EXPLORING THE DROUGHT TOLERANCE IN RICE (*ORYZA SATIVA* L.) GERMPLASM: INSIGHTS FROM MORPHOLOGICAL AND MOLECULAR SCREENING

Ali Abbas[1], Attique Ur Rehman[1], Muhammad Arshad Javed[1], Qurban Ali[1], Shahbaz Ahmad[2], Shabnum Shaheen[4], Muhammad Ali*[2], Mukhdoom Shahjahan Bukhari[3], Urooj Mubashar[5] and Muhammad Ashfaq*[1]

[1]Department of Plant Breeding and Genetics, University of the Punjab Lahore, Pakistan; [2]Department of Entomology, University of the Punjab, Lahore, Pakistan; [3]Agricultural Research Station, Bahawalpur, Pakistan; [4]Department of Plant Sciences, Lahore College for Women University (LCWU) Lahore, Pakistan;
[5]Government Girls High School No2 Ghakkhar, Gujranwala, Pakistan
Corresponding authors email: ashfaq.iags@pu.edu.pk; ali.iags@pu.edu.pk

ABSTRACT

Rice (*Oryza Sativa* L.) is the most consumed staple diet for billions of the people in the world. Water stress is one of the primary issues for sustainable rice productivity in a climate change scenario. Drought causes severe damages to both vegetative and reproductive stage which subsequently effects a yield ultimately reduces grain weight. A total of 35 rice genotypes sown under drought stress with checked variety. RCBD design, two-way ANOVA factorial analysis, Tukey test, Genetic advance and Heritability were used for experiment. Screened the drought tolerance lines according to the standard Index of IRRI lines identify morphological/molecular processing in Rice Research Institute Kala shah Kaku, Pakistan. The objectives of the study were to screen the rice germplasm against drought stress on morphological and molecular basis. Fifteen rice genotypes performed better in stress condition. Five out of sixteen polymorphic markers namely RM-109, RM-22, RM-135, RM-232, and RM-261 were identified as potential markers linked to drought stress. These linked markers can be further used as a tool for screening of rice genotypes against drought stress. These genotypes such as Nonabokra, Vehari, Pokkoli, Hossooli, Kalomonk, CSR13, PK8892Tol-19, Chenab Tol-19, Basmati 515 Tol-19, Shaheen Tol-19, Basmati 370, Basmati 385, Super Basmati, Basmati 515, PK1121 aromatic show good performance under drought condition and these genotypes can be grown in drought stress areas of Pakistan. Overall, this study provides important information about the genetic basis for drought tolerance in rice and the possible approaches to improving it.

Keywords: drought stress, paddy length, paddy weight, SSR markers

INTRODUCTION

Rice (*Oryza Sativa* L.) is the most consumed staple diet for billions of the people in the world. About 90% world's rice is grown in Asia. About 50 to 80% people are having their everyday calorie intake by rice (Mohidem *et al*., 2022). In 2019, Pakistan has the 10th largest rice producer in all over the world and importer. Rice contributed 3.2% value addition in agriculture Pakistan and 0.7% GDP of Pakistan. Total yield of rice was 7.5 million tones/hac according to Pakistan Economic Survey (2022-23). During 2019 total rice production in Punjab was reportedly 39.8 million tones/hac. In Pakistan, the total cultivated area of rice is 57%. Rice has diploid with 2n=2x 24 chromosomes and size of genome of ssp. Indica is about 466Mb (mega base pairs) (Serba *et al*., 2020).

Human population is increasing very rapidly worldwide and it is need of the hour to produce 87% more food as compared to today especially food crop like wheat, rice, soy and rice by 2050 (Hemathilake & Gunathilake, 2022). Among different stresses, drought is causing serious hindrance to normal rice production and it affects almost 23 million hectares in Asia (Fahad *et al*., 2019). The present and expected global food demand needs a significant increase in crop production output. With declining agricultural water supplies, drought adaptation of rice is becoming crucial (Hussain *et al*., 2020). In Pakistan, reasons behind this water shortage are global climate change, wastage of agricultural water due to poor management and political issues regarding Indus-basin treaty. Pakistan ranks third among the countries facing water shortages, according to an International Monetary Fund (IMF) report (Mahfooz, 2024).

Screening of resistant rice genotypes is becoming critically important due to depleting agricultural water resources. Irregularity of climate patterns and complexity of response mechanism by rice plant have made it problematic to depict component traits needed for batter performance, thus reducing crop improvement to increase drought tolerance. Complexity of drought stress tolerance occurs to various factors like biochemical, morphological, physiological and molecular characters. So in order to attain complete understanding of mechanism of drought stress and to produce drought tolerant rice genotypes, there is need to produce data under these stresses (Panda *et al*., 2021). Data taken from different studies will help to construct a figure that how different yield constitutive traits respond to drought stress (Osmolovskaya *et al*., 2018). Growth and development of rice is very susceptible to drought due to less uptake of water by roots (Kim *et al*., 2020). Upland rice has adapted in drought stress due to deep and thick root system to expand its root hydraulic properties and get water from deeper soil profiles. There is great difference found in yield of upland rice genotypes as compared to low land rice under drought stress condition (Vijayaraghavareddy *et al*., 2020). Water stress given at vegetative phase especially at time of booting phase, flowering time and on later stage can inhibit the floret initiation which causing sterile spikelet and during grain filling causing lower grain weight, ultimately reduced paddy yield .

Drought tolerance exhibited complex heritability. Low heritability and lack of effective selection criteria are the main constrains in development of drought tolerant genotypes of rice (Sahebi *et al*., 2018). Different response can be observed by different genotypes of rice against drought stress and easiest way to tackle these problems is development of stress tolerant cultivar which is economically feasible.

The genetic diversity of rice germplasm provides a valuable resource for developing drought-tolerant rice varieties. Germplasm collections can be a source of useful traits that can be incorporated into breeding programs to develop drought-tolerant varieties. Rice germplasm from diverse regions, ecosystems, and genetic backgrounds exhibit different levels of drought tolerance and may contain useful genes and alleles for drought tolerance. Therefore, the study of rice germplasm for drought tolerance can contribute to the development of new and improved rice varieties that can withstand drought stress (Panda *et al*., 2021). The objectives of the study were to screen the rice germplasm against drought stress on morphological and molecular basis.

A powerful way to find superior genotypes with better drought tolerance is to do a molecular screening of rice germplasm for drought tolerance (Oladosu *et al*., 2019). It involves using morphological and molecular markers to find better genotypes that can handle stress from drought and perform better when water is scarce (Younis *et al*., 2020). The goal of this research is to find out how to use morphological and molecular markers to find drought-tolerant rice germplasm.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted in the field of Rice Research Institute, Kala Shah Kaku Lahore Pakistan (31.72140 N, 74.27020E). The Randomized Complete Block Design (RCBD) was used to conduct this experiment. The seeds of 35 varieties were sown under drought stress condition to check their productivity. After 34 days, one set of plants was relocated to a field with normal environmental conditions, while the other was transferred to a controlled environment with limited water availability and shelter against precipitation.

Stress Application

Following the adaption of genotypes, fourteen days of drought stress were applied at the panicle initiation stage only under rain out shelter to avoid precipitations during the drought period, in order to assess its effect on yield optimization in a study conducted International Rice Research Institute (IRRI) under the protocol of the Rice Evaluation System.

Traits Measurement

Different parameters were recorded at the appropriate time like Plant height (cm), number of tillers per plant, stem diameter (mm), panicle length (cm), total grains, filled grains, empty grains survival plants, flag leaf length (cm), flag leaf width (cm), Paddy length (mm), Paddy width (mm), Paddy thickness (mm), and 1000 Paddy weight (g).

