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Evaluation of mutant lines of rice (*Oryza sativa* L.) for drought tolerance at seedling and reproductive stages

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Abstract

Rice productivity severely decreases due to the drought stress at seedling and reproductive stage. This study evaluated drought tolerance in 20 rice mutant lines at seedling and reproductive stages, comparing them to tolerant (Nagina-22) and susceptible (IR-64) varieties. Experiments were conducted in tunnels and fields using an Augmented Randomized Complete Block Design during the Kharif season 2023. Seedlings were subjected to 15 days of drought stress by withholding irrigation, and 14 traits were recorded. In the field, reproductive-stage drought stress was imposed for 30 days by ceasing irrigation. Analysis of variance revealed significant ($p<0.05$) and highly significant ($p<0.01$) differences among mutant lines for seedling traits under drought stress. Highly significant ($p<0.01$) differences were also observed for all traits during reproductive-stage drought stress. Seedling vigor is positively and strongly correlated with other traits. Seedling recovery was strongly and positively correlated with seedling height, leaf rolling, seedling dryness, and seedling vigor. Grain yield strongly and positively correlated with productive tillers per plant, panicle length, primary branches per panicle, number of spikelets per panicle, 1000 grain weight, harvesting index, filled spikelets per plant, chlorophyll content, photosynthesis rate and Stomata conductance rate. In quarter 1, quarter 2, quarter 3 and quarter 4 in the biplot, the traits which were closed to each other are correlated and same color of the mutant lines were similar on the bases of traits. Cluster Analysis on the basis of traits recorded after drought stress at seedling stage and reproductive stage divided the mutant lines into 3 clusters and 4 clusters, respectively. Results of the research indicated that mutant lines such as 54, 58-1, 129-1, 130-2, 582-1, 631, 931, 1227-1 and 1230-1 showed drought tolerance during drought stress at seedling and reproductive stage.

Keywords: Augmented, Correlation, Drought tolerance, Principle component analysis, Rice

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Introduction

Rice is the staple food of 17 countries in the Asian region, 9 countries in North and South America and 8 countries in the African region (FAO, 2022). It nourishes one third of the world's population (Ibrahim et al., 2016; Zhang et al., 2014; Madhu et al., 2023). According to the available energy in the world, rice is at the top with 20 percent, wheat at second with 19 percent and maize is third with 5 percent (FAO, 2022; Roheen et al., 2023). Rice is the second staple food among cereals after wheat in Pakistan (Ali et al., 2019; Mehmood et al., 2021; Qasim et al., 2022; Hassan et al., 2022). The geographic zone of Pakistan is 79.61 million hectares and cultivated area is 22.1 million hectares. The added value of rice is 1.9% in agriculture and 0.4% in Gross Domestic Product (GDP). The total area of rice decreased by nearly 15.87% between 2020-2021 and 2022-23, from 3537 thousand hectares to 2976 thousand hectares. Due to this reduction in area, the target of production of 9323 thousand tones was not achieved and remained short by 21.45%, standing at 7322 thousand tones. The climatic imbalance and water deficit have also reduced the production of rice (Pakistan Agriculture Survey, 2022). The rice crop needs 3000 liters of water for the production of 1 kg of rice in Pakistan. Pakistan is placed among the world's highest water-deficient countries due to low rainfall. According to the previous report of the commission of Pakistan planning, water availability was reduced from 5,650 m^3 to 1,000 m^3 from 1951 to 2010. It also observed that it will decrease by up to 800 m^3 in 2025 and the population will be 241 million (Farooq et al., 2011; Shaikh & Tunio, 2015).

Drought influences the different phenotypical or morphological traits and severely affects the Crop productivity (Abbas et al., 2013; Mehmood et al., 2020; Ahmad et al. 2022). In drought conditions, rice utilizes energy sources for survival and not for production (Casartelli et al., 2018; Zampieri et al., 2023; Hassan et al., 2023). Drought at the seedling stage adversely affects the growth and activates the enzyme activities to tolerate the drought stress (Sohag et al., 2020). Drought stress at the stages of panicle development, anthesis, and grain filling decreases yield by 30%, 60% and 40% (Nokkoul and Wichitparp, 2014; Shafqat et al., 2019). Drought stress at the vegetative, flowering, or reproductive stages reduces yield by 50% to 90% (Guan et al., 2010, Dixit et al., 2012, Shamsudin et al., 2016, Swamy et al., 2017). Physiological traits such as the total phenolic content, methylglyoxal and proline level are changed according to the environment, stress and duration. In drought, their level increases and allows the plant toward tolerance (Hossain et al., 2009; Bunnag and Pongthai, 2013; Sun et al. 2020; Priyanthi and Sivakanesan, 2021).

In rice, diversification plays an important role in the selection of plants according to the goal (Donde et al., 2019; Zaid et al., 2022). High yielding rice varieties can be developed by using conventional and nonconventional methods for biotic and abiotic stresses (oladosu et al., 2019; Hassan et al., 2023). Drought is one of the most significant abiotic stress factors affecting rice production globally. With the increasing unpredictability of weather patterns due to climate change, the need to develop drought-tolerant rice varieties has become paramount. Drought not only reduces crop yield but also threatens food security, particularly in regions heavily dependent on rice as a staple food. Therefore, it is essential to identify and develop drought-tolerant rice lines to mitigate the adverse effects of water scarcity on rice production. Statistical tool Such as ANOVA help the scientist to evaluate the variation in the germplasm and correlation analysis helps understand the relationships between different traits and their impact on drought tolerance. Principal Component Analysis (PCA) is a powerful statistical tool that reduces the dimensionality of data while retaining important information. PCA can identify which agronomic, physiological, or genetic traits are most strongly associated with drought tolerance. This helps breeders focus on the most relevant traits. PCA can be applied to genetic data to identify genetic markers associated with drought tolerance. This assists in marker-assisted breeding programs. PCA can group rice lines with similar trait profiles, aiding in the selection of diverse. The objective of our research is to evaluate the variation in the mutant lines and their response against drought at seedling and reproductive stage.

