. Haritha *et al.*, (2017) reported that above ground biomass was significantly correlated with total dry matter and harvest index. HI showed significant negative correlations with most of the contributing traits like days to fifty percent flowering, plant height, productive tiller number, total tiller number, unfilled grains and biomass; followed by filled and unfilled grains, biomass, grain length and grain perimeter in *Rabi* 2018/19. In *Kharif* 2019 days to fifty percent flowering (with thousand grain weight, filled grains and harvest index), plant height (with productive tiller number, total tiller number and filled grains), thousand grain weight (with days to fifty percent flowering, productive tiller number and total tiller number) and harvest index (with days to fifty percent flowering, unfilled grains and biomass) showed the significant and negative correlations with the majority of the traits under study. HI showed significant and positive association with thousand grain weight, number of filled grains, and single plant yield. It showed significant and negative association with days to fifty percent flowering, unfilled grains and biomass. Balakrishnan *et al.,* (2016) observed that single plant yield exhibited a highly significant association with panicle weight, biomass and harvest index in the introgression lines derived from *Oryza sativa* cv. Swarna /*Oryza nivara*.

Grain shape traits (GL, GW, LW, GA and GP) were interrelated with each other significantly across the seasons. Grain length had significant and positive association with length: width, grain area and grain perimeter. The positive correlation between grain length and length by width ratio was also reported by Abdala *et al.*, (2016) and Golam *et al.*, (2014). Grain width had a significant and negative association with LW. Abdala *et al*, (2016) also reported a similar association, but observed a strong correlation between brown rice shape and paddy rice width. In the present study, there was no correlation between grain length and grain width as reported by Abdala *et al*, (2016). Length/ width ratio had a negative and significant association with grain width and grain area while it had a positive and strong association with grain perimeter and grain length. Grain area had a significant, moderate and positive association with grain length and grain perimeter, but significant negative association with grain length/width ratio.

In this study most of the trait associations were consistently significant in both *Rabi* 2018/19 and *Kharif* 2019 seasons. Days to fifty percent flowering had a significant positive association with plant height, panicle length, panicle weight, unfilled grains and biomass and had a significant negative association with harvest index and number of filled grains. Balakrishnan *et al.*, (2016) reported that days to fifty percent flowering showed a negative correlation with number of filled grains, harvest index and days to fifty percent flowering while highly significant association observed between panicle length and panicle weight. Following associations showed the significant correlation, in same direction across the seasons; single plant yield with biomass, harvest index, grain length and grain weight; grain length/width ratio with grain area and grain perimeter; grain length showed significant association with grain length/width ratio, grain area and grain perimeter in both the seasons. Plant height showed significant, positive relationship with days to fifty percent flowering, panicle length, panicle weight and biomass. This corroborates with the findings of Immanuel *et al.*, (2011) for panicle length. Productive tiller number and total tiller number had a strong and positive association with each other and moderate positive association with single plant yield and biomass. Immanuel *et al*, (2011), and Sudhakar *et al.*, (2012) also reported the significant and positive association of total tiller number with productive tiller number. Yusuff, *et al.*, (2018) revealed that tillers per hill and grain weight per hill possessed positive association with yield per hectare.

For some traits, significant associations were observed only in one season (Table 1). Following associations were significant and positive in *Rabi* 2018/19 season only. Days to fifty percent flowering with productive tiller number and single plant yield; plant height with single plant yield; productive tiller number with panicle length; panicle length with single plant yield; panicle weight with biomass; unfilled grains with biomass and grain length; harvest index with grain length/width ratio; grain weight with grain area and grain perimeter. Following associations were significant and negative, productive tiller number with harvest index and grain length; total tiller number with harvest index, grain length and grain perimeter; number of filled grains with biomass and grain length. In *Kharif* 2019 only association among plant height with unfilled grains; panicle weight with number of filled grains and harvest index; number of filled grains with single plant yield were significant and positive, while following associations were significant and negative, days to fifty percent flowering with thousand grain weight; plant height with productive tiller number, total tiller number and number of filled grains; productive tiller number with panicle weight, thousand grain weight; total tiller number with panicle weight, thousand grain weight; panicle weight with unfilled grains; thousand grain weight with biomass; unfilled grains with single plant yield; single plant yield with grain perimeter. Bagati *et al*, (2016) reported panicle length exhibited significant positive and negative association with grain yield during 2014 and 2015, respectively. The major reason for this difference in trait association is mainly the varying environmental conditions. After transplanting, the 21 days old seedlings in the field were exposed to total submergence for few days and partially for about ten days due to heavy rains in *Kharif* 2019. In general, the observed disparity in cultivation conditions, the inconsistencies in individual genotype expression, are reasonable causes for fluctuations in environmental indices (Adesola, 2013). Trait associations showed seasonal variation mainly due to the fact that genotypic performance depends on environmental conditions (Bose *et al.*, 2014: Balakrishnan *et al.*, 2016). Similar observations on GEI were made by Tariku *et al*., (2013) and Akter *et al*., (2014) on multi-environment studies using rice genotypes.

Conclusion

In the present study, association analysis was done using 177 RILs derived from a cross between Swarna x 166s for seventeen yield and grain shape-related traits in *Rabi* 2018/19 and *Kharif* 2019 seasons. Biomass showed significant positive correlations with the majority of traits, followed by days to fifty percent flowering in both seasons. Among all the traits, highest significant and strong positive correlation was observed between grain length and grain perimeter followed by productive tiller number and total tiller number in *Rabi* season. When harvest index showed significant negative correlations with majority of the yield contributing traits followed by filled grains in *Rabi* season, days to fifty percent flowering, plant height, 1000 grain weight and harvest index showed the same correlations in *Kharif* season. Grain shape traits were interrelated with each other significantly in both seasons. Grain length had a significant, positive and moderate association with grain length/width ratio, grain area and a high association with grain perimeter. Single plant yield showed a moderate positive association with both productive and total tillers per plant, panicle weight, biomass and harvest index in both seasons. According to our results, the focus on productive tiller number and panicle weight will be most helpful while selecting the genotypes in the field for higher yield in this population.

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