Statistical Analysis

All the phenotypic data was analyzed by using statistical analysis Software Statista8.1 to see the genetic variability among the genotypes on the basis of various traits. The mean values are very highly significant at $P = 0.01$, significant at $P = 0.05$ and non-significant at $p > 0.05$ using Factorial Design, Tukey Test, Heritability and Genetic Advance. The heritability was estimated from this formula $H2=Vg/Vp$

Here Vg is genetic variation and Vp is phenotypic variation H is the heritability estimate, and genetic advance was calculated by this formula

$GA\ \%=(\sqrt{(PV*H2b)}/(G.\ M)*1.755)*100$

Primers Design

Primers were designed on the following SSR markers associated with *OsMS-L locus, q PRO3.1*, *qDTY 11.1*, and *bp* using Gramene Markers Database. Five Primer Sequences, temperature, and band size of SSR markers in Table1.Various SSR primers were used for PCR analysis

RESULTS

Number of Tillers

Results from Table 2 showed significant differences among genotypes, drought stress application, and their interaction. Average number of tillers per plant was 8.2745±0.07 with low coefficient of variation (2.4%). Table 3 showed more panicles per plant recorded for Nonabokra (11.167), Vehari (9.667), Basmati 385 (9), and Kalomonk (9) while fewer for Shaheen Basmati (7), PK 2021 (7), and Pusa 1718 (6.5) and show in Figure1. Heritability of number of tillers was 25.132%, with a genetic advance value of 21.059% (Table 4).

Total Grain

Results (Table 2) demonstrate significant differences among genotypes, drought stress application, and the interaction between the two. Mean total grains yielded 99.873±2.07 with a coefficient of variation (2.82%), indicating high reliability and consistency. Table 3 reveals Nonabokra yielded the most grains (183.33), followed by Vehari (155.33), Pokkoli (142) and Hossooli (135.33), while OP-50 (65.67), PK2021 (56.33), and Pusa (49.5) yielded the least and show in Figure 2. Heritability of total grain was 84.529%, and genetic advance value was 367.830% (Table 4).

Table 1. Five Primer Sequences, temperature, and band size of SSR markers

Sr. No.	SSR Marker	Primer Sequence (5'-3')	Annealing Temperature	Band Size
1	RM-22_F RM-22_R	GGTTTGGGAGCCCATAATCT CTGGGCTTCTTTCACTCGTC	51.3 °C	194
2	RM-109_F RM-109_R	GCCGCCGGAGAGGGAGAGAGAG CCCCGACGGGATCTCCATCGTC	57.8 °C	97
3	RM-135_F RM-135_R	CTCTGTCTCCTCCCCCGCGTCG TCAGCTTCTGGCCGGCCTCCTC	63 °C	131
4	RM-232_F RM-232_R	CCGGTATCCTTCGATATTGC CCGACTTTTCCTCCTGACG	54.3 °C	158
5	RM-261_F RM-261_R	CTACTTCTCCCCTTGTGTCG TGTACCATCGCCAAATCTCC	53.3 °C	125

Table 2. Variance analysis of different morphological traits of rice genotypes regarding drought stress.

Source	DF	NT	TG	FG	GL	GW	GTH	SUR	THOUS
RP	2	0.0619	36.53	37.95	0.068	0.00433	0.0029	0.3	0.6699
Genotype	34	**0.40299	**315.94	**446.27	*0.377	**0.02408	**0.01274	**10816.7	**4.1665
Stress	1	**5.56335	**5602.48	**5783.71	*11.364	**0.67248	**0.10035	**7.4	**95.7344
Genotype* Stress	34	**0.08427	**31.76	**56.74	*0.009	**0.00344	**0.00206	**9	**0.129
Error	140	0.03959	7.94	7.82	0.0059	0.00156	0.00093	1.4	0.064

Level of significant: 1) P value is significant because P value is less than 0.05 and greater than 0.01 and its show single *
2) If P value is highly significant because P value is less than 0.01 and its show double **
DF= degree of freedom, NT= Number of tillers, TG= total grain, FG=filled grain, GL= grain length, GW= grain width, GTH= grain thickness, SUR= survival plants, THOUS= thousand grain weight

Table 3. Tukey test of different rice genotypes.

Genotype	NT	TG	FG	GL	GW	GT	SUR	THOUS
Nonabokra	11.167 A	183.33 A	172.83 A	13.05 A	3.0833 A	2.1667 A	21.667 B	32.992 A
Vehari	9.667 B	155.33 B	147.83 B	12.767 B	2.6833 B	2.05 B	20.833 B	31.297 B
Pokkoli	9 C	142 C	128 C	12.483 C	2.6 BC	2 BC	21.5 B	30.62 C
Hossooli	9 C	135.33 D	122.67 CD	12.3 D	2.55 CD	2 BC	20.833 B	29.751 D
Kalomonk	9 C	132.5 DE	119 DE	12.117 E	2.5 DE	1.9333 CD	22 B	28.901 E
CSR 13	9 C	128.83 EF	115.83 EF	11.933 F	2.45 EF	1.9 D	20.833 B	27.641 F
PK8892Tol-19	9 C	125.33 FG	112.17 FG	11.667 G	2.4 F	1.9 D	21.167 B	26.631 G
Chenab Tol-19	9 C	122.83 FGH	110 FGH	11.533 GH	2.3 G	1.9 D	21 B	25.944 H
Bas. 515 Tol-19	9 C	121.83 GH	107.67 GHI	11.467 HI	2.3 G	1.9 D	21.5 B	25.43 HI
Shaheen Tol-19	9 C	119.33 GHI	105.17 HIJ	11.4 HIJ	2.3 G	1.9 D	21.333 B	24.921 IJ
Basmati 370	9 C	117.67 HI	102.67 IJK	11.3 IJ	2.3 G	1.9 D	21.333 B	24.424 JK
Bas. 385	9 C	114.17 IJ	101.5 JK	11.283 J	2.2 H	1.9 D	20.833 B	24.046 KL
Super Basmati	8.5 D	102.33 LMN	90.58 MN	10.968 KL	2.1 I	1.8333 E	21.417 B	23.078 MN
Basmati 515	9 C	109.33 JK	96.83 KL	11.085 K	2.2 H	1.8 E	20.833 B	23.49 LM
PK1121aromatic	8.833 CD	107.33 KL	93.17 LM	11 KL	2.1167 HI	1.8 E	21.333 B	23.267 M
Kissan Basmati	8 E	104.33 KLM	90.5 MN	11 KL	2.0667 IJ	1.8 E	20.833 B	23.061 MN
Punjab Basmati	8 E	99.83 MN	87 NO	10.9 L	2 JK	1.8 E	22 B	22.636 NO
Chenab Basmati	8 E	97.33 N	84.33 O	10.833 LM	2 JK	1.8 E	21 B	22.496 O
Super Basmati 2019	8E	103.32MN	96.76KL	10.981 KLM	2.1 I	19.03 CD	22.045 B	22.568 O
Super Gold	8 E	91 O	78.17 P	10.683 MN	1.9333 KL	1.8 E	21.167 B	22.166 OP
PK10029	8 E	88.83 O	76.17 P	10.567 NO	1.9 L	1.8 E	21.5 B	21.866 PQ
PK9966	8 E	85.5 OP	72.5 PQ	10.5 O	1.9 L	1.8 E	21.333 B	21.589 Q
PK386	8 E	82.5 PQ	68.5 QR	10.433 OP	1.9 L	1.8 E	20.833 B	21.413 QR
PK434	8 E	80.33 PQR	67.67 QRS	10.317 PQ	1.9 L	1.8 E	20.833 B	21.313 QRS
PK10683	8 E	78.67 QRS	65.67 RST	10.217 Q	1.8667 LM	1.8 E	20.667 B	20.973 RST
PK10324	8 E	77.83 QRST	63.67 RST	10.017 R	1.8 M	1.7833 E	21.833 B	20.798 STU
KS282	8 E	75.67 RSTU	61.83 STU	9.883 R	1.8 M	1.69 FG	21.667 B	20.587 TUV
N22	7.667 E	73.33 STUV	60 TUV	9.7 S	1.8 M	1.7 F	27.333 A	20.376 UVW
Shaheen Basmati	7 F	72 TUV	57.5 UVW	9.4 T	1.8 M	1.54 J	21.833 B	20.231 VW
Basmati 2000	7 F	70.33 UVW	56.5 UVW	9.233 T	1.8 M	1.67 H	21.167 B	20.018 WX
Alkhalid Basmati	7 F	68.83 VW	55.5 VW	8.983 U	1.8 M	1.53 JK	21 B	19.637 XY
OP-50	7 F	65.67 WX	53 WX	8.717 V	1.8 M	1.63 HI	21.167 B	19.318 Y
PK10436	7 F	60.33 XY	48 XY	8.233 W	1.7 N	1.52 L	21 B	19.139 Y
PK2021	7 F	56.33 Y	43.5 Y	7.717 X	1.7 N	1.51 M	21.667 B	18.357 Z
Pusa 1718	6.5 G	49.5 Z	36.67 Z	7.333 Y	1.6833 N	1.5 N	21.667 B	15.403 a