Materials and Methods

Experiments' location and plant materials

The experiments were carried out in tunnel and field conditions at the Nuclear Institute for Agriculture and Biology (NIAB) (31.3989° N, 73.0331° E), Faisalabad, Pakistan. Twenty mutant lines developed through mutation breeding from the parent IR-6 (Table 1) were used for the screening.

Table 1 Mutant lines evaluated for the drought tolerances under drought stress at seedling and reproductive stage

Symbols	Mutant lines	Symbols	Mutant lines	Symbols	Mutant lines	Symbols	Mutant lines
G-1	54	G-6	233-2	G-11	911	G-16	631
G-2	58-1	G-7	311	G-12	928	G-17	688-1
G-3	122-2	G-8	324-1	G-13	931	G-18	815
G-4	129-1	G-9	507-4	G-14	1227-1	G-19	864-1
G-5	130-2	G-10	582-1	G-15	1230-1	G-20	871-1

In the first experiment, Seeds of twenty genotypes were sown in the tunnel in an Augmented Randomized Completed Block Design. Tolerant (Nagina-22) and susceptible (IR-64) varieties with three replications were used. It was sown on the bed with one square foot for one genotype having 25 seedlings. The distance between the nursery was maintained to reduce the chance of mixing. After sowing, seeds were covered with farmyard manure and wheat straws to reduce the evaporation, heat and bird attack. Tap water at 2 inches per square meter was applied for seed germination. Before stress, all water was removed. Drought stress of 15 days was applied to 30-day old seedlings and covered with polythene sheet to avoid rainfall during the period of stress. Seedling traits such as Seedling height (cm), leaf rolling, seedling vigor (counting green seedling after stress per mutant line) and dry seedling were recorded after stress according to Table 2 (IRRI, 1976). But total phenolic content (mg/gFw) (Priyanthi et al., 2021), methylglyoxal (mg/gFw) (Hossain et al., 2009) and proline content (umole of proline /g of FW) (Bunnag and pongthi, 2013) were recorded before stress, during stress and after stress. Seedlings were irrigated and seedling recovery data was recorded after 15 days of irrigation according to Table 2 (IRRI, 1976).

Table 2 Scale used for measuring leaf rolling, seedling leaf dry and seedling recovery

Scale No.	Leaf Rolling	Seedling dryness	Recovery percentage of plants
0	Leaves are healthy	Complete leaf dry	
1	Leaves start to be folded	More than half leaf dry	0%–19% of plants recovered
3	Leaves are folded (Deep-V-shaped)	Half leaf dry	20%–39% of plants recovered
5	Leaves are fully cupped (U-shaped)	One third leaf dry	40%–69% of plants recovered
7	Leaves margins touching (O-shaped)	Only tip dry	70%–89% of plants recovered
9	Leaves are tightly rolled	Leaves are healthy	90%–100% of plants recovered

Soil and water analysis

The fertility of soil and fitness of water for irrigation were analyzed by the Soil and Water Testing Laboratory in Hafiz Abad, Punjab, Pakistan. Soil was taken from three different places and two different depths 0-15 and 15-30 cm. Make a separate mixture of depth 0-15 cm and 15-30 cm. Both mixtures were used for the analysis shown in Table 3a and Table 3b. The canal and tube well water was mixed and used for analysis. According to the report water and soil are fit for the experiment.

Table 3a Soil analysis of field

Soil depth (cm)	pH	Electric conductivity (dSm^{-1})	Organic matter (%)	Available phosphorous (P) (ppm)	Available potash (K) (ppm)	Saturation (%)	Texture
0-15	8.18	0.61	0.59	8	140	34	Loam
15-30	8.40	0.66	0.32	5	140	36	Loam

Table 3b Water analysis for field irrigation

Electric conductivity (dSm^{-1})	Ca+Mg ($meqL^{-1}$)	Na ($meqL^{-1}$)	Co_3 ($meqL^{-1}$)	HCO_3 ($meqL^{-1}$)	Cl ($meqL^{-1}$)	Sodium Adsorption Ratio	Residual sodium carbonate (meL^{-1})
950	5.0	4.5	0	7.2	1.2	2.85	2.2

Growth conditions of nursery for field experiment

In the second experiment, seeds of the same twenty mutant lines were used and the same condition was provided as discussed above. Tube well water was used for irrigation. Standing water was applied for nursery and field transplantation.

Transplanting

Thirty-five-day old seedlings were transplanted into the Augmented Randomized Complete Block Design (RCBD). The soil was well puddled with a cultivator and levelled with plank. Plant to plant and row to row distances (9 inches) were maintained and one plant per hole was transplanted. In this experiment, the recommended fertilizer application consists of 1.75 bags of DAP for phosphorous, 2.25 bags of Urea for Nitrogen, and 1 bag of Potash for acre. The application should be divided, with all DAP and Potash applied as a basal dose, along with half of the Urea. The remaining half of the Urea should be applied 30 days after transplanting. Tolerant (Nagina-22) and susceptible (IR-64) varieties with three replications were transplanted as standard. Average temperature and humidity (%) are shown in Fig. 1 and 2.

