Genetic variability and association analysis in rice (Oryza sativa L.) germplasm based on biometrical and floral traits

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Abstract
The present experiment was carried out to evaluate 150 rice germplasm accessions for biometrical and floral traits to assess the amount of variability and association among them. Eight biometrical and thirteen floral characters were recorded. Analysis of variance showed that there was a significant variation observed for all the characters studied at a 5% probability level. High PCV and GCV was recorded for spikelet sterility percentage (28.78 %, 21.61 %), number of pollen grains per anther (22.25 %, 21.08 %) and pollen load (31.31 %, 31.12 %). High heritability along with high genetic advance was recorded for all biometrical traits except spikelet sterility percentage and six floral traits viz., stigma length, anther length, style length, apical pore, number of pollen grains per anther and pollen load. Single plant yield showed a positive correlation with thousand grain weight. Mean performance of traits studied indicated that, higher yield per plant was observed in Thilainayagam and a high 1000 grain weight in Sembala. The high number of productive tillers per plant was observed in Kodai. Four genotypes, Thooyamalli, IG 23, IG 24 and Vellai chithiraikar were recorded higher panicle length and the least spikelet sterility percentage was observed in IG 7. Hence, the identified germplasm with distinct biometrical and floral characters could be utilized for further hybridization programme.

Key words: Variability, Correlation, Germplasm, Rice

INTRODUCTION
Rice (Oryza sativa L.) is one of the major staple food crops in the world (Abodolereza and Racionzer, 2009). About ninety per cent of the world’s rice was produced and consumed in Asia, out of which 20 per cent is produced in India. The cultivated area of rice in India is 43.8 m.ha with a production of 116.4 m.t and productivity of 2.6 t/ha during 2018-2019 (www.agricoop.nic.in). In Tamil Nadu, the area under rice cultivation is 1.72 m. ha with a production of 6.1 m.t and productivity is 3.1 t/ha (www.indiastat.com). The exploitation of germplasm and designing suitable plant breeding programmes is necessary to augment yield increase. First hand knowledge on variability and genetic diversity in the germplasm is essential to initiate any breeding programme. The germplasm lines are a reservoir for various important traits including earliness, quality characters but none of the studies harnessed the floral characters of germplasm. Variability helps in better understanding of breeding behaviour of various traits and to assess the character of importance for choosing the best cultivars. Correlation studies help in knowing the type of association between the characters. Hence, the present study was attempted to measure the variability, heritability, genetic advance besides association studies in rice.
MATERIALS AND METHODS
The field experiment was laid out with 150 rice germplasm accessions at the Department of Rice, TNAU, Coimbatore during 2016-2017. The design, RBD was followed with a spacing of 20×20 cm in 4 rows with 1.5 m each. All the recommended agronomic practices were followed. Observations were recorded on eight biometrical traits namely, days to fifty per cent flowering, the number of productive tillers, the number of grains per panicle, spikelet sterility percentage (%), panicle length (cm), 1000 seed weight (g), plant height (cm) and single plant yield (g) along with 13 floral traits namely, anther length (mm), anther breadth (mm), style length (mm), style breadth (mm), stigma length (mm), stigma breadth (mm), apical pore (mm), basal pore (mm), the number of pollen grains per anther, pollen fertility percentage (%), glume opening angle (°), anthesis duration and pollen load (Matsui et al., 2003) were measured by using fluorescence microscope by scope image V 9.1 build 90225p. Heritability in a broad sense (h²), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and genetic advance (GA) and genetic advance as a percentage over mean was calculated by using the formula illustrated by Burton (1952) and Lush (1940). The association analysis was illustrated by using the formula given by Johnson et al. (1955).

