

Genetic Analysis And Traits Association Study In Marker-Assisted Multi-Drought-Traits Pyramided Genotypes Under Reproductive-Stage Moisture Stress In Rice (*Oryza Sativa L.*)

Ashvinkumar Katral (✉ ashokkatral@gmail.com)

ICAR-Indian Agricultural Research Institute, New Delhi <https://orcid.org/0000-0002-4000-0481>

Hanamareddy Biradar

SPESS, Louisiana State University, Baton Rouge, LA 70803, United States

Yallappa Harijan

Central Sericultural Research and Training Institute Berhampore, West Bengal, India

Aruna Y. R.

University of Agricultural Sciences, GKVK, Bengaluru, India

Jagadeesh Hadimani

University of Agricultural Sciences, GKVK, Bengaluru, India

Shailaja Hittalmani

University of Agricultural Sciences, GKVK, Bengaluru, India

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Abstract

Reproductive-stage drought-stress is a major production constraint in rainfed rice ecosystem. Emergence of marker-assisted breeding strategies for developing drought-tolerant rice varieties are being optimized through exploiting adaptive-trait for their increased contribution towards grain-yield under recurring-drought. Grain-yield is a complex-trait; requires knowledge of genetics and association among yield contributing component-trait. Current study was undertaken using 21 marker-assisted multi-drought-trait pyramided genotypes responses for genetic variability and association of traits for grain-yield under aerobic and reproductive-stage drought conditions. Field evaluation was carried-out in two seasons and data was collected on various parameters. Path-coefficient analysis was used as a selection criterion to select yield contributing-trait and found nine phenotypic traits were having a positive direct-effect on grain-yield during both and/or at least one season under both moisture-regimes. The data from summer and *Kharif* seasons have been pooled within their respective moisture-regimes due to the non-significance of Levene's test of homogeneity of variances and estimated BLUP values. ANOVA based on BLUP values revealed significant differences for moisture-conditions and also among genotypes. Phenotypic variation via. box-plots and histogram depicted mean phenotypic differences of traits under two moisture-regimes. Majority of the traits possessed high PCV and GCV with high heritability and GAM indicating higher trait expression and additive gene action lead to effectiveness of selection under drought/moisture stress. Grain-yield possessed a positive correlation with all the component-trait under consideration during both moisture-regimes. Selection of genotypes based on these component-trait were rewarding and seems to be better selection-criteria. Finally, we can end-up with superior-genotypes suitable for intermittent-drought conditions.

Introduction

Grain production is the basis of global food security and is indispensable for feeding mankind worldwide; it needs a significant increase in output in the coming decades to sustain the food and feed requirements (Mesterhazy et al. 2020). As sustainability is the key factor in the production, globally rice, wheat, and maize are the three leading food crops; together they directly supply more than 50% of all calories consumed by the entire human population (FAO 2016). Among these, rice is a major cereal food crop in Asia and feeds more than two-third of a global population (Dowling et al. 1998; Fahad et al. 2019). Asia is the biggest producer and consumer of rice, accounting for 90% of the world's production and consumption (Khahani and Hittalmani 2015). About 55% of the rice area is irrigated and accounts for 75% of total rice production across the globe (Fahad et al. 2019). It signifies that rice is a water intensive crop and requires 5000 litres of water per kg rice grain production (Hittalmani 2010; Jana et al. 2018). Rice production is dwindling due to many abiotic and biotic factors (Korrens et al. 2017; Rawte et al. 2021). To meet the growing food demands of the population, it is critical to increase the productivity levels of rice by combating abiotic and biotic stresses (Roberts and Mattoo 2018).

Among the abiotic stresses, drought is the period of no rainfall or irrigation that affects plant growth and is a major production constraint for about 50% of the world production area of rice (Fahad et al. 2017). It is a major prevailing limiting factor affecting rice productivity in the Asian-Pacific region and reported to affect the area of 56 million hectares under different rice ecosystems (46 Mha: rainfed lowland and 10 Mha: rainfed upland) (Pandey et al. 2007; Bhandari et al. 2020). Varying intensities of drought stress at different developmental stages of crop via. seedling, vegetative and reproductive stages are quantified (Price and Courtois 1999; Tripathy et al. 2000; Nguyen and Bui 2008; Xu et al. 2011). In Asia itself, drought is reported to affect approximately 42 Mha of rice production (34 Mha: rainfed lowland and 8 Mha: rainfed upland) (Huke and Huke 1997). Drought causes more damage during reproductive stage moisture stress (RMS), especially during flowering stage (Venuprasad et al. 2007; Serraj et al. 2009) reducing the number of grains per panicle and increasing grain sterility; thereby reducing the grain yield of 34–53% under moderate stress and 65–88% under severe stress in comparison to irrigated condition (O'Toole 1982; Kumar et al. 2008; Torres and Henry 2018; Bhandari et al. 2020). Globally, rice production is predicted to be further challenged by an erratic and increasing frequency; even more severe drought due to climate change (Wassmann et al. 2009). Targeted higher productivity with climate resilience is thereby essential to stabilize rice production by creating climate-smart varieties for adverse ecologies.

The efforts are needed to address the problem of water scarcity by breeding new genotypes tolerance to moisture stress conditions. The alternate option is the growing of aerobic rice varieties, which can be grown in rainfed conditions (Hittalmani and Shivashankar 1987; Atlin et al. 2004; Zhao et al. 2010; Babu et al. 2011; Liu et al. 2019). Moreover, before taking any breeding

programme, a breeder should have a thorough knowledge of nature and magnitude of genetic variability, heritability, genetic advance as *per cent* mean (GAM) and traits associations with grain yield in crop species under different moisture regimes (Lone et al. 2019). The multi-trait pyramided genotypes are thought to serve the problem of moisture stress conditions to a greater extent. Most of the economically important traits are complex and are the result of the interaction of a number of component traits. Understanding the relationship between grain yield and its component traits is most important step for making the best use of these relationships in selection. Grain yield is a complex trait; the direct selection of genotypes based on yield is misleading (Eltaher et al. 2021). Hence, the selection of genotypes suitable for reproductive stage moisture stress conditions based on component traits is most meaningful. The genotypes possessing higher genetic divergence would be responsible for improvement as they were likely to produce required recombinants upon crossing of such genotypes.