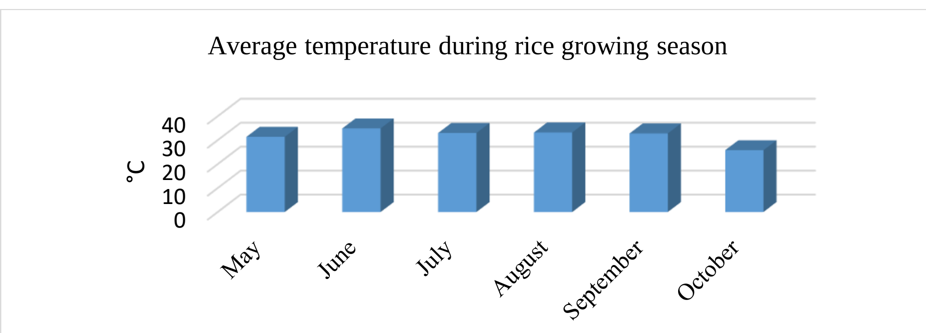

Fig. 1 Average temperature during rice growing season

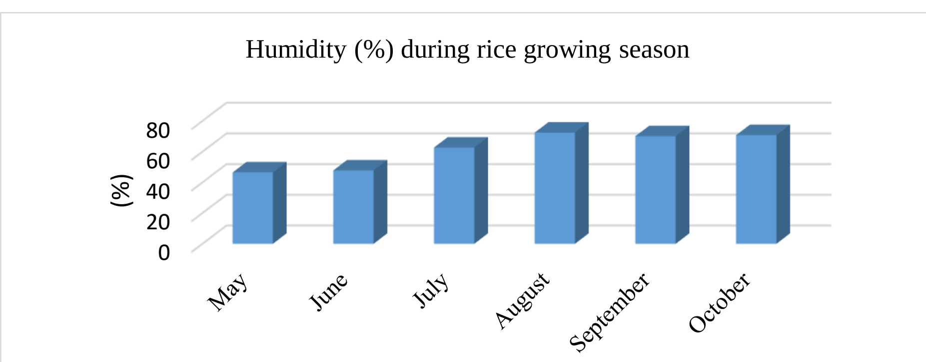

Fig. 2 Humidity (%) during rice growing season

Stress conditions and traits recorded at maturity

Before the stress, each genotype was isolated from the other with the help of bunds because of the difference in days to flowering stage. Standing water conditions were applied before the reproductive stage. Drought stress was applied at the reproductive stage of rice for 30 days. After 30 days of drought stress, the field was irrigated. Data of traits like chlorophyll content recorded with SPAD-502plus, stomata conductance rate (C) (mmol/m^2/s), photosynthesis rate (mmol/m^2/s) and transpiration rate (E) (mmol/m^2/s) were recorded with Infra-Red Gas Analyzer (IRGA CI-340 Handheld) and other traits such as days to flowering, length of flag leaf (cm), plant height (cm), productive tillers per plant, biological yield (g), main panicle length (cm), number of primary branches per plant, number of spikelets per panicle, unfilled spikelets per panicle, filled spikelets per panicle, weight of biomass (g), grain yield per plant (g), 1000 grain weight (g), and harvesting index (%) were recorded at maturity stage.

Statistical analysis

The different responses against drought was analyzed with R-software for the analysis of variance (ANOVA). ANOVA is a statistical method used to compare means among groups and determine whether there are statistically significant differences between them (Federer and Raghavarao, 1975). Pearson correlation is a statistical measure used to assess the linear relationship between two continuous variables by R-software. Principle Component analysis (PCA) is a multivariate statistical technique used for dimensionality reduction and to explore patterns or relationships in complex datasets by Mini tab software. PCA was used to analyze the similarity and dissimilarity among the mutant lines.

Results

Analysis of variances of traits recorded during and after drought stress at seedling stage

The results of our study involved the evaluation of twenty mutant rice lines, including both the drought-tolerant variety Nagina-22 and the susceptible variety IR-64, under drought stress during the seedling stage, provided valuable insights into the potential for developing drought-tolerant mutant lines. The analysis of variance showed significant differences ($p<0.05$) among the mutant lines for several key traits, including seedling height, leaf rolling, seedling dryness, seedling vigor, seedling recovery and proline levels after stress as shown in Table 4a and 4b. But there were more significant ($p<0.01$) differences among mutant lines on the bases of total phenolic contents before stress, total phenolic contents in stress, total phenolic contents after stress, methylglyoxal before stress, methylglyoxal in stress, methylglyoxal after stress, proline before stress and proline in stress. These significant differences highlighted the genetic diversity among the mutant lines in their responses to drought stress during the seedling stage. The study confirmed highly significant differences ($p<0.01$) between the tolerant check variety Nagina-22 and the susceptible check variety IR-64 across all the studied traits. This validated the choice of these two varieties as references for evaluating the mutant lines and demonstrates their distinct responses to drought stress. Check varieties vs mutant lines shown highly significant ($p<0.01$) differences on the bases of traits as shown in Table 4a and 4b. Mutant line 54 stands out as significant and exhibits high mean values for drought-tolerant traits under drought stress at the seedling stage. This suggested that mutant line 54 possessed favorable characteristics for drought tolerance and may be a promising candidate for further breeding program.