RESULTS AND DISCUSSION
ANOVA for biometrical traits and floral traits (Tables 1 & 2) showed significant differences among all genotypes and characters studied. The presence of high variability among all the genotypes for all traits is revealed. Variability helps to choose potential parents in a breeding programme. High PCV was observed for spikelet sterility percentage (28.78 %) and number of grains per panicle (22.25 %) among the biometrical traits and stigma width (23.38 %), basal pore (26.78 %), number of pollen grains per anther (24.73 %) and pollen load (31.31 %) among the floral traits studied (Tables 3 & 4). These results were in agreement with Singh et al. (2012), Mahalingam et al. (2013), Augustina et al. (2013) and Nath et al. (2016). Moderate PCV was observed for days to 50 per cent flowering (14.0 %), plant height (18.09 %), number of productive tillers per plant (19.43 %), panicle length (12.71 %), thousand grain weight (18.34 %), single plant yield (19.77 %), anther length (14.92 %), anther width (15.47 %), stigma length (18.51 %), style length (20.0 %), style width (14.33 %), apical pore (15.08 %), glume opening angle (11.34 %) and anthesis duration (15.83 %). Similar reports of moderate PCV were reported for a number of productive tillers per plant and panicle length by Priyanka et al. (2019). Low PCV was observed for pollen fertility percentage (3.31 %) and a similar finding was reported by Sheeba et al. (2006).

High GCV values were observed for a number of spikelet sterility percentages (21.61 %), pollen grains per anther (21.08 %) and pollen load (31.12 %). The studies of

Table 1. ANOVA for biometrical traits of 150 germplasm accessions of rice

| Sources of variation | df | Mean sum of squares |
|----------------------|----|---------------------|
|                      |    | DFF | PHT | NPT | PL | GPP | SSP | TGW | SPY |
| Replication          | 2  | 6.17 | 12.26 | 0.11 | 3.03 | 515.52 | 43.01 | 0.57 | 0.77 |
| Genotype             | 149| 487.8294** | 1359.85** | 29.44** | 23.20** | 1461.72** | 94.99** | 56.02** | 72.99** |
| Error                | 298| 3.48 | 12.24 | 2.21 | 1.08 | 214.02 | 19.48 | 1.65 | 2.23 |

**Significant at % level, DFF – Days to fifty per cent flowering, PHT– Plant height, NPT- Number of productive tillers per plant, PL – Panicle length, GPP – Number of grains per panicle, SSP – Spikelet sterility percentage, TGW – Thousand grain weight, SPY – Single plant yield

Table 2. ANOVA for floral traits of 150 germplasm accessions of rice

| Sources of variation | df | Mean sum of squares |
|----------------------|----|---------------------|
|                      |    | AL | AW | SGL | SGW | STL | STW | BP | AP | NPG | PF | POL | GOA | AD |
| Replication          | 2  | 0.0891 | 0.0009 | 0.0258 | 0.0006 | 0.0044 | 0.0005 | 0.0005 | 0.0123 | 147856.2 | 7.9683 | 12.8892 | 3.5356 | 1.1022 |
| Genotype             | 149| 0.29* | 0.01* | 0.10* | 0.02* | 0.05* | 0.001* | 0.03* | 0.05* | 478313.70* | 19.68* | 1212.40* | 31.41* | 1.99* |
| Error                | 298| 0.0326 | 0.0022 | 0.0154 | 0.0032 | 0.0069 | 0.0002 | 0.0065 | 0.0041 | 53215.68 | 5.239 | 5.003 | 2.3812 | 0.534 |

*Significant at 5% level, AL – Anther length, AW – Anther width, SGL – Stigma length, SGW – stigma width, STL – Style length, STW – Style width, BP – Base pore, AP – Apical pore, NPG – Number of pollen grain per anther, PF – Pollen fertility, POL – Pollen load, GOA – Glume opening angle, AD – Anthesis duration