NT= Number of tillers, TG= total grain, FG=filled grain, GL= grain length, GW= grain width, GTH= grain thickness, SUR= survival plants, THOUS= thousand grain weight

Table 4. Different morphological character of rice shows genotypic variance, genotypic coefficient variance%, phenotypic variance, phenotypic coefficient variance%, error coefficient variance%, heritability% and genetic advance %.

Traits	M.S	G.M	GV	GCV %	PV	PCV %	EV	ECV %	h2bs%	GA%
Number of Tiller	1.25	8.275	0.209	15.897	0.832	31.71	0.623	27.437	25.132	21.059
Total Grain	2453.9	100	770.933	277.657	912.033	301.999	141.1	118.786	84.529	367.83
Filled Grain	2509.7	86.784	787.8	301.292	934.1	328.078	146.3	129.838	84.338	399.141
Paddy Length	7.607	10.612	2.419	47.739	2.77	51.09	0.351	18.197	87.314	63.243
Paddy Width	0.272	2.1	0.084	20.042	0.019	9.405	0.019	9.558	454.137	26.551
Paddy Thickness	0.04	1.82	0.01	7.538	0.009	7.094	0.009	6.882	112.918	9.987
Survival Plants	7.4	21.439	2	30.543	1.4	25.554	1.4	25.554	142.857	40.462
Thousand Grain Weight	81.539	23.386	27.048	107.546	0.723	17.583	0.394	12.977	3741.134	142.473

M.S= mean Square, G.M= grand mean, GV= Genotypic variance, GCV%= Genotypic coefficient variance%, PV= Phenotypic variance, PCV%= Phenotypic coefficient variance%, ECV= Error coefficient variance%, h2bs%= Heritability% and GA%= Genetic Advance%.

Filled Grain

Results (Table 2) showed significant differences between the genotypes, drought stress application and their interaction. Mean filled grain was 86.841±2.10; CV was 3.22%, indicating reliable results with consistent grain filling. Nonabokra had highest value (172.83) followed by Vehari (147.83), Pokkoli (128) while PK10436 (48), PK2021 (43.5) and Pusa 1718 (36.67) had lowest (Table 3) and show in Figure 3. Heritability was 84.338% and genetic advance was 399.141% (Table 4).

Paddy Length

Results from Table 2 showed significant differences among genotypes, drought stress and their interaction. The average paddy length was 10.618±0.09mm with low coefficient of variation (0.72%). Table 3 revealed Nonabokra had the highest paddy length (13.05cm), followed by Vehari, Pokkoli and Hossooli, whereas PK10436, PK2021, and Pusa had the lowest and show in Figure 4. Heritability of paddy length was 87.314%, and genetic advance was 63.243% (Table 4).

Paddy Width

Results (Table 2) showed significant differences between genotypes, drought stress, and their interaction; mean paddy width was 2.0951±0.02mm, with lowest coefficient of variation (1.88%), indicating reliable results. Table 3 revealed Nonabokra had highest paddy width (3.0833cm), followed by Vehari, Pokkoli, Hossoli, lowest was PK 10436, PK 2021, Pusa 1718 and show in Figure 5. Heritability and genetic advance value of paddy width was 454.134% and 26.551% (Table 4).

Paddy Thickness

Results showed significant differences in genotype, drought-stress application, and genotype-drought interaction (Table 2). Average paddy thickness was 1.8191 ± 0.01mm, with low coefficient of variation (1.68%) indicating reliable results. Nonabokra, Vehari, Pokkoli and Hossooli had highest paddy thickness (2.1667, 2.05, 2.00 and 2.00mm) and PK10436, PK2021 and Pusa 1718 had lowest (1.52, 1.51 and 1.50mm) (Table 3) and show in Figure6. Heritability of paddy thickness was 112.918%, genetic advance of 9.987% (Table 4).

Flag Leaf Altitude of Blade

Flag leaf angle was noted at the maturity stage. Based on the altitude of blade, flag leaf was categorized in four categories i.e., Erect, Semi-Erect, and Horizontal etc. (Fig. 7).

Survival Plant

Results from Table 2 showed significant differences between genotypes, drought stress and their interaction. On average, 21.439±0.52 plants survived (CV=5.57%), suggesting reliable results. Table 3 showed N22 had the highest survival rate of 27.33, followed by Punjab Basmati (22), Kalomonk (22) and Shaheen Basmati (21.833). Vehari, Basmati 385 and PK 10683 had the lowest survival rates of 20.833, 20.833, and 20.667 respectively and show in Figure 8. Heritability of survival plants was 142.857%, with a genetic advance of 40.462% (Table 4).

Thousand Paddy Weight

Results from Table 2 indicated significant differences among genotypes, drought stress application and their interactions, with a mean thousand paddy weight of 23.347±0.27 and the lowest coefficient of variation of 1.08%, suggesting reliable data with highest consistency. Table no.03 showed Nonabokra (32.992), Vehari (31.297), Pokkoli (30.62), and Hossooli (29.751) as highest, while PK 10436 (19.139), PK 2021 (18.357), and Pusa 1718 (15.403) had lowest thousand paddy weight and show in Figure 9. Heritability value of thousand paddy weight was 3741.134% (Table 4), with a genetic advance value of 142.473%.

Five drought- linked polymorphic SSR markers, RM-109, RM-22, RM 135, RM 231, and RM 261 have been used for PCR analysis, and bands were compared with positive check variety N-22. Genotypes/Varieties with similar banding pattern of positive check variety were considered tolerant, however bands other than positive check variety considered susceptible.

RM 109

For marker RM-109, these 13 genotypes Nonabokra, Vehari, Pokkoli, Hossooli, Kalomonk, CSR-13, Basmati 515 Tol-19, Basmati 370, Basmati 385, Super Basmati, Basmati 515, and PK1121aromatic showed similar banding pattern of positive check variety N-22 and were found tolerant against drought stress. While the varieties PK8892 Tol-19, Chenab Basmati Tol-19, and Shaheen Tol-19 have a band size of 95bp. That is different from positive check variety N-22 and were found susceptible genotypes against in drought stress (Figure 10).

RM-22

For RM-22, these all genotypes Nonabokra, Vehari, Pokkoli, Hossooli, Kalomonk, CSR-13, PK8892 Tol-19, Chenab Basmati Tol-19, Basmati 515 Tol-19, Shaheen Tol-19, Basmati 385, Super Basmati, Basmati 515, and PK1121aromatic with similar banding pattern of positive check variety N-22 and were found tolerant against drought stress. And did not find any susceptible in genotypes. N-22 is a resistant variety against drought stress (Figure 11).