Analysis of variances of traits recorded after drought stress at reproductive stage

The results of our experiment evaluated mutant rice lines for drought tolerance under drought stress during the reproductive stage provided valuable insights into the potential for breeding drought-tolerant rice varieties. The analysis revealed that there were significant ($p<0.01$) differences among the mutant lines for all studied traits during the reproductive stage under drought stress. This indicated a high degree of genetic diversity among the mutant lines in their responses to drought stress, offering opportunities for selecting lines with improved drought tolerance. IR-64 and Nagina-22, which served as reference varieties, had exhibited highly significant differences ($p<0.01$) in several key traits under drought stress conditions as shown in Table 4a and 4b. The comparison between the check varieties (IR-64 and Nagina-22) and the mutant lines highlighted significant differences in multiple critical traits shown in Table 5a and 5b. Several mutant lines, including 54, 58-1, 129-1, 130-2, 582-1, 631, 931, 1227-1, and 1230-1, exhibited higher mean values compared to other tolerant standards. This suggested that these specific mutant lines possess favorable traits related to drought tolerance, making them potential candidates for further breeding and selection.

Correlation among the traits recorded after drought stress at seedling stage

Seedling vigor and seedling height have shown a strong positive correlation (0.59). This indicated that seedlings with greater vigor tended to exhibit taller heights during the seedling stage. Leaf rolling and seedling vigor have shown a very strong positive correlation (0.85). This suggested that seedlings with vigorous growth were less likely to exhibit leaf rolling, a common stress response. Seedling vigor positively correlated with proline levels before stress (0.57) and proline levels during stress (0.53). Seedling vigor was associated with higher proline levels, indicating a potential role of proline in enhancing seedling vigor during stress conditions. Seedling height strongly and positively correlated with leaf rolling (0.64), seedling dryness (0.75), and seedling vigor (0.68). Leaf rolling and seedling dryness positively and strongly correlated with seedling vigor (0.61). The seedling recovery was strongly and positively correlated with seedling height (0.53), leaf rolling

(0.66), seedling dryness (0.61), and seedling vigor (0.64). These strong positive correlations among seedling traits suggested that they were interrelated and tended to co-occur. For example, vigorous seedlings are less likely to exhibit leaf rolling or dryness, and they recover well from stress as shown in Fig. 3.

A genotype by trait biplot helped to understand the relationships among traits (according to breeding objectives) and helped to identify traits that were positively or negatively associated, traits that are redundantly measured, and traits that can be used in indirect selection for another trait. In quarter 1, quarter 2, quarter 3 and quarter 4, the traits which were closed to each other are correlated and same color of the mutant lines were similar on the bases of traits. Traits recorded after drought stress at seedling stage were observed in quarter 1 and 4. Same direction of the traits indicated that had correlation among each other as shown in Fig. 4. All genotypes were scattered in all quarters but genotypes in quarter 4 indicated good response for drought tolerant traits.

Correlation among the traits recorded during and after drought stress at reproductive stage

In our research, there were two main characteristics such as grain yield and 1000 grain weight which play a vital role in the yield and grain quality. Grain yield strongly and positively correlated with productive tillers per plant (0.70), panicle length (0.88), primary branches per panicle (0.91), number of spikelets per panicle (0.77) that suggested that longer panicles with more primary branches and spikelets lead to higher grain yield. It also positively correlated with 1000 grain weight (0.51), harvesting index (0.97), filled spikelets per plant (0.82), Chlorophyll content (0.69), photosynthesis rate (0.66) and Stomata conductance rate (0.88). It indicated that larger grains were associated with higher grain yield. Grain yield negatively correlated with the day to flowering (-0.30), suggesting that delayed flowering might lead to the lower grain yield. Weak negative correlations with plant height (-0.03) and biological yield (-0.12) indicated that taller plants with higher overall biomass may not necessarily result in higher grain yield. Unfilled spikelets per panicle (-0.79) negatively affected grain yield, emphasizing the importance of minimizing unfilled spikelets. Negative correlation with biomass yield (0.26) suggested that excessive biomass may divert resources away from grain production. Negative correlation with transpiration rate (-0.87) indicated that excessive water loss through transpiration might reduce grain yield. 1000 grain weight strongly and positively correlated with panicle length (0.43), primary branches per panicle (0.42), and number of spikelets per panicle (0.30), suggested that these traits contributed to larger grain size. Positive correlation with harvesting index (0.49) indicated that efficient resource allocated towards grain production also leads to heavier grains. Positive correlations with filled spikelets per panicle (0.35), chlorophyll content (0.52), photosynthesis rate (0.34), and stomata conductance (0.54) as shown in Fig. 5 suggested that optimal physiological processes enhance grain weight. The fact that traits in the same quarter (quarter 1, quarter 2, quarter 3, and quarter 4) were close to each other on the biplot suggests that these traits were correlated or similar patterns of variation across the genotypes as shown in Fig. 6. Traits that were close to each other on the biplot are likely positively associated, meaning that when one trait increases, the other tends to increase as well. Conversely, when one trait decreases, the other tends to decrease. The biplot can help identify which traits were positively or negatively associated with each other. Traits that are far apart on the biplot are likely negatively correlated, meaning that when one trait increases, the other tends to decrease. Similar colors for mutant lines indicated that they shared common trait patterns or responses across the quarters, which could be indicative of specific genetic characteristics or breeding outcomes.