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Seetharamaiah et al. (2001) and Mahalingam et al. (2013) were in agreement with this finding. Moderate GCV values were recorded by days to 50 per cent flowering (13.85 %), plant height (17.85 %), the number of productive tillers per plant (17.43 %), panicle length (11.86 %), the number of grains per panicle (18.08 %), thousand grain weight (17.56 %), the number of grains per panicle (18.08 %), single plant yield (18.90 %), anther length (12.73 %), anther width (11.71 %), stigma length (14.79 %), stigma width (17.71 %), style length (16.32 %), basal pore (19.59 %), apical pore (13.22 %), glume opening angle (10.16 %) and anthesis duration (10.93 %). Low GCV was observed for style width (9.11 %) and pollen fertility percentage (2.29 %). These results were found similar to Verma et al. (2010) and Seyoum et al. (2012). The data showed that there is a significant level of variability for most of the traits in this germplasm. Hence, selection can be carried out for those traits with high and moderate GCV percentages. 

The heritability and genetic advance provide the proportion of heritable variation and the genetic gain to be obtained in subsequent generations. High heritability was recorded for 50 per cent flowering (97.89 %), plant height (97.35 %), the number of productive tillers per plant (80.41 %), panicle length (87.11 %), the number of grains per panicle (66.02 %), thousand grain weight (91.65 %), single plant yield (91.36 %), anther length (72.71 %), stigma length (63.80 %), style length (66.60 %), apical pore (76.91 %), the number of pollen grains per anther (72.70 %), pollen load (98.77 %) and glume opening angle (80.25 %). These results were coinciding with the findings of Mahalingam et al. (2013), Parikh et al. (2012), Ekka et al. (2015), Table 3. Estimates of variability, heritability and genetic advance of biometrical traits in 150 germplasm accessions of rice

| Traits | Phenotypic variance | Genotypic variance | PCV (%) | GCV (%) | Heritability (%) | GAM |
|--------|---------------------|-------------------|---------|---------|-----------------|-----|
| DFF    | 164.93              | 161.45            | 14.00   | 13.85   | 97.89           | 28.24 |
| PHT    | 461.46              | 449.21            | 18.09   | 17.85   | 97.35           | 36.28 |
| NPT    | 11.29               | 9.08              | 19.43   | 17.43   | 80.41           | 32.19 |
| PL     | 8.46                | 7.37              | 12.71   | 11.86   | 87.11           | 22.81 |
| GPP    | 629.93              | 415.91            | 22.25   | 18.08   | 66.02           | 30.26 |
| SSP    | 44.65               | 25.17             | 21.61   | 18.80   | 56.37           | 33.42 |
| TGW    | 19.78               | 18.13             | 17.56   | 17.56   | 91.65           | 34.63 |
| SPY    | 25.82               | 23.59             | 18.90   | 18.90   | 91.36           | 37.20 |

DFF – Days to fifty per cent flowering, PHT– Plant height, NPT- Number of productive tillers per plant, PL – Panicle length, GPP – Number of grains per panicle, SSP – Spikelet sterility percentage, TGW – Thousand grain weight, SPY – Single plant yield

Table 4. Estimates of variability, heritability and genetic advance of floral traits in 150 germplasm accessions of rice

| Traits | Phenotypic variance | Genotypic variance | PCV (%) | GCV (%) | Heritability (%) | GAM |
|--------|---------------------|-------------------|---------|---------|-----------------|-----|
| AL     | 0.120               | 0.087             | 14.92   | 12.73   | 72.71           | 22.35 |
| AW     | 0.005               | 0.003             | 15.47   | 11.71   | 57.33           | 18.27 |
| SGL    | 0.043               | 0.027             | 18.51   | 14.79   | 63.80           | 24.33 |
| SGW    | 0.008               | 0.004             | 23.38   | 17.71   | 57.35           | 27.63 |
| STL    | 0.021               | 0.014             | 20.00   | 16.32   | 66.60           | 27.44 |
| STW    | 0.000               | 0.000             | 14.33   | 9.11    | 40.40           | 11.93 |
| BP     | 0.014               | 0.007             | 26.78   | 19.59   | 53.49           | 29.51 |
| AP     | 0.018               | 0.014             | 15.08   | 13.22   | 76.91           | 23.88 |
| NPG    | 194915.03           | 141700.93         | 24.73   | 21.08   | 72.70           | 37.03 |
| PF     | 10.049              | 4.816             | 3.31    | 2.29    | 47.92           | 3.27 |
| POL    | 407.469             | 402.465           | 31.31   | 31.12   | 98.77           | 63.71 |
| GOA    | 12.058              | 9.677             | 11.34   | 10.16   | 80.25           | 18.74 |
| AD     | 1.020               | 0.486             | 11.13   | 10.93   | 47.66           | 15.54 |