Breeding efforts at University of Agricultural Sciences, GKVK, Bengaluru has led to the development of set of F₉ generation marker-assisted multi-trait pyramided (MAP) lines with different combinations of gene/QTLs for drought tolerance, bacterial blight and blast disease resistance. However, these lines have not been characterised at phenotypic level under aerobic and reproductive stage drought stress. The present investigation was thus aimed to (i) assess the genetic variability among the MAP genotypes under aerobic and RMS conditions (ii) study the traits association for grain yield and related traits in MAP genotypes under aerobic and RMS conditions.

Material And Methods

Plant material used and design of the experiment

The present study comprised of 21 F₉ generation marker assisted multi-trait pyramided genotypes obtained by crossing divergent parental lines 'RB6' (derived from cross RpBio × BL 122, resistant to leaf blast and bacterial blight diseases with fine grain quality) and 'QRT25' (derived from the cross IR64 × Azucena, drought-tolerant, high water use efficiency, high yielding with medium bold grain quality). The list containing the gene/QTLs possessed by the parents (RB6 and QRT25) have been presented in Table S1. F₁ plants were obtained and forwarded through marker assisted selection (MAS), genotypes were selected in each generation based on grain yield per plant (Haradari 2013; Uday 2016). Twenty-one marker assisted multi-trait pyramided genotypes along with the parents (RB6 and QRT25) and check (MAS 946-1) were evaluated for grain yield and other component traits under two moisture regimes namely aerobic (A) as control and reproductive stage moisture stress (RMS) as test environment during summer-2017 and Kharif-2017-18 seasons with two replications in randomized complete block design (RCBD) at experimental plots of Genetics and Plant Breeding, 'K' block, UAS, GKVK, Bengaluru.

Reproductive stage moisture stress (RMS)

The standard crop management practices were followed to grow the crop during both seasons. The crop management practices were the same till 75 days after sowing (DAS) in both moisture regimes. The water supply was withheld from 75 DAS in RMS condition to drain out available soil moisture and reproductive stage moisture stress was imposed at 80 DAS for the period of 15 days (Fig. 1) but in the aerobic condition, need based irrigation has been given throughout crop cycle in both the seasons (Fig. 1).

Phenotypic evaluation

The observations were recorded on five randomly selected plants from each marker assisted pyramided genotype in each replication from both aerobic and RMS conditions. Mean values were utilized for statistical analysis and the traits observed for eliciting the information are: plant height- PH (cm), days to 50% flowering- DFF, days to maturity- DM, productive tillers per plant- PT, panicle length- PL (cm), panicle exertion- PE (cm), flag leaf length- FL (cm), spikelet fertility- SF (%), 1000-grain weight- 1000-GW (g), biomass yield- BM (kg/ha), harvest index- HI (%) and grain yield- GY (kg/ha).

Statistical analysis:

Path analysis was carried out for both the season data (summer-2017 and Kharif-2017-18) and both moisture regimes (aerobic and RMS) separately via Windostat v8.1 statistical software. Levene's test has been carried-out using SPSS v26 software to test

the homogeneity of variances among summer and *Kharif* seasons data under two different moisture regimes. If the test is significant, the variances of both the season's differ significantly; if it is non-significant then both the season's variances are approximately equal or same. Hence, the decision on combining/pooling of data from both the seasons can be done. Due to non-significance of Levene's test, the summer and *Kharif* season data have been pooled within their respective moisture regimes and the Best Linear Unbiased Predictor (BLUP) values have been estimated via. Multi Environment Trail Analysis with R (META-R) statistical software. Further statistical analyses have been carried-out using BLUP values. BLUP-based analysis of variance (ANOVA) was done further to decide whether both the conditions (aerobic and RMS) data can be pooled or not and also to know statistical significance among the genotypes for various characters using Cropstat v7.2 statistical software. Phenotypic variation *via.* box plots, frequency distribution curves, estimation of genetic variability parameters [Phenotypic and Genotypic Coefficient of Variation (PCV and GCV), heritability (h^2) and genetic advance as percent mean (GAM)] and correlation plots have been deduced using 'R v4.1.0' statistical tool.

Formulae used for estimation of variability parameters are:

a. PCV and GCV were estimated as per Burton and Devane (1953)

$$PCV (\%) = \frac{\sqrt{\sigma^2_P}}{\bar{X}} \times 100$$

$$GCV (\%) = \frac{\sqrt{\sigma^2_G}}{\bar{X}} \times 100$$

b. Heritability in broad sense (h^2 (b)) was estimated as per Allard (1960)

$$h^2 (bs) = \frac{\sigma^2_G}{\sigma^2_P} \times 100$$

c. Genetic advance was estimated as per Johnson et al. (1955)

$$GA = K h^2 \sigma_P$$

Further, the GA as percent of mean (GAM) was estimated

$$GAM = \frac{GA}{\bar{X}} \times 100$$

where, σ^2_P = Phenotypic variance, σ^2_G = Genotypic variance, \bar{X} = General mean of character, K = Selection differential at 5% (2.06) selection intensity, h^2 = Broad sense heritability estimate, σ_P = Phenotypic standard deviation

Results And Discussion

Drought stress has emerged as an essential part of research and improvement of drought tolerance in plants is a thought-provoking task due to the complexity of these traits. Yield is one of the most important and complex trait; primarily affected by drought and leads to greater reduction in grain yield. It is both regulated by genes *via.* quantitative trait loci (QTLs) and even influenced by external environmental factors (Wang et al. 2012; Zeng et al. 2017). Selection of genotypes for prevailing environment solely based on yield is misleading. To overcome this complexity of decision, it is necessary to know association of traits for grain yield and it can act as strong evidence and most precise selection criteria.