Cluster analysis on the traits recorded after drought stress at seedling stage

Similarity in mutant lines on the bases of traits recorded under drought stress at seedling stage shown in dendogram. The clusters were divided into two groups: Group A and Group B. The presence of a single cluster (Cluster 1) in Group A suggested that the mutant line IR-64 had distinct traits under drought stress compared to the other mutant lines analyzed. IR-64 might possess unique genetic characteristics or responses to drought stress that differentiate it from the rest of the mutants in this study. Group B included cluster 2 and cluster 3. Cluster 2 included mutant lines such as 233-2, 311, 122-2, 688-1, 507-4, 1227-1, 1230-1, 324-1 and 631. There might be shared genetic factors or mechanisms that contributed to their similar performance under drought conditions. Cluster 3 included on mutant lines named 58-1, 129-1, 815, 582-1, 871-1, Nagina-22, 130-2, 864-1, 54, 911, 928 and 931 as shown in Fig. 7. The formation of Cluster 3 indicated that these mutant lines also displayed common traits in response to drought stress.

Table 4a F-value of traits recorded during and after drought stress at seedling stage

Sources of variance	Degree of freedom	Seedling height (cm)	Leaf rolling	Seedling dryness	Seedling vigor	Seedling recovery	Total phenolic content before stress (mg/g Fw)	Total phenolic content in stress (mg/g Fw)	Total phenolic content after stress (mg/g Fw)
Mutant lines adjusted	21	792.63*	25.234*	28.206*	31.14*	30.86*	204.76**	502.7**	204.76**
Check varieties (Nagina-22 vs IR-64)	1	703.53**	49**	196**	327.97**	108**	181.93**	1559.1**	181.93**
Check varieties VS Mutant lines	20	797.09**	24.046**	19.817**	16.29**	27.00**	205.90**	449.9**	205.90**
Residuals	2								

** p < 0.01, * p < 0.05 and p> 0.05 Non-significant = n.s

Table 4b F-value of traits recorded during and after drought stress at seedling stage

Sources of variance	Degree of freedom	Methylglyoxal before stress (µmol/g FW)	Methylglyoxal in stress (µmol/g FW)	Methylglyoxal after stress (µmol/g FW)	Proline before stress (µmole of proline /g of FW)	Proline in stress (µmole of proline /g of FW)	Proline after stress (µmole of proline /g of FW)
Mutant lines adjusted	21	3044**	344.10 *	3044**	40.61*	125.54**	40.61*
Check varieties (Nagina-22 vs IR-64)	1	2721**	31.97**	2721**	126.41**	814.58**	126.41**
Check varieties vs Mutant lines	20	3060**	359.70**	3060**	36.32**	91.09**	36.32**
Residuals	2						

** p < 0.01, * p < 0.05 and p> 0.05 Non-significant = n.s

Table 5a F-Value of traits recorded after drought stress at reproductive stage

Sources of variance	Degree of freedom	Days to flowering	Flag leaf length (cm)	Chlorophyll (nmol/cm)	Photosynthesis rate (mmol/m^2/s)	Stomata conductance rate (mmol/m^2/s)	Transpiration rate (mmol/m^2/s)	Plant height (cm)	Productive tiller per plant	Biological yield (g)
Mutant lines adjusted	21	25.198**	584.36**	150.76**	204.96**	111.81**	60.026**	309.78**	25.198 **	1603.0 **
Check varieties (Nagina-22 vs IR-64)	1	226.138**	22.292*	326**	1728**	147.84**	216.75**	672.01**	226.1378 **	22.292 **
Check varieties vs Mutant lines	20	15.152*	612.463**	142**	128.81**	110.01**	52.19*	291.67**	15.152 *	1682.1**
Residuals	2									

** p < 0.01, * p < 0.05 and p> 0.05 Non-significant = n.s

Table 5b F-Value of traits recorded after drought stress at reproductive stage

Sources of variance	Degree of freedom	Main Panicle length (cm)	Primary branches per plant	Number of Spikelets per panicle	Unfilled spikelets per panicle	Filled spikelets per panicle	Biomass yield (g)	Grain yield (g)	1000 Grain weight (g)	Harvesting index (%)
Mutant lines Adjusted	21	31.27**	802.91**	337.5**	309.78**	337.5**	1866.5 **	197.31 **	107.937**	802.91**
Check varieties (Nagina-22 vs IR-64)	1	24.143*	127.16**	252.68**	672.01 **	252.6**	1243.0 **	213.00 **	484**	127.16**
Check varieties vs Mutant lines	20	31.626*	836.7**	341.75**	291.67 **	341.7**	1897.6 **	196.52 *	89.134*	836.7**
Residuals	2									