RM-135

For RM-135 these 14 genotypes Nonabokra, Vehari, Pokkoli, Hossooli, Kalomonk, CSR-13, PK8892 Tol-19, Basmati 515 Tol-19, Shaheen Tol-19, Basmati 370, Basmati 385, Super Basmati, Basmati 515, and PK1121 aromatic showed similar banding pattern of positive check variety N-22 and were found tolerant against drought stress. While 1 variety Chenab Basmati Tol-19 has different band from positive check variety N-22. And Chenab Basmati Tol-19 was found susceptible genotype against drought stress (Figure 12).

RM-232

For RM-232, these all genotypes Nonabokra, Vehari, Pokkoli, Hossooli, Kalomonk, CSR-13, PK8892 Tol-19, Chenab Basmati Tol-19, Basmati 515 Tol-19, Shaheen Tol-19, Basmati 385, Super Basmati, Basmati 515, and PK1121aromatic showed similar banding pattern of positive check variety N-22. These all genotypes were found tolerant against drought stress. And did not find any susceptible in genotypes. N-22 is a resistant variety against drought stress (Figure 13).

RM-261

For RM-261, these 12 genotypes Pokkoli, Hossooli, Kalomonk, CSR-13, PK8892 Tol-19, Chenab Basmati Tol-19, Chenab Basmati Tol-19, Basmati 515 Tol-19, Basmati 385, Super Basmati, Basmati 515, and PK1121aromatic showed similar banding pattern of positive check variety N-22. While varieties Nonabokra, Vehari, Shaheen Tol-19 have a band size of positive check variety N-22. These 12 genotypes were found tolerant against drought stress. N-22 is a resistant variety against drought stress (Figure 14).

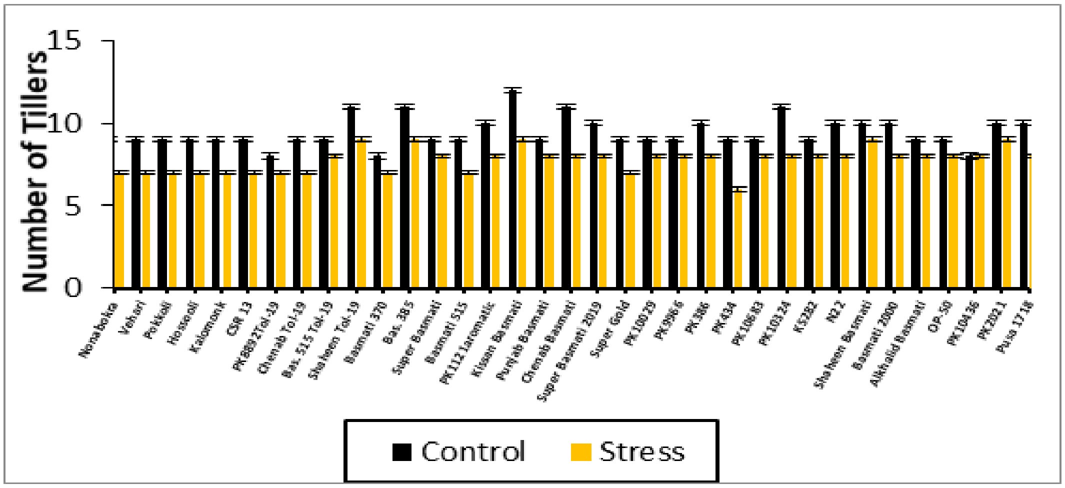

Fig. 1. In 35 varieties, these varieties Nonabokra, Vehari, Basmati515, and Kalomonk best perform in more number of tillers while these varieties Shaheen Basmati, PK2021, and Pusa1718 show less number of tillers.

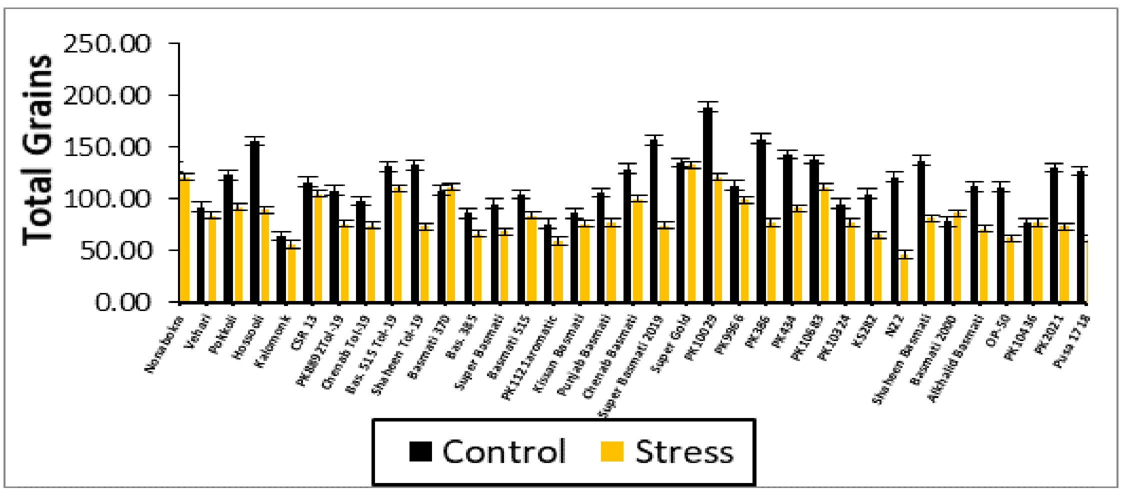

Fig. 2. In 35 varieties, these varieties Nonabokra, Vehari, Pokkoli, and Hossooli Kalomonk best perform in more number of total grain while these varieties PK 10436, PK 2021, and Pusa 1718 show less number of total grain.

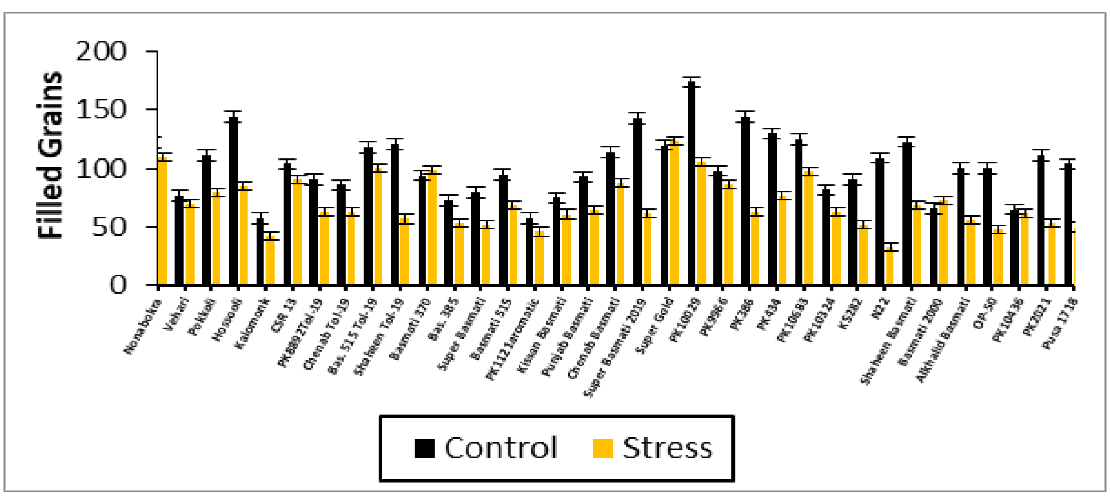

Fig. 3. In 35 varieties, these varieties Nonabokra, Vehari, Pokkoli, and Hossooli best perform in more number of filled grains while these varieties PK 10436, PK 2021, and Pusa 1718 show less number of filled grains.