Path coefficient analysis as a selection criterion for growth and yield contributing traits:

Path analysis was carried out for both the seasons' data under both the moisture regimes (Aerobic and RMS) separately for all the studied characters using phenotypic coefficient to ascertain the direct and indirect effect of the yield components on grain yield as suggested by Wright (1921) and Dewey and Lu (1959). Path analysis is a powerful tool to understand the association

among different variables more clearly as recorded by simple correlation values by partitioning the overall association of particular variables with dependent variables into direct and indirect effects. The results of path coefficient analysis for studied traits under aerobic (A) and reproductive stage moisture stress (RMS) during summer-2017 (Table S2) and *Kharif*-2017-18 (Table S3) have been presented. Positive direct effect on grain yield was exhibited by productive tillers, biomass yield and harvest index under both moisture conditions during both the seasons. The similar results have been reported earlier by Venkataramana and Hittalmani 1999; Manickavelu et al. 2006; Uday 2016. Biomass yield has highest direct effect [summer (A = 0.632 and RMS = 0.579) and *Kharif* (A = 0.704 and RMS = 0.778)] followed by harvest index [summer (A = 0.348 and RMS = 0.424) and *Kharif* (A = 0.230 and RMS = 0.220)]. The component traits like days to maturity (A = 0.009 and RMS = 0.002) and productive tillers (A = 0.031 and RMS = 0.068) were contributing positively to grain yield during the summer season. The other traits like days to 50% flowering (A = 0.021; RMS = 0.013), panicle exertion (A = 0.002; RMS = 0.004), spikelet fertility (A = 0.009; RMS = 0.015) and 1000-grain weight (A = 0.006; RMS = 0.004) also contributing positive towards grain yield during *Kharif* season. The spikelet fertility (A= -0.022; RMS= -0.063) has highest negative direct effect on grain yield and days to 50% flowering has lowest negative direct effect (A= -0.009; RMS= -0.001) during summer season. Grain yield is a result of other component traits contributing directly or indirectly (Eltaher et al. 2021). The direct selection of genotypes based on component traits viz., harvest index and productive tillers can be adopted for enhancement of grain yield as they showed positive direct effect on grain yield (Khahani and Hittalmani, 2015). The lower residual effect under both moisture regimes during both the seasons [summer (A = 0.156; RMS = 0.199) and *Kharif* = A = 0.102; RMS = 0.138) indicated that different characters other than the characters considered in this study influence the grain yield negligible.

By considering the positive contribution of component traits towards grain yield during both and/or either of the seasons under both the moisture regimes have been selected and further analysis have been carried out for these nine selected traits (viz., DFF, DM, PT, PE, SF, 1000-GW, BM, HI and GY) hereafter; mainly to focus on core contributing traits and to restrict our discussion to emphasise more on these traits.

Levene's test of homogeneity of variances

The results of Levene's test for selected traits have been presented in Table 1. Levene statistic in the table (1) represents F-test value at df1 and df2 degrees of freedom. Non-significance of Levene's statistic based on p-value (> 0.005) has been observed for all the phenotypic traits i.e., DFF, DM, PT, PE, SF, 1000-GW, BM, HI and GY across the seasons in both aerobic and RMS conditions separately. The positive sign of acceptance of Levene's homogeneity of variances, the trait variances of summer and *Kharif* seasons found to have equal variances in their respective moisture regimes (aerobic and RMS) (Levene 1960; O'Neill et al. 2002; Kim et al. 2018). Hence, the data from the summer and *Kharif* seasons have been pooled within their respective moisture regimes separately.

Table 1

Levene's test of homogeneity of variances for yield and yield contributing traits under aerobic and reproductive stage moisture stress (RMS)

| Traits | Aerobic | | | | RMS | | | |
|---------|------------------|-----|-----|---------|------------------|-----|-----|---------|
| | Levene statistic | df1 | df2 | p-value | Levene statistic | df1 | df2 | p-value |
| DFF | 0.647 | 1 | 40 | 0.426 | 1.236 | 1 | 40 | 0.273 |
| DM | 0.128 | 1 | 40 | 0.722 | 0.021 | 1 | 40 | 0.886 |
| PT | 4.124 | 1 | 40 | 0.050 | 4.724 | 1 | 40 | 0.036 |
| PE | 0.851 | 1 | 40 | 0.362 | 1.343 | 1 | 40 | 0.253 |
| SF | 0.865 | 1 | 40 | 0.358 | 0.826 | 1 | 40 | 0.369 |
| 1000-GW | 0.279 | 1 | 40 | 0.601 | 2.468 | 1 | 40 | 0.127 |
| BM | 0.357 | 1 | 40 | 0.553 | 0.031 | 1 | 40 | 0.861 |
| HI | 0.592 | 1 | 40 | 0.446 | 4.662 | 1 | 40 | 0.037 |
| GY | 0.726 | 1 | 40 | 0.399 | 0.025 | 1 | 40 | 0.876 |

d.f.- degrees of freedom, DFF- Days to 50% flowering, DM- Days to maturity, PT- Productive tillers plant⁻¹, PE- Panicle exertion (cm), SF- Spikelet fertility (%), 1000-GW- 1000-grain weight (g), BM- Biomass yield (kg/ha), HI-Harvest index (%), GY- Grain yield (kg/ha)

Estimation of BLUP value for pooled data

BLUP, a standard method for estimating random effects of a mixed model (Robinson 1991); was used for estimating genetic merits. Due to the non-significance of Levene's test, the pooled data has been used to estimate combined BLUP values for all the phenotypic traits i.e., DFF, DM, PT, PE, SF, 1000-GW, BM, HI and GY under aerobic and RMS conditions separately (Table S3 and S4 respectively). The estimated BLUP values have been used for further analyses hereafter.

ANOVA based on BLUP values for yield and yield attributing traits

The results of ANOVA based on BLUP values under aerobic and RMS conditions revealed the existence of highly significant differences between aerobic and RMS conditions for all the phenotypic traits i.e., DF, DM, PT, PE, SF, 1000-GW, BM, HI and GY (Table 2). The prevailing moisture conditions for yield and yield attributes were adversely affected on the genotypic performance and indicating significant variability among the pyramided genotypes (Uday 2016; Bhandari et al. 2020). The mean performance among the genotypes varying significantly for all the traits deducing the presence of higher variability; the presence of variability is a pre-requisite factor for any plant breeding activity (Uday 2016; Bhandari et al. 2020; Harijan et al. 2021). Even the genotype × condition interaction also showed significant differences for the traits i.e., PT, PE, 1000-GW, HI and GY indicating the genotypic performance is better restricted to particular moisture condition (Uday 2016) (Table 2). Significant differences among the genotypes due to differential expression of gene/QTLs in MAP genotypes under aerobic and RMS conditions (Haradari 2013; Uday 2016; Bhandari et al. 2020). Selection can be operated on available variability to select the suitable MAP genotype for prevailing/intermittent drought conditions; thereby achieving the sustainable production of grain yield under adverse climatic conditions. The significant results of BLUP-based ANOVA for the condition is strong evidence to conclude that the data on aerobic and RMS conditions have to be maintained separately and hence further analysis is carried-out individually for aerobic and RMS conditions.