** p < 0.01, * p < 0.05 and p> 0.05 Non-significant = n.s

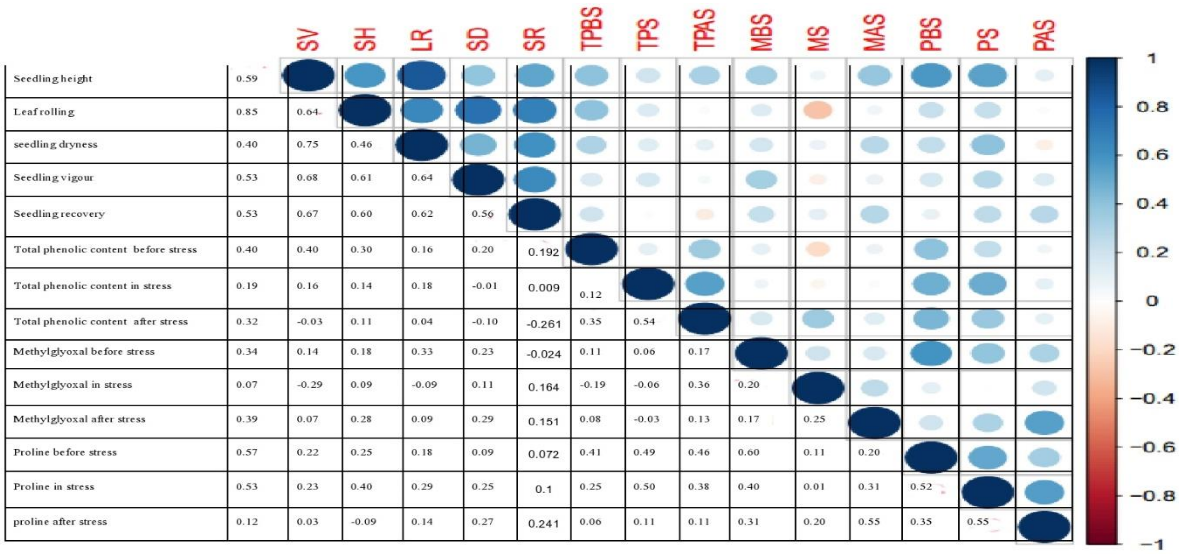

Fig. 3 Correlation among the traits recorded after drought stress at seedling stage

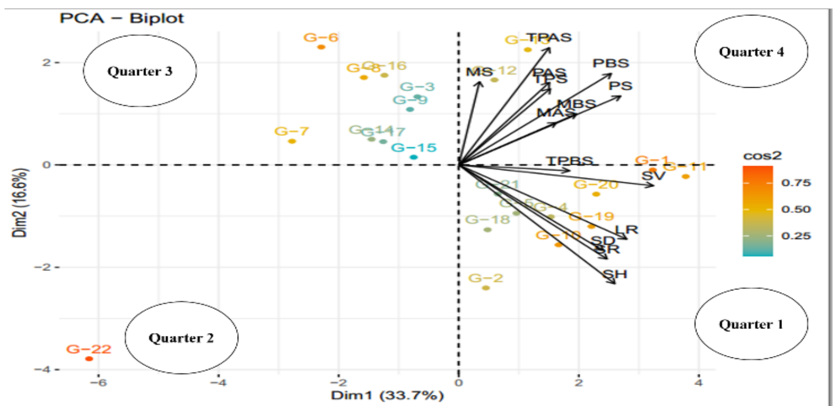

Fig. 4 Biplot of mutant lines on the bases of traits recorded after drought stress at seedling stage

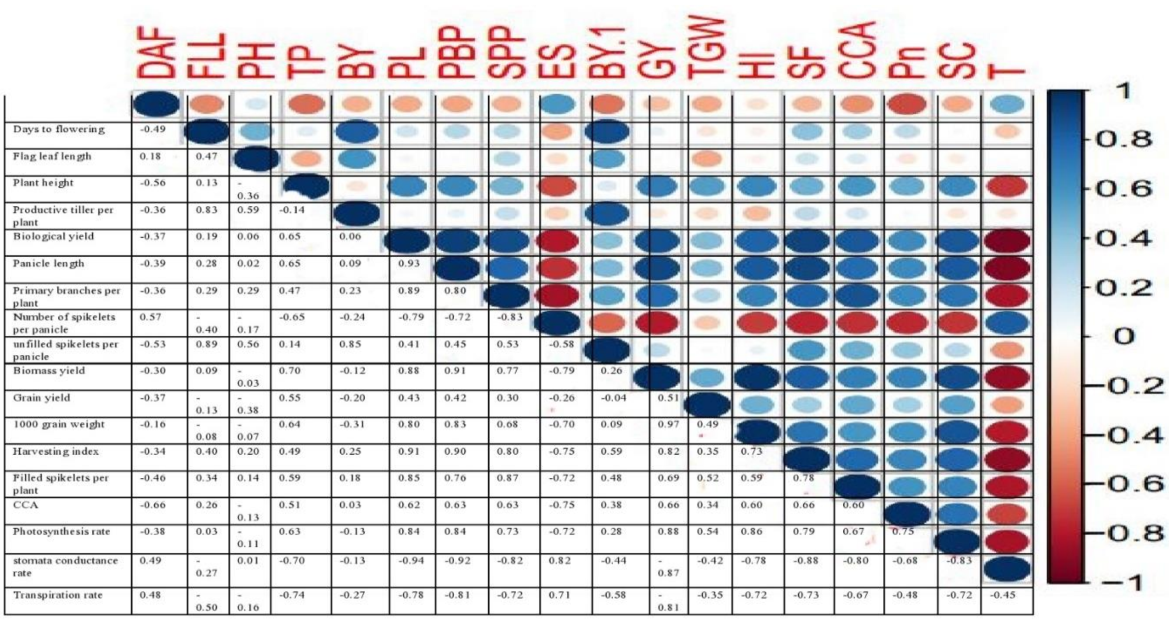

Fig. 5 Correlation among the traits recorded under drought stress at reproductive stage

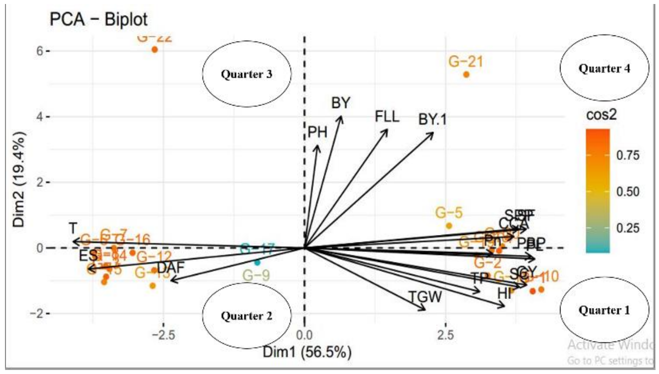

Fig. 6 Biplot among the traits recorded under drought stress at reproductive stage