AL – Anther length, AW – Anther width, SGL – Stigma length, SGW – stigma width, STL – Style length, STW – Style width, BP – Base pore, AP – Apical pore, NPG – Number of pollen grain per anther, PF – Pollen fertility, POL – Pollen load, GOA – Glume opening angle, AD – Anthesis duration

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Hemalatha et al. (2019) and Kujur et al. (2019). Moderate heritability was observed for spikelet sterility percentage (56.37 %), anther width (57.33 %), stigma width (57.35 %), style width (40.40 %), basal pore (53.49 %), pollen fertility percentage (47.92 %) and anthesis duration (47.66 %). A low heritability value was not observed for any trait. High heritability and high genetic advance as percentage of mean were recorded for the traits, 50 per cent flowering (28.24 %), number of productive tillers per plant (32.19 %), panicle length (22.81 %), number of grains per panicle (30.26 %), thousand grain weight (34.63 %), plant height (36.28 %) single plant yield (37.20 %), anther length (22.35 %), stigma length (24.33 %), style length (27.44 %), apical pore (23.88 %), number of pollen grains per anther (37.03 %) and pollen load (63.71 %). The preponderance of additive gene action to control these traits was revealed by high heritability and genetic advance as percentage of mean and were in line with findings of Singh et al. (2012) and Sritama et al. (2015). Moderate heritability and low genetic advance as per cent of gain were recorded for pollen fertility percentage (3.27 %).

Association among the traits is of primary importance for the selection of desirable characters and selecting associated traits aids in speeding up the breeding programmes. Single plant yield is dependent on many independent traits. The selection efficiency for yield is dependent on the direction and magnitude of association between yield and its associated characters. Single plant yield showed a significant and direct association with thousand grain weight (Tables 5). The results were similar to the findings of Ekka et al. (2011) and Moukombi et al. (2011). Inter correlation among component traits was estimated and days to 50 per cent flowering showed a significant and direct association with panicle length, the number of grains per panicle, plant height but a negative

Table 5. Correlation coefficient among different biometrical traits with single plant yield

| Traits | DFF | PHT | NPT | PL | GPP | SSP | TGW | SPY |
|--------|-----|-----|-----|----|-----|-----|-----|-----|
| DFF    | 1   | 0.37** | -0.10 | 0.32** | 0.54** | -0.33** | -0.06 | 0.04 |
| PHT    | 1   | 0.24** | 0.46** | 0.29** | -0.17* | 0.03 | 0.03 |
| NPT    | 1   | 0.03 | -0.04 | 0.00 | -0.15 | 0.04 |
| PL     | 1   | 0.55** | -0.19 | 0.04 | -0.04 |
| GPP    | 1   | -0.66** | -0.34** | -0.14 |
| SSP    | 1   | 0.20* | 0.05 |
| TGW    | 1   | 0.34** |
| SPY    | 1   | | | | | | |

*Significant at 5%, **Significant at 1%

DFF – Days to fifty per cent flowering, PHT– Plant height, NPT- Number of productive tillers per plant, PL – Panicle length, GPP – Number of grains per panicle, SSP – Spikelet sterility percentage, TGW – Thousand grain weight, SPY – Single plant yield