Table 2
BLUP-based analysis of variance (ANOVA) for yield and yield attributing traits

| Source of variation | d.f | Mean sum of squares | | | | | | | | |
|----------------------|-----|---------------------|----------|---------|---------|----------|---------|------------|----------|------------|
| | | DF | DM | PT | PE | SF | 1000-GW | BM | HI | GY |
| Replication | 1 | 8.68 | 9.33 | 0.54** | 7.90** | 1.65 | 0.75 | 635795 | 0.0038** | 2426.89 |
| Condition | 1 | 16.30* | 131.25** | 87.61** | 18.37** | 595.24** | 18.52** | 97710000** | 0.039** | 32670500** |
| Genotype | 20 | 51.85** | 95.73** | 12.84** | 14.90** | 173.95** | 13.59** | 6369000 | 0.0051** | 1476770** |
| Genotype x Condition | 20 | 1.35 | 1.59 | 3.49** | 1.54** | 28.86 | 0.91** | 875283 | 0.0006** | 141167** |
| Error | 41 | 3.80 | 8.67 | 0.092 | 0.70 | 23.33 | 0.20 | 4299050 | 0.0007 | 3462.12 |
| Total | 83 | 14.99 | 29.43 | 5.04 | 4.62 | 67.59 | 3.83 | 5054130 | 0.0019 | 785224 |

* = significant at 5% level and ** = significant at 1% level,

d.f.- degrees of freedom, DFF- Days to 50% flowering, DM- Days to maturity, PT- Productive tillers plant⁻¹, PE- Panicle exertion (cm), SF- Spikelet fertility (%), 1000-GW- 1000-grain weight (g), BM- Biomass yield (kg/ha), HI-Harvest index (%), GY- Grain yield (kg/ha)

Phenotypic variation of the traits among MAP genotypes under aerobic and RMS conditions

Distribution of the measured phenotypic traits via. Box plots

Box-plots based on BLUP values for the phenotypic traits i.e., DF, DM, PT, PE, SF, 1000-GW, BM, HI, and GY presented in Fig. 2. The plots depicted the mean phenotypic differences under two moisture regimes (i.e., aerobic and RMS) and the performance of genotypes varied significantly except for days to 50% flowering and days to maturity (Fig. 2). Overall, the traits range was higher in aerobic compared to RMS; the traits PT, PE, 1000-GW, and GY exhibited symmetric distribution while the traits DF, DM, SF, BM, and HI exhibited skewed distributions under aerobic condition (Bhandari et al. 2020). Under RMS, all the traits values decreased in comparison to aerobic condition; the traits PT, 1000-GW and GY exhibited symmetric distribution under RMS also (Fig. 2). The mean consistent performance of genotypes under aerobic and RMS conditions is due to the presence of drought responsive gene/QTLs in the marker assisted pyramided genotypes (Haradari 2013; Uday 2016). The traits like PT, 1000-GW and GY distributed symmetrically under both moisture regimes can serve as strong selection criteria to select the genotypes for adverse conditions.

Distribution of the measured phenotypic traits via. histogram

Phenotypic traits variation via. histogram analysis among the MAP genotypes under aerobic and RMS conditions have been depicted separately using BLUP values and also represented the parental (RB6 and QRT25) and check MAS946-1 values in the histograms (Figs. 3 and 4). The frequency distributions for all the studied traits are found to be normal and following a standard normal distribution. The number of days required for flowering and maturity were found to be quite less as compared to parents (RB6 and QRT25) and check (MAS946-1) under both moisture regimes (Fig. 3and 4). The earliness in flowering and maturity is due to the recovery of transgressive segregants among the MAP genotypes (Haradari 2013). Earliness is one of the mechanism for drought escape and/or tolerance, hence these MAP genotypes can serve as a good source of breeding for adverse conditions (Haradari 2013; Uday 2016; Torres and Henry 2018). From among the parents and check, the mean performance of QRT25 is found to be better in aerobic as well as in RMS conditions since it is possessing higher mean values for the majority of the traits. Overall, the frequency distribution for all the traits is normal indicating to possess all kinds of combinations of gene/QTLs among the MAP genotypes (Haradari 2013). Hence, the histogram based selection of MAP genotypes for adverse conditions is additional support to the results of box plots.

Genetic variability parameters for yield and yield associated traits

In any crop improvement programme, the availability of genetic variability is a first and foremost prerequisite (Venkataramana and Hittalmani 2003; Uday 2016; Bhandari et al. 2020; Harijan et al. 2021). The economically important characters like grain yield; a well-established complex character and influenced by the large number of genes that are greatly influenced by environmental factors. The genetic variability observed is the average of total hereditary effects of concerned genes as well as the environmental influence. Hence, the variability is partitioned into heritable and non-heritable components with suitable genetic variability components such as phenotypic coefficient of variation (PCV %), genotypic coefficient of variation (GCV %), heritability in a broad sense (h^2 bs %) and genetic advance as *per cent* mean (GAM). The estimation of these variability parameters assists the breeder in achieving the projected crop improvement by operating selection on available variation.

In the current study, the genetic variability parameters viz., PCV, GCV, heritability and GAM for the phenotypic traits i.e., DF, DM, PT, PE, SF, 1000-GW, BM, HI and GY under both aerobic and RMS conditions have been presented in Table 3. Among all the studied characters, the highest PCV is observed than GCV; which indicated the role of environment on the expression of characters (Manjappa et al. 2014; Uday 2016; Harijan et al. 2021). The traits like productive tillers, panicle exertion, 1000-grain weight, biomass yield, harvest index and grain yield possessed high PCV and GCV with moderate to high heritability and high GAM under both moisture regimes. The spikelet fertility possessed moderate to high PCV and GCV with high heritability and GAM. This high heritability coupled with high GAM indicates that most likely the heritability is due to additive gene effects and the selection for aforesaid characters in drought/moisture stress conditions is found to be highly effective (Courtois et al. 2003; Singh and Narayan 2017). These traits are highly amenable for selection leading to high genetic variability and transmissibility while taking efforts towards crop improvement for grain yield under reproductive stage moisture stress condition (Singh et al. 2011; Haradari 2013; Manjappa et al. 2014).