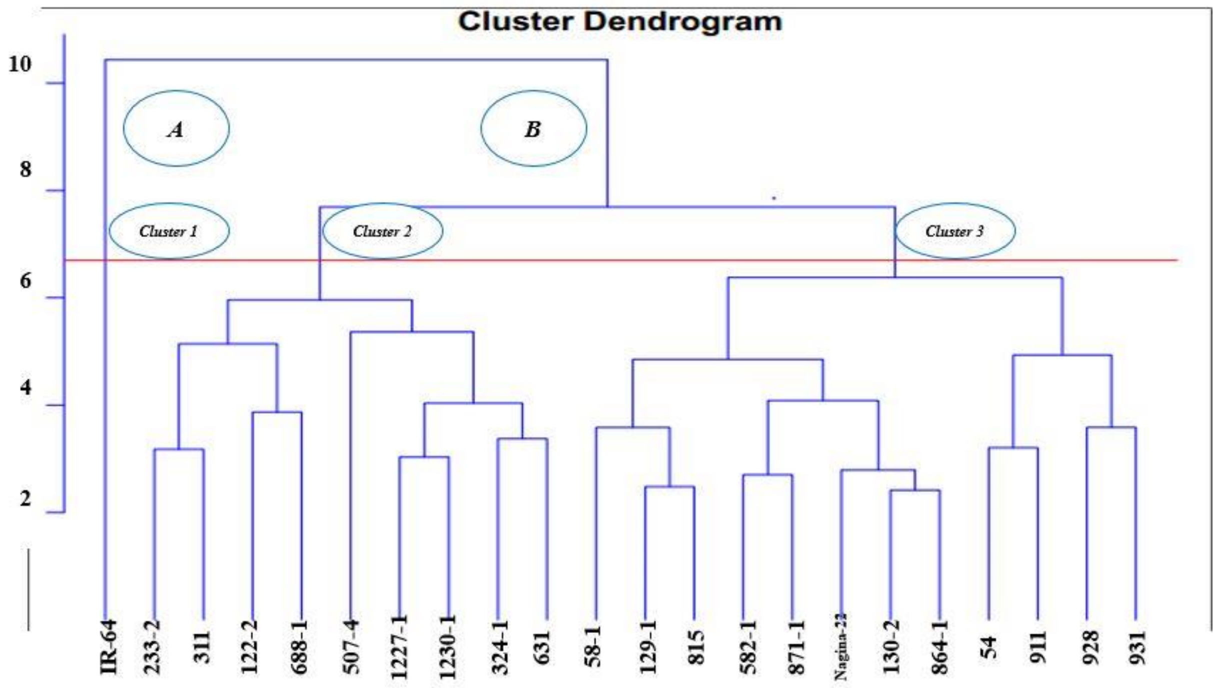

Fig. 7 Cluster analysis of twenty mutant lines with standards on the bases of traits recorded after drought stress at seedling stage

Cluster Analysis on the traits recorded during and after drought stress at reproductive stage

The dendrogram analysis had grouped the mutant lines into clusters based on their similarities in traits recorded during and after drought stress at the reproductive stage. While IR-64 and Nagina-22 stand out as distinct in Group A, the mutant lines within Clusters 3 with mutant lines130-2, 582-1,54,58-1,911, 129-1, 864-1,815,871-1 and cluster 4 with mutant lines 507-4, 688-1,233-2,324-1,122-2,1227-1, 931,1230-1928, 311,631 in Group B display shared traits and responses to drought stress as shown in Fig. 8. These findings provide valuable information for understanding the genetic and physiological factors influencing drought tolerance in these mutant lines and can guide further research, create more variation by crosses between two different cluster lines and breeding efforts for crop improvement.