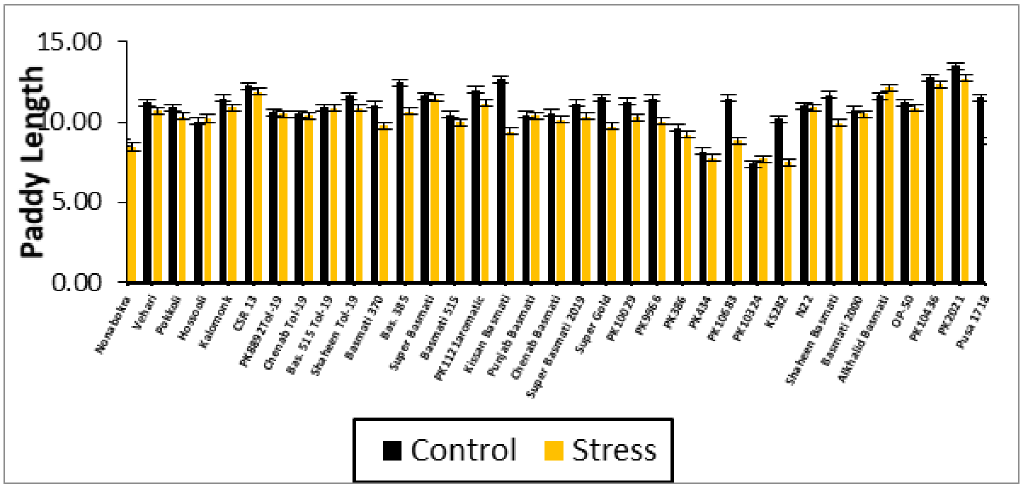

Fig. 4. In 35 varieties, these varieties CSR 13, PK2021, PK10436, and PK1121aromatic best perform in higher paddy length while these varieties KS282, PK 324, and Nonabokra show less paddy length.

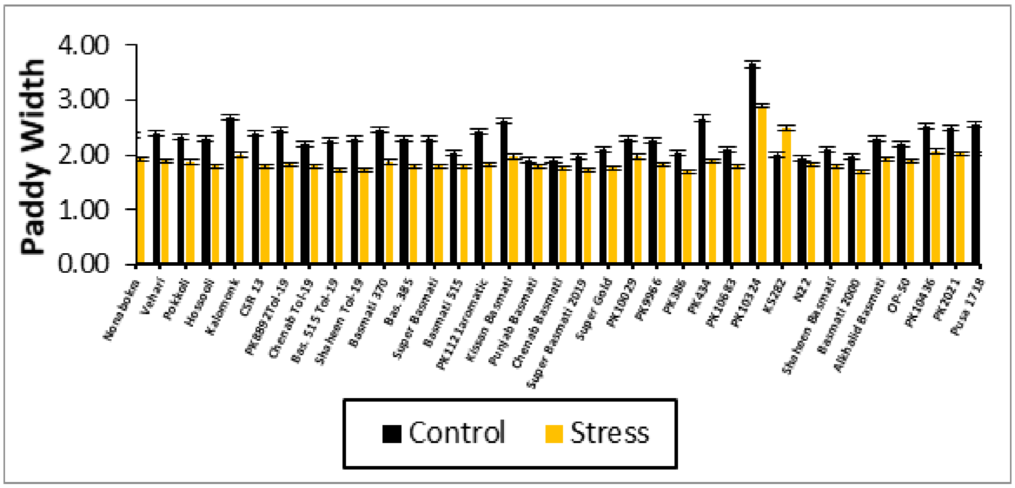

Fig. 5. In 35 varieties, these varieties PK10324, KS282, Kalomonk, and Kissan Basmati best perform higher paddy width while these varieties PK386, Basmati 2000, and Hossooli show less paddy width.

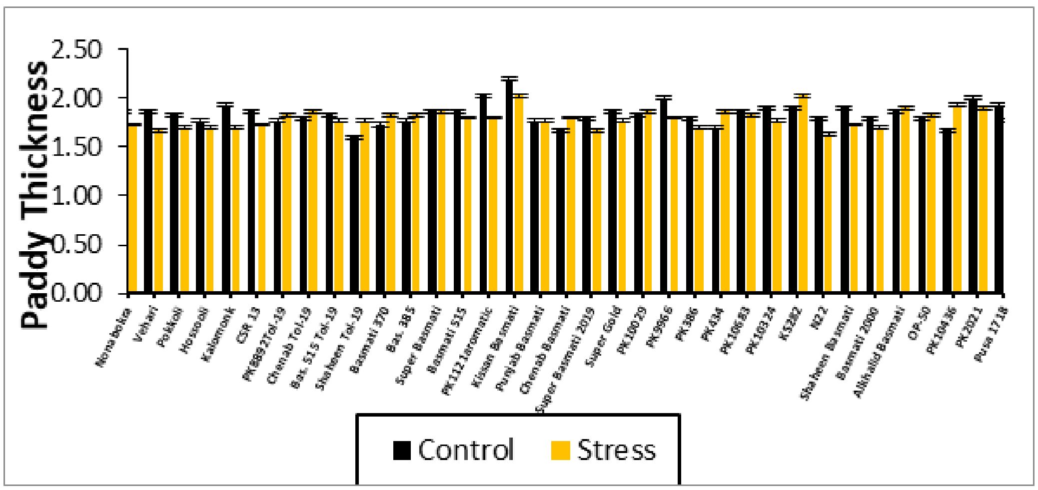

Fig. 6. In 35 varieties, these varieties Kissan basmati, KS282, OP-50, and Alkhalid Basmati best perform in higher paddy thickness while these varieties N22, PK386, and Vehari show less paddy thickness.

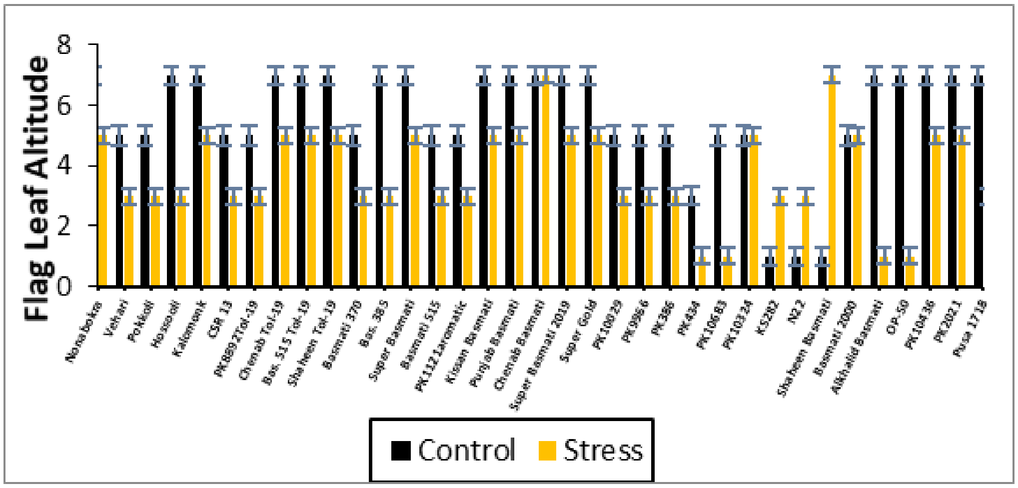

Fig. 7. In 35 varieties, these varieties Shaheen basmati, Chenab basmati, super basmati 2019, Super Gold best perform while these varieties OP-50, Alkhalid Basmati, and Pk434 show less perform.