Table 3

Estimates of components of genetic variability parameters for yield and yield attributing traits under aerobic and RMS conditions

| Traits | PCV % | | GCV% | | h^2 bs (%) | | GAM % | |
|----------------|---------|-------|---------|-------|--------------|-------|---------|-------|
| | Aerobic | RMS | Aerobic | RMS | Aerobic | RMS | Aerobic | RMS |
| DFF | 4.59 | 4.87 | 4.41 | 4.53 | 91.50 | 87.50 | 8.71 | 8.72 |
| DM | 4.43 | 3.72 | 4.33 | 3.68 | 95.50 | 97.00 | 8.76 | 7.48 |
| PT | 25.35 | 31.96 | 18.38 | 24.96 | 52.00 | 61.00 | 26.75 | 40.16 |
| PE | 120.05 | 64.26 | 101.41 | 42.17 | 72.50 | 44.00 | 176.81 | 59.29 |
| SF | 16.60 | 25.65 | 12.81 | 23.23 | 62.00 | 80.50 | 20.59 | 43.60 |
| 1000-GW | 20.05 | 26.72 | 14.03 | 24.73 | 50.50 | 83.00 | 20.56 | 47.26 |
| BM | 31.19 | 37.59 | 22.14 | 26.90 | 51.50 | 53.00 | 32.60 | 40.41 |
| HI | 22.47 | 31.89 | 15.55 | 22.40 | 50.00 | 51.00 | 22.36 | 32.49 |
| GY | 36.48 | 46.77 | 25.20 | 35.74 | 47.00 | 57.50 | 35.89 | 56.26 |

DFF- Days to 50% flowering, DM- Days to maturity, PT- Productive tillers plant⁻¹, PE- Panicle exertion (cm), SF- Spikelet fertility (%), 1000-GW- 1000-grain weight (g), BM- Biomass yield (kg/ha), HI-Harvest index (%), GY- Grain yield (kg/ha)

Association of yield and yield attributing traits under aerobic and RMS conditions among the MAP genotypes

The correlation coefficient estimates the degree and direction of association between pair of characters and helps simultaneous improvement of the correlated traits through selection. The results of the correlation analysis have been depicted in Fig. 5a and

5b under aerobic and RMS conditions respectively. Grain yield was possessing a positive correlation with all the phenotypic traits i.e., DF, DM, PT, PE, SF, 1000-GW, BM, and HI during both the moisture regimes except for 1000-GW in RMS condition. Similar results have been reported by Haradari 2013; Khahani and Hittalmani 2015; Uday 2016; Bhandari et al. 2020. The phenotypic trait 1000-GW is having a negative correlation with DF, DM, and PE under both moisture conditions (Fig. 5a and 5b). The existence of negative correlation among these traits is due to the prolonged period of flowering and maturity. Non-emergence of panicle from leaf sheath has an adverse effect on spikelet fertility, 1000-GW thereby on grain yield (Chakrabort and Chaturvedi 2014; Khahani and Hittalmani 2015). The selection of genotypes based on these component traits (DF, DM, PT, PE, SF, 1000-GW, BM, and HI) in addition to grain yield was rewarding; we can robust the selection criteria and end up with superior genotypes suitable for an adverse climatic condition to attain sustainability in production.

Conclusions

Drought is particularly damaging during the reproductive stage and reduces the grain yield drastically. Hence, the selection of genotypes solely based on grain yield is misleading. In our findings, path analysis identified nine phenotypic traits (days to 50% flowering, days to maturity, productive tillers, panicle exertion, spikelet fertility, 1000-grain weight, biomass yield, harvest index and grain yield), one can rely upon these traits to select drought tolerant genotypes under moisture stress conditions.. Non-significance of Levene's test of homogeneity of variances among the data from summer and *Kharif* seasons lead to data pooling within their respective moisture regimes and estimation of BLUP values. ANOVA based on BLUP values revealed significant differences for moisture conditions and genotypes for all the nine phenotypic traits. Phenotypic variation of traits via. box plots and histograms depicted the mean phenotypic differences for all the nine phenotypic traits among the MAP genotypes under both moisture regimes. The transgressive segregants have been observed among the MAP genotypes for early flowering and maturity. Majority of the traits possessed moderate to high GCV and PCV with moderate to high heritability and high GAM under both moisture regimes. Grain yield possessed a positive correlation with all the phenotypic traits under consideration. The selection based on these component traits is rewarding and we can end up with superior genotypes suitable for diverse climatic conditions to attain sustainability in production.

Declarations

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Authors' contribution

Design of the experiment: AK and SH, Conduct of the experiments: AK, JH and AYR, Statistical analyses: YH and HB, Drafting of manuscript: AK, HB and SH

Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

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Figures

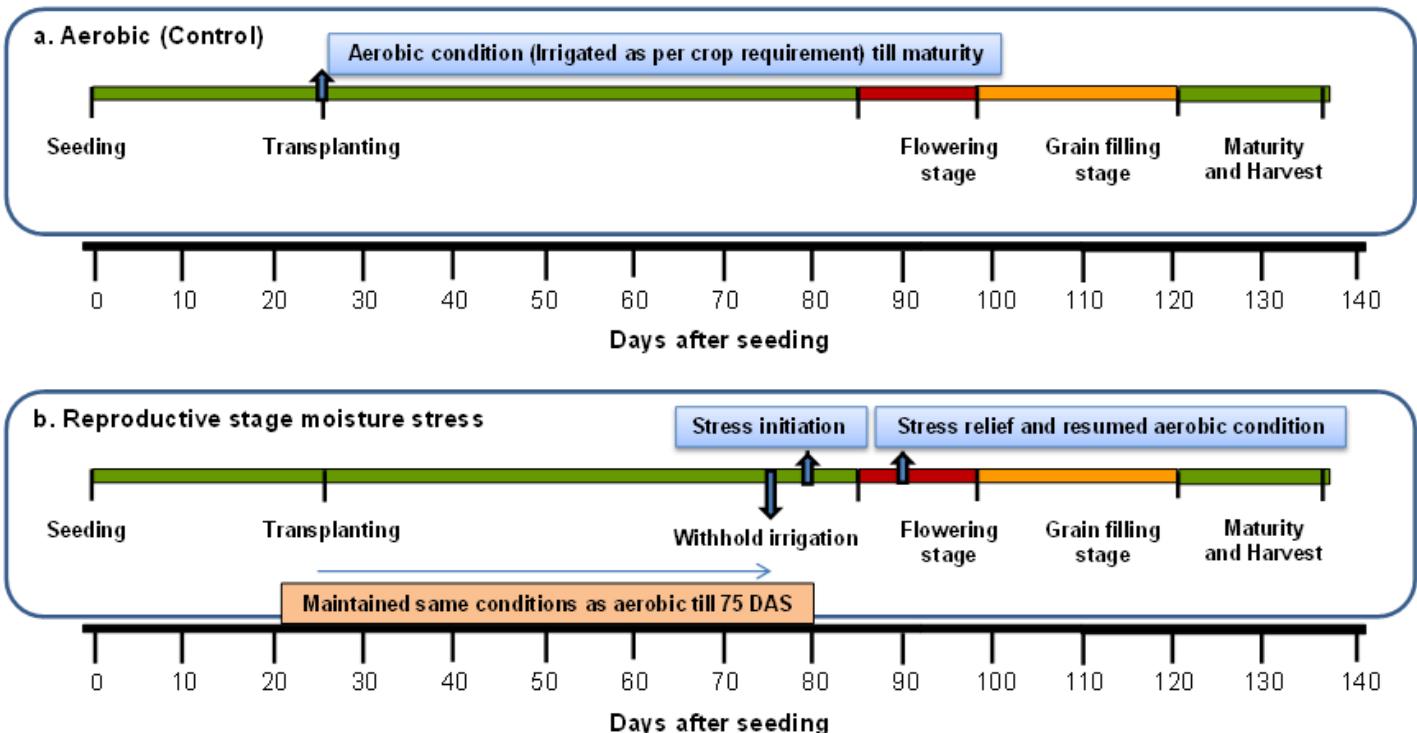


Figure 1

Protocol to impose moisture stress; a. Aerobic as normal, b. Reproductive moisture stress (RMS) as stress environment

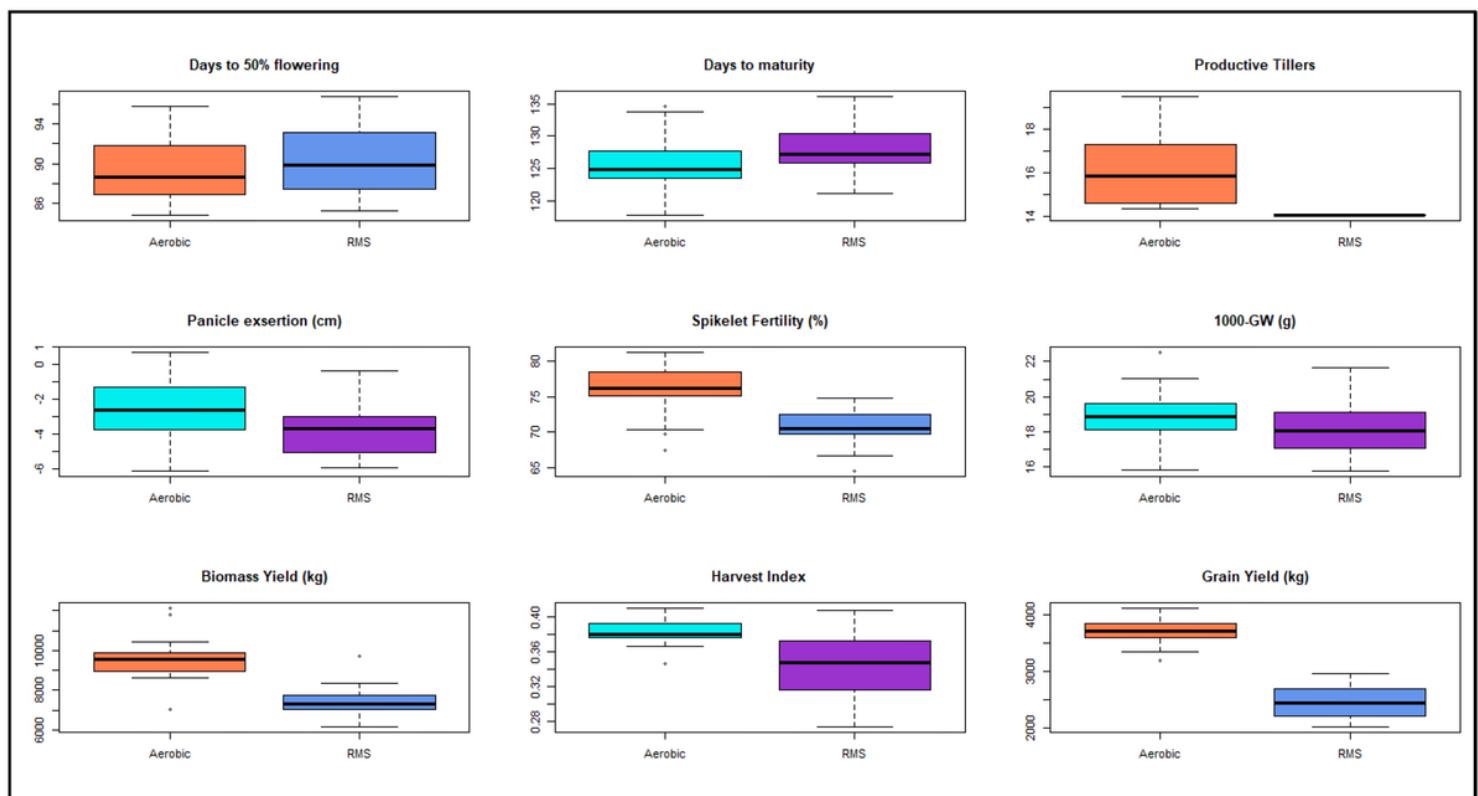


Figure 2

Boxplots of nine phenotypic variables within MAP genotypes under aerobic and RMS conditions; Days to 50% flowering, Days to maturity, Productive tillers per plant, Panicle exertion (cm), Spikelet fertility (%), 1000-grain weight (g), Biomass yield (kg/ha),

Harvest index and Grain yield (kg/ha). The boxplots are divided into aerobic and RMS for each trait. Within the boxplots, bold line represents the median, box edges represent upper and lower quantiles, and whiskers are 1.5 times the quantile of the data. Open dots represent the outliers