Table 6. Correlation coefficient among different floral traits

| Traits | AL | AW | SGL | SGW | STL | STW | BP | AP | NPG | PF | POL | GOA | AD |
|--------|----|----|-----|-----|-----|-----|----|----|-----|----|-----|-----|----|
| AL     | 1  | 0.07 | 0.11 | 0.16 | 0.08 | 0.18 | 0.21** | 0.23** | 1.00** | 0.03 | 0.01 | -0.05 | 0.10 |
| AW     | 1  | 0.06 | 0.12 | 0.01 | 0.03 | 0.24** | 0.15 | 0.07 | -0.06 | -0.06 | -0.04 | 0.07 |
| SGL    | 1  | 0.47** | 0.62** | 0.42** | 0.18* | 0.07 | 0.11 | 0.05 | 0.10 | -0.10 | -0.12 |
| SGW    | 1  | 0.27** | 0.16 | 0.11 | 0.04 | 0.16 | 0.08 | 0.04 | 0.04 | 0.07 | 0.13 |
| STL    | 1  | 0.42** | -0.09 | -0.13 | 0.08 | 0.00 | 0.08 | -0.21* | 0.10 |
| STW    | 1  | -0.24** | -0.15 | 0.18* | 0.05 | -0.01 | -0.25** | 0.17* |
| BP     | 1  | 0.31** | 0.21** | -0.12 | -0.06 | -0.07 | -0.15 |
| AP     | 1  | 0.23** | 0.05 | 0.01 | 0.02 | -0.07 |
| NPG    | 1  | 0.03 | 0.01 | -0.05 | 0.10 |
| PF     | 1  | 0.12 | -0.08 | 0.05 |
| POL    | 1  | -0.06 | 0.01 |
| GOA    | 1  | -0.19* |
| AD     | 1  | | | | |

*Significant at 5%, **Significant at 1%

AL – Anther length, AW – Anther width, SGL – Stigma length, SGW – stigma width, STL – Style length, STW – Style width, BP – Base pore, AP – Apical pore, NPG – Number of pollen grain per anther, PF – Pollen fertility, POL – Pollen load, GOA – Glume opening angle, AD – Anthesis duration

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with spikelet sterility. Plant height showed a significant correlation with the number of productive tillers, panicle length and the number of grains per panicle. Panicle length showed a positive correlation with the number of grains per panicle. The number of grains per panicle was significant and negative with spikelet sterility percentage and thousand grain weight. The anther length had a positive correlation with basal pore, style width, basal pore, apical pore and number of pollen grains per anther. (Table 6.) A significant positive correlation was found between anther width and basal pore. Stigma length had a positive inter correlation with stigma width, style length, style width and basal pore. Stigma width was positively correlated with style length. Style length exhibited a positive association with style width and significant and negative association with glume opening angle. Style width had a significant and negative association with basal pore, glume opening angle and positively correlated with a number of pollen grains per anther and anthesis duration. Basal pore had a positive significant association with apical pore and number of pollen grains per anther. Apical pore had positively correlated with a number of pollen grains per anther. Stigma length exhibited a negative association with anthesis duration. Similar results were also found by Nandan et al. (2010) and Singh et al. (2012).

Out of 150 germplasm, a higher yield per plant was observed in Thilainayagam and high thousand grain weight in Sembala. A high number of productive tillers per plant was observed in Kodai and the least spikelet sterility percentage was observed in IG 7. High anther length was observed in sorna kuruvai and high stigma length in IG 23. Pollen fertility percentage was higher in IG 38 and high stigma length was observed in IG 7. High anther length was observed in Kodai and the least spikelet sterility in Sembala. A high number of productive tillers per plant were also found by Nandan et al. (2010) and Singh et al. (2012). Hence the present study concluded that there is high variability among all the genotypes for the biometrical and floral traits. Accordingly, this germplasm can be exploited for improving the specific traits in a breeding programme.

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