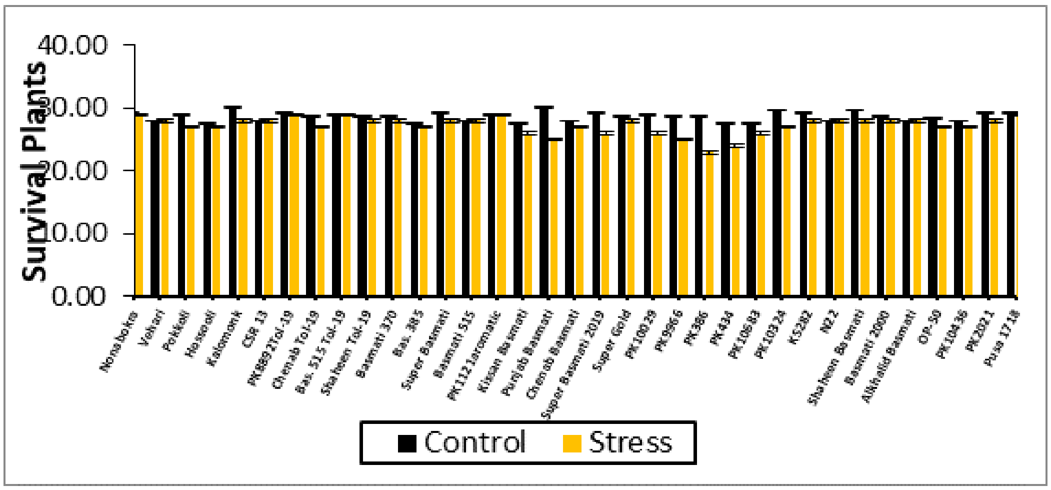

Fig. 8. In 35 varieties, these Nonabokra, PK1121aromatic, Super Golden, PK2021 best perform in more number of plant survived while these varieties PK386, PK434, and Punjab Basmati show less number of plant survived.

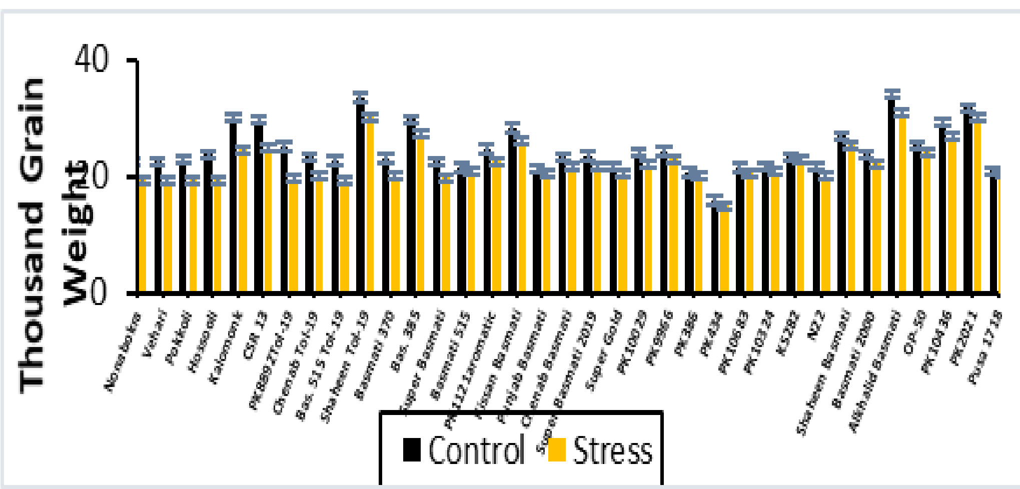

Fig. 9. In 35 varieties, these varieties Nonabokra, Vehari, Pokkoli, and Hossooli best perform in more thousand weights while these varieties PK10436, PK2021, and Pusa 1718 show less thousand weights.

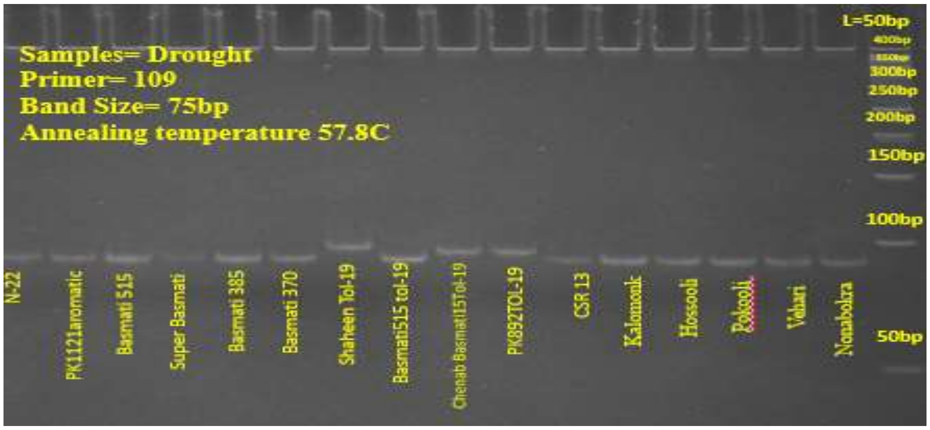

Fig. 10. RM-109 Marker, Resistance: These 12 genotypes Nonabokra, Vehari, Pokkoli, Hossooli, Kalomonk, CSR-13, Basmati 515 Tol-19, Basmati 370, Basmati 385, Super Basmati, Basmati 515, and PK1121aromatic showed tolerance with reference to positive check variety N-22 against drought stress. Susceptible: While these 3 varieties shown PK8892 Tol-19, Chenab Basmati Tol-19, and Shaheen Tol-19 showed different banding pattern with reference to positive check variety, and were found susceptible.

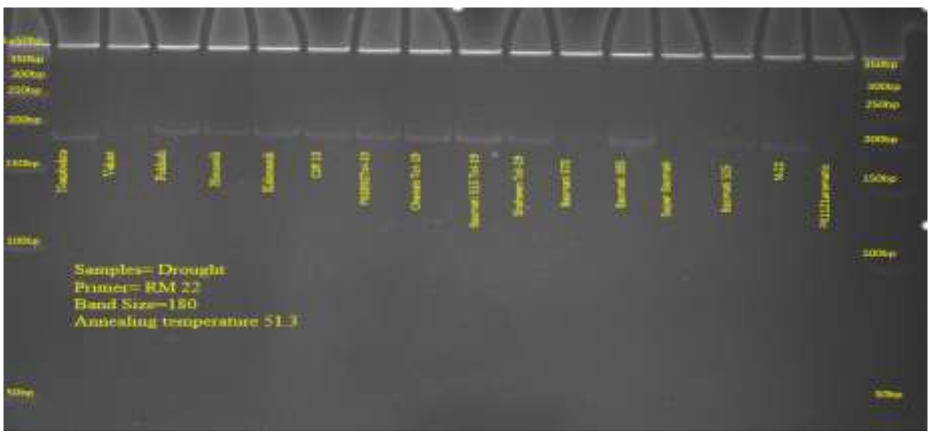

Fig. 11. RM-22 Marker, Resistance: Nonabokra, Vehari, Pokkoli, Hossooli, Kalomonk, CSR-13, PK8892 Tol-19, Chenab Basmati Tol-19, Basmati 515 Tol-19, Shaheen Tol-19, Basmati 385, Super Basmati, Basmati 515, and PK1121aromatic showed tolerance with reference to positive check variety N-22 against drought stress. Susceptible: no variety found susceptible.