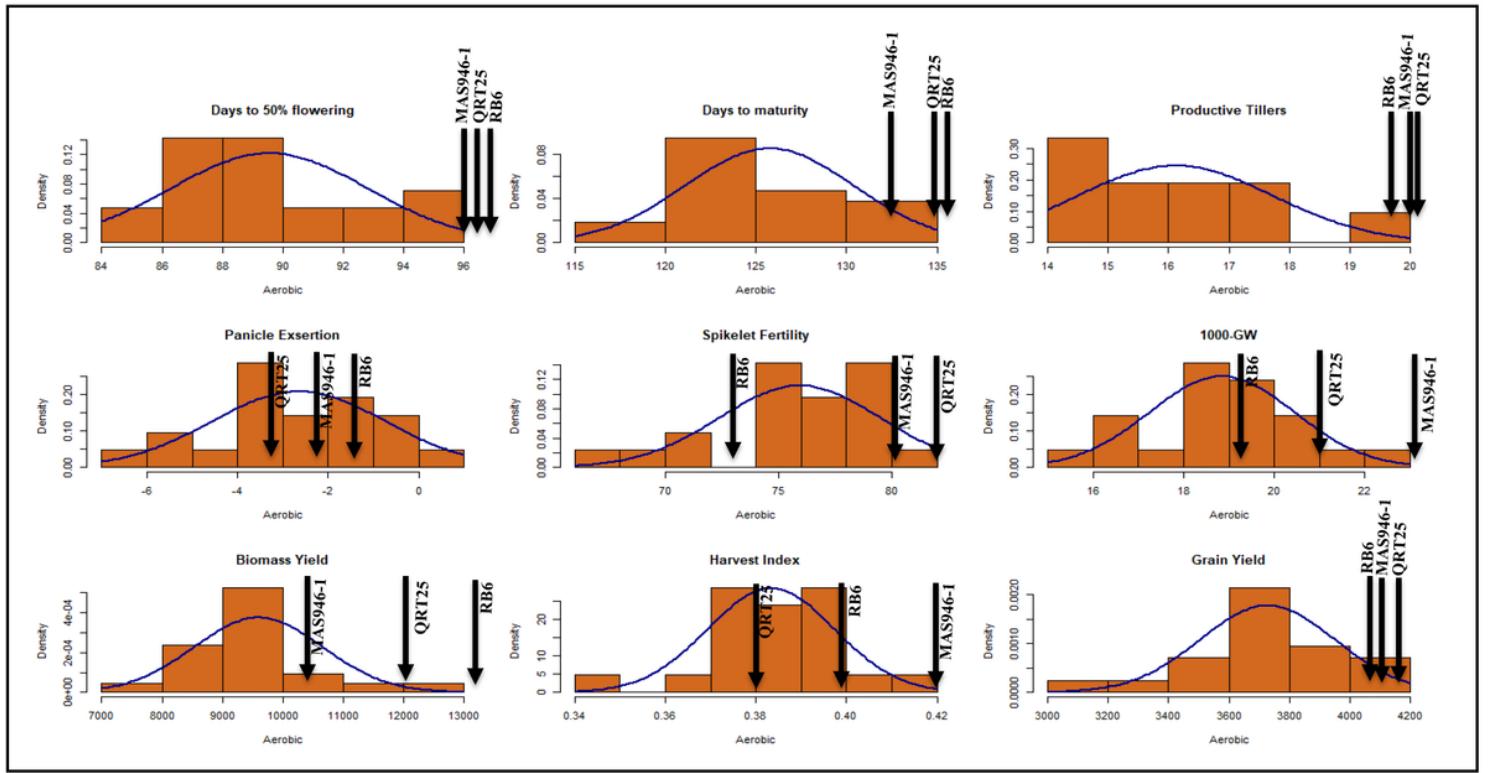


Figure 3

Frequency distribution plots of nine phenotypic variables within MAP genotypes under aerobic condition; Days to 50% flowering, Days to maturity, Productive tillers per plant, Panicle exertion (cm), Spikelet fertility (%), 1000-grain weight (g), Biomass yield (kg/ha), Harvest index and Grain yield(kg/ha). RB6 and QRT25 are the parents of the MAP genotypes and MAS946-1 is used as check.