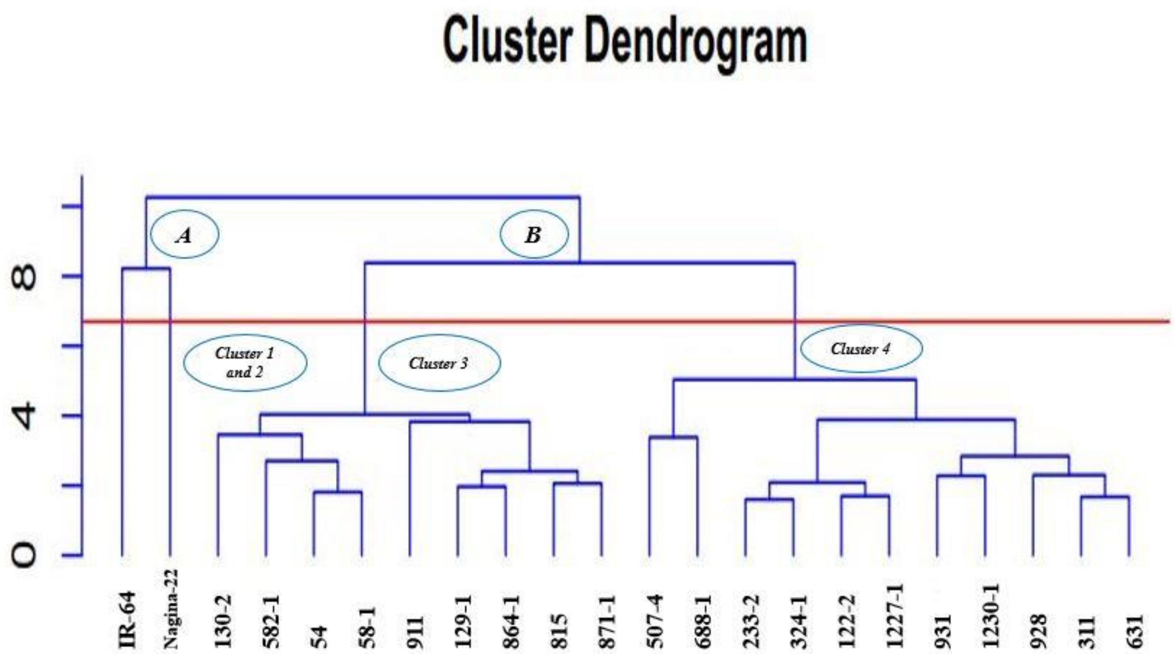

Fig. 8 Cluster analysis of twenty mutant lines with standards on the bases of traits recorded after drought stress at reproductive stage

Discussion

The present research was divided into two distinct segments. Seeds of twenty different mutant lines were sown in an augmented RBCD design. In the first segment, drought stress was applied during the seedling stage within controlled tunnel conditions. Subsequently, in the second phase, drought stress was induced during the reproductive stage of rice in open-field conditions. Various parameters were meticulously recorded throughout the duration of both the experiments. Mutation breeding plays a vital role in enhancing genetic variation for breeding programs (Zafar et al. 2020). Mutant lines shown significant different on the bases of traits under the drought stress at seedling and reproductive stage as shown in Table 4a, 4b, 5a and 5b. Drought stress significantly affects the plant traits because water reduction also reduces the enzymatic reaction activity and growth (Ashfaq et al., 2012; Swain et al., 2014; Islam et al., 2018). Highlight the distinct characteristics of IR-64 and Nagina-22, as reported (Akram et al., 2013; Donde et al., 2019). The importance of using these two varieties as variety checks were used in various rice studies as reported (Zafar et al., 2020; Ahmad et al., 2022. Seedling vigor shows strong positive correlation with seedling height, leaf rolling, proline level before stress, proline level during stress as shown in Fig. 3. This indicated that seedlings with greater vigor tended to exhibit taller heights, less likely to exhibit leaf rolling, potential role of proline in enhancing seedling vigor during the seedling stage. Seedling recovery was strongly and positively correlated with seedling height, leaf rolling, seedling dryness, and seedling vigor. These strong positive correlations among seedling traits suggested that they were interrelated and tended to co-occur. For example, vigorous seedlings are less likely to exhibit leaf rolling or dryness, and they recover well from stress. Grain yield strongly and positively correlated with productive tillers per plant, panicle length, primary branches per panicle, number of spikelets per panicle that suggested that longer panicles with more primary branches and spikelets lead to higher grain yield as shown in Fig. 5. It also positively correlated with 1000 grain weight, harvesting index, filled spikelets per plant, Chlorophyll content, photosynthesis rate and Stomata conductance rate and results similar with (Gaballah et al., 2022; Ata-ul-karim et al., 2022).

Cluster Analysis on the bases of traits recorded after drought stress at seedling stage and reproductive stage divided the mutant lines into 3 cluster. The presence of a single cluster (Cluster 1) in Group A suggested that the mutant line IR-64 had distinct traits under drought stress compared to the other mutant lines analyzed. IR-64 might possess unique genetic characteristics or responses to drought stress that differentiate it from the rest of the mutants in this study. Cluster 2 included mutant lines such as 233-2, 311, 122-2, 688-1, 507-4, 1227-1, 1230-1, 324-1 and 631. Cluster 3 included on mutant lines named 58-1, 129-1, 815, 582-1, 871-1, Nagina-22, 130-2, 864-1, 54, 911, 928 and 931 as shown in Fig. 7. Mutant lines within cluster shown similar behavior against drought stress (Rajiv et al., 2010). Previous research reported from (Verma et al., 2017; Widyawan et al., 2020) dendrogram on the bases of molecular data which is totally different from our experiment. The dendrogram analysis had grouped the mutant lines into clusters based on their similarities in traits recorded during and after drought stress at the reproductive stage. While IR-64 and Nagina-22 stand out as distinct in Group A, the mutant lines within Clusters 3 and 4 in Group B display shared traits and responses to drought stress as shown in Fig. 8. Emphasize the potential of mutant line 54 as a promising candidate for further breeding and selection due to its favorable drought tolerance characteristics. Highlight other mutant lines (e.g., 58-1, 129-1, 130-2, 582-1, 631, 931, 1227-1, 1230-1) that exhibited high recovery percentage and yield under drought stress at seedling and reproductive stage.

Conclusion

Results of the research indicated that mutant lines such as 54, 58-1, 129-1, 130-2, 582-1, 631, 931, 1227-1 and 1230-1 showed drought tolerance in seedling and reproductive stage stress experiment. Emphasize the potential of mutant line 54 as a promising candidate for further breeding and selection due to its favorable drought tolerance characteristics. Highlight other mutant lines (e.g., 58-1, 129-1, 130-2, 582-1, 631, 931, 1227-1, 1230-1) that exhibited high recovery percentage and yield under drought stress at seedling and reproductive stage. A higher recovery percentage under drought stress on seedling stage in mutant lines also resulted in a higher yield under drought stress at reproductive stage stress.

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