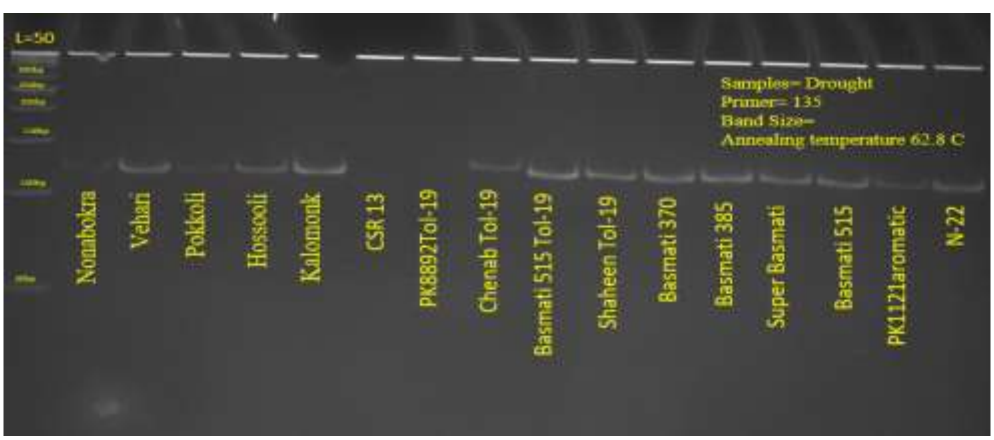

Fig. 12. RM-135, Resistance: these 14 genotypes Nonabokra, Vehari, Pokkoli, Hossooli, Kalomonk, CSR-13, PK8892 Tol-19, Basmati 515 Tol-19, Shaheen Tol-19, Basmati 370, Basmati 385, Super Basmati, Basmati 515, and PK1121 aromatic showed tolerance with reference to positive check variety N-22 against drought stress. Susceptible: Chenab Basmati Tol-19 was found susceptible genotype against drought stress.

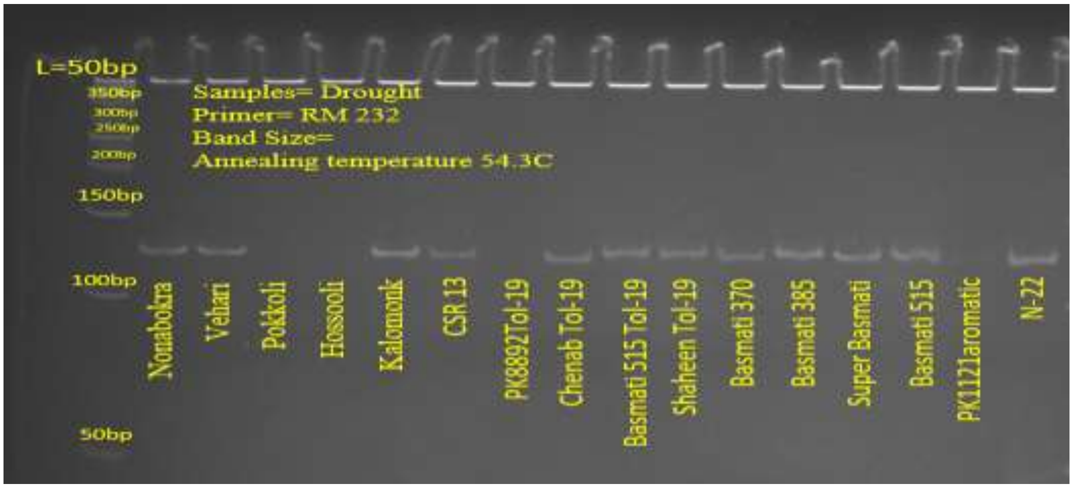

Fig. 13. RM-232, Resistance: these all genotypes Nonabokra, Vehari, Pokkoli, Hossooli, Kalomonk, CSR-13, PK8892 Tol-19, Chenab Basmati Tol-19, Basmati 515 Tol-19, Shaheen Tol-19, Basmati 385, Super Basmati, Basmati 515, and PK1121aromatic showed tolerance with reference to positive check variety N-22 against drought stress. Susceptible: no variety found susceptible.

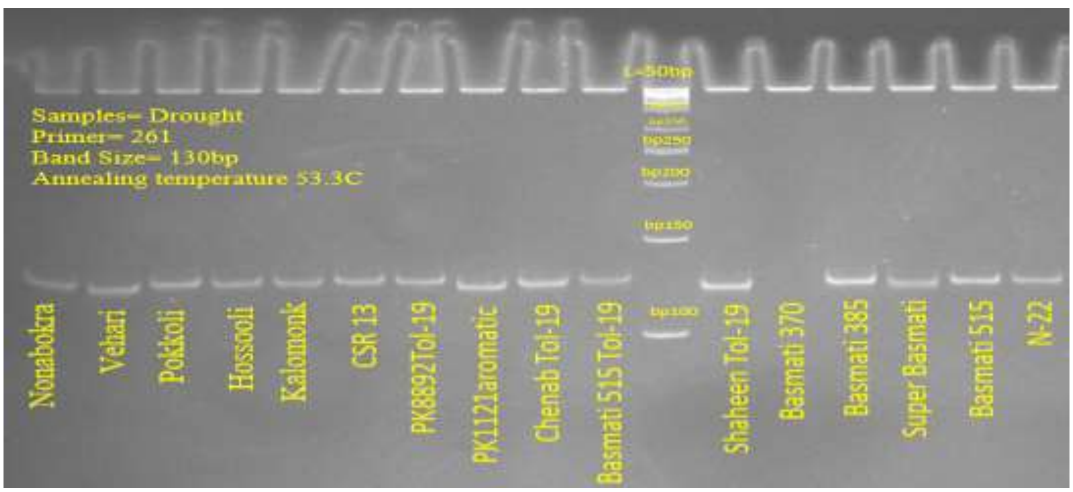

Fig. 14. RM-261 Marker, Resistance: these 12 genotypes Pokkoli, Hossooli, Kalomonk, CSR-13, PK8892 Tol-19, Chenab Basmati Tol-19, Basmati 515 Tol-19, Basmati 385, Super Basmati, Basmati 515, and PK1121aromatic showed tolerance with reference to positive check variety N-22 against drought stress. Susceptible: While these varieties Nonabokra, Vehari, Shaheen Tol-19 have a different band size as compared to positive check variety N-22.

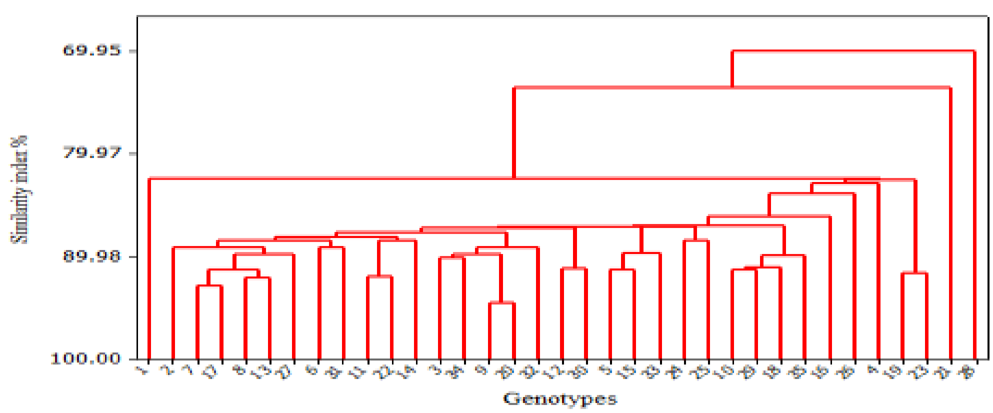

Fig. 15. Dendrogram showing rice genotype genetic interaction of control condition.