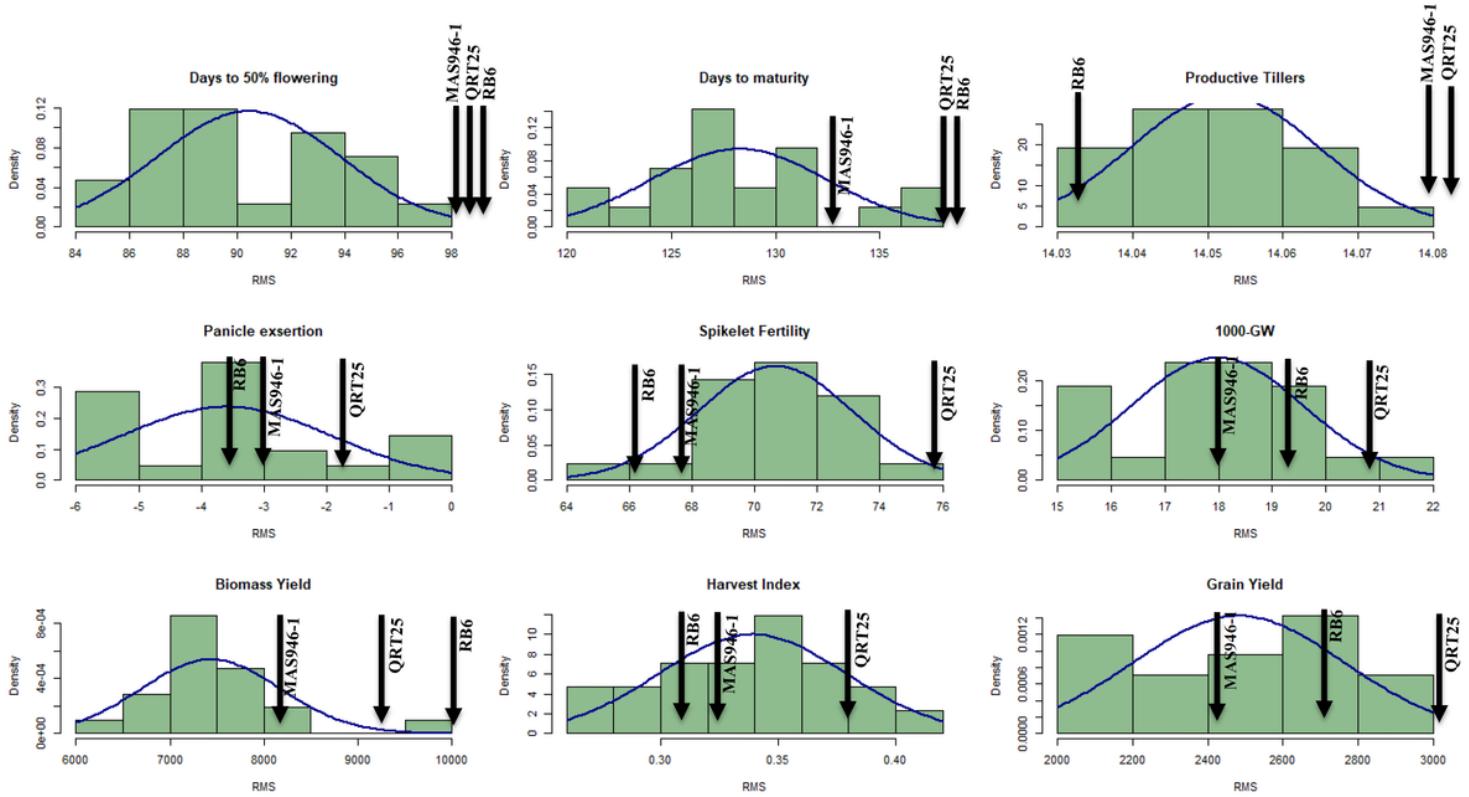


Figure 4

Frequency distribution plots of nine phenotypic variables within MAP genotypes under RMS condition; Days to 50% flowering, Days to maturity, Productive tillers per plant, Panicle exertion (cm), Spikelet fertility (%), 1000-grain weight (g), Biomass yield (kg/ha), Harvest index and Grain yield (kg/ha). RB6 and QRT25 are the parents of the MAP genotypes and MAS946-1 is used as check.

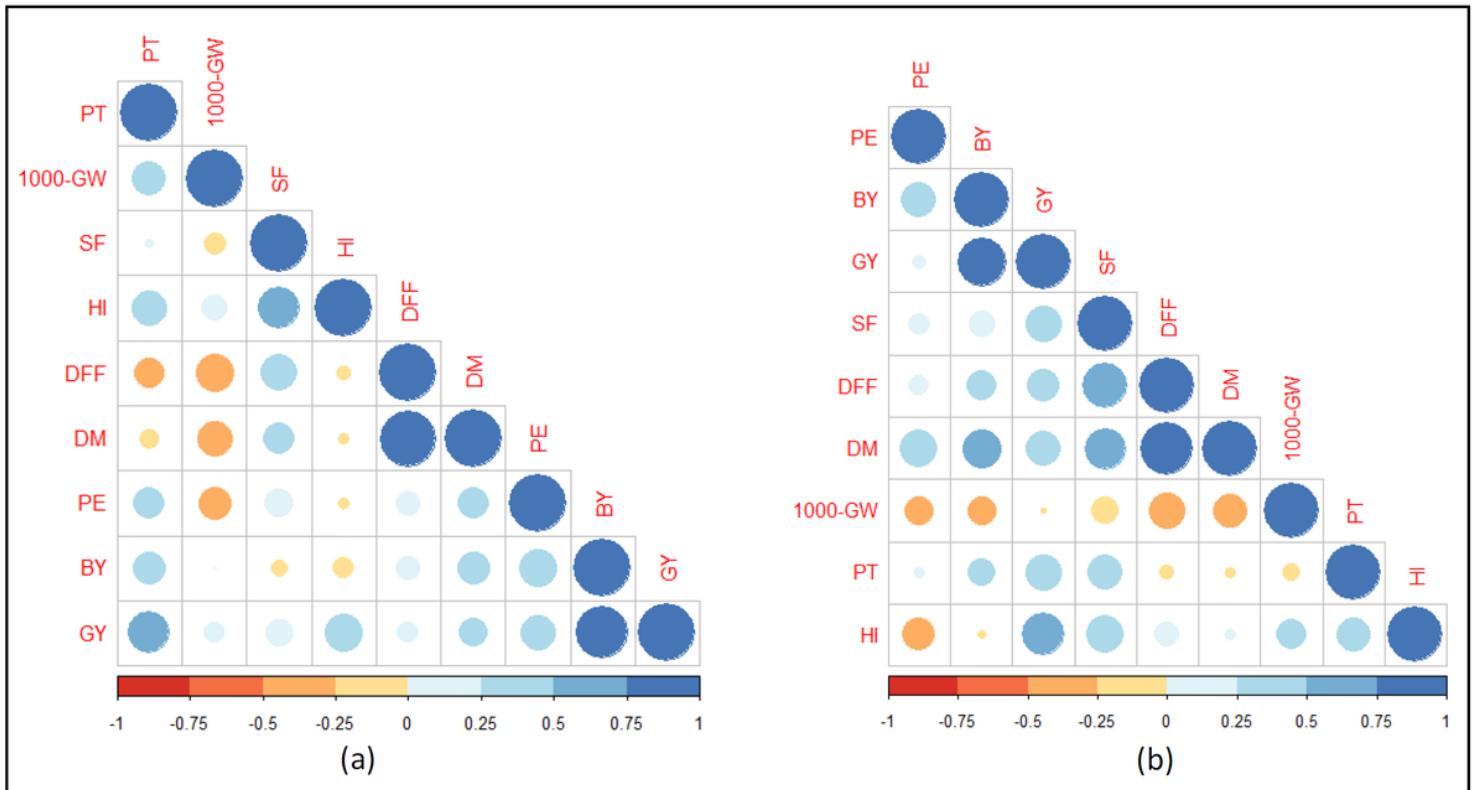


Figure 5

a Plot of correlation between each of the nine phenotypic traits under aerobic condition; b. Plot of correlation between each of the nine phenotypic traits under RMS condition; Days to 50% flowering (DFF), Days to maturity (DM), Productive tillers per plant (PT), Panicle exertion (cm) (PE), Spikelet fertility (%) (SF), 1000-grain weight (g) (1000-GW), Biomass yield (kg/ha) (BM), Harvest index (HI) and Grain yield (kg/ha) (GY). Blue color indicates positive correlation and red color indicates negative correlation among different traits, with color intensity variance depicting the strength of correlation.

Supplementary Files

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