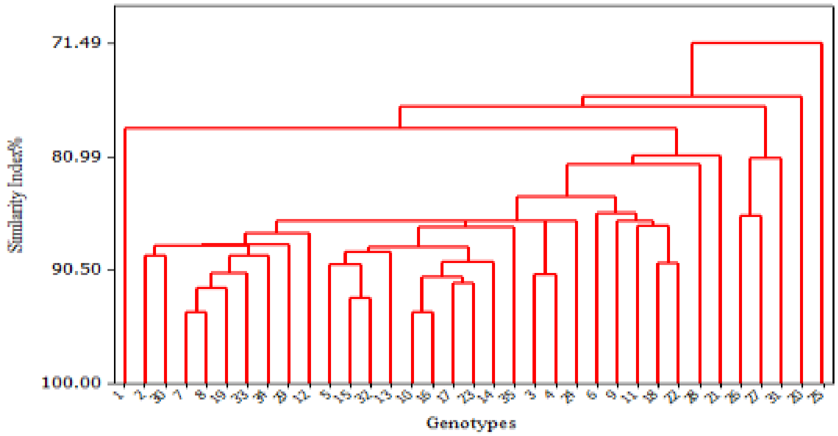

Fig. 16. Dendrogram showing rice genotype genetic interaction of stress condition.

Dendrogram

The dendrogram showed genetic interaction of control and stress condition (Figure 15, and Figure 16) in rice genotypes **(**1)Nonabokra (2) Vehari (3) Pokkoli (4) Hossooli (5) Kalomonk (6) CSR-13 (7) PK8892 Tol-19 (8) Chenab Tol-19 (9) Basmati 515 Tol-19 (10) Shaheen Tol-19 (11) Basmati 370 (12) Basmati 385 (13) Super Basmati (14) Basmati 515 (15) PK1121 aromatic (16) Kissan Basmati (17) Punjab Basmati (18) Chenab Basmati (19) Super Basmati 2019 (20) Super Gold (21) PK10029(22) PK 9966 (23) PK386 (24) PK434(25) (26) PK10683 (27) KS282 (28)N22 (29) Shaheen Basmati (30) Basmati 2000 (31) Alkhalid Basmati (32) OP-50 (33) PK10436 (34) PK2021 (35) Pusa 1718.

DISCUSSION

Number of Tillers

Genotypes with more tillers showed better drought resistance and potential for improving grain yield under such conditions, as indicated by higher heritability and genetic advance values. It suggests that selection of drought tolerant varieties is feasible in low-yield environments.

Total Grain

The results suggest that genotypes with more number of total grains indicate higher drought tolerance, which can be leveraged to develop new rice varieties and hybrids for improved grain yield under drought conditions (Sabar *et al*., 2024). Analysis of heritability and genetic advance values further reveals that drought stress resistance is heritable, with significant variation in the gene pool that can be selected through breeding programs. Thus, creating drought-resistant varieties in a relatively short period of time is possible (Oladosu *et al*., 2019).

Filled Grain

Results suggest that genotypes with a greater number of filled grains may be used to develop drought-tolerant rice varieties and hybrids (Abd El-Aty *et al*., 2022). The trait has a high heritability and genetic advance, making it a suitable subject for selection and improvement (Ahmad *et al*., 2020; Oladosu *et al*., 2019). This suggests potential for developing strategies for breeding resilient rice varieties with greater drought tolerance.

Paddy Length

Higher paddy length indicated tolerance to drought stress and a normal growth pattern for the genotypes tested (Hussain *et al*., 2021). Results suggest that these genotypes may be used for developing rice varieties and hybrids to increase grain yield under drought conditions. The high heritability of paddy length and genetic advance suggests that selective breeding may be an efficient way of improving drought stress tolerance in the tested rice germplasm.

Paddy Width

The genotypes with highest paddy width suggested tolerance against drought stress (Anupama *et al*., 2018). This implies that these genotypes could be used to develop higher-yielding drought-tolerant rice varieties and hybrids. The heritability and genetic advance data for this trait show considerable potential for improvement (Ahmad *et al*., 2020; Oladosu *et al*., 2019). Stability analysis and gene network exploration can help determine the most effective crossing scheme to maximize yield.

Paddy Thickness

Genotypes with highest paddy thickness showed high drought tolerance and consistent growth patterns (Ramamoorthy *et al*., 2018). These variants at the genetic level are likely to have a significant effect on the observed phenotypes, leading to a heritability value of high transmissibility of this trait. Targeted selection of mutants with high paddy thickness is desirable to develop high yielding, drought tolerant varieties

Flag Leaf Altitude of Blade

The analysis of qualitative parameters of flag leaf altitude, showed varying patterns among the genotypes as were found in (Kumar *et al*., 2021). This qualitative trait can have implications for the visual appearance and market preferences of rice grains. Further investigations into the genetic basis of this trait can provide insights into the underlying mechanisms and potential breeding strategies.

Survival Plant

Results showed significant differences in all genotypes. Genotypes with more plants survived showed tolerance to drought stress and normal growth, while those with less plants survived were less resistant (Larkunthod *et al*., 2018). These more survived plants genotypes may be used to create improved drought-resistant varieties and hybrids to enhance grain yields (Luo *et al*., 2019). High heritability values suggest strong genetic correlations among generations for survival, and high genetic advance values suggest potential for creating improved drought-tolerant genetics (Ahmad *et al*., 2020; Oladosu *et al*., 2019). Selection of superior genotypes could therefore lead to the development of improved drought-resistant varieties.

Thousand Paddy Weight

Genotypes with highest thousand paddy weight displayed tolerance to drought stress and maintained normal growth (Kamarudin *et al*., 2018). Those with highest thousand paddy weight showed resistance tolerance, which can be utilized to improve cereal yield under drought conditions (Wang *et al*., 2022). Heritability value demonstrates that the dry weight of grain is largely determined genetically and high genetic improvement potential exists for the studied germplasm, making drought tolerant varieties more likely to be developed through simple breeding methods (Ahmad *et al*., 2020; Oladosu *et al*., 2019). Thus, this study provides insight into the gene structure of the studied germplasm's drought stress response.

In addition to the phenotypic analysis, molecular marker screening was conducted. The molecular marker results provide valuable insights into the genetic diversity and potential association with drought stress tolerance among the studied genotypes (Gaballah *et al*., 2020). Several markers, including RM 109, RM 135, RM 22, RM 232, and RM 261 were screened using PCR analysis to assess their polymorphism and ability to differentiate genotypes (Hossain *et al*., 2024).

RM -109

RM 109 SSR marker demonstrated polymorphism and effectively divided genotypes based on band positions. It had similar results compared to Positive check variety N-22 (Palumbo, Galvao, Nicoletto, Sambo, & Barcaccia, 2019). 13 genotypes showed a band at 90 bp, indicating their drought tolerance, but 3 varieties had a different band size at 95 bp than N-22. Thus, this marker could serve as a useful marker for distinguishing drought tolerance among rice genotypes (Verma *et al*., 2019).

RM-22

RM22, SSR marker, was found to be polymorphic and effective in dividing different genotypes based on band sizes, similar to Positive check variety (Verma *et al*., 2019). N-22, a resistant variety, possessed a band at 180 bp, indicating drought tolerance. The same band size was seen in other rice genotypes - Nonabokra, Vehari, Pokkoli, Hossooli, Kalomonk, CSR-13, PK8892 Tol-19, Chenab Basmati Tol-19, Basmati 515 Tol-19, Shaheen Tol-19, Basmati 385, Super Basmati, Basmati 515, and PK1121aromatic - thus suggesting that RM22 is a reliable marker for distinguishing drought tolerance in them.

RM-135

The SSR marker RM 135 was found to effectively divide genotypes based on band positions, and the results similar to Positive check variety N-22 (Nayana, 2020). 14 genotypes had the same band size of 180 bp, while Chenab Basmati Tol-19 demonstrated a band of 135 bp, suggesting its drought tolerance. This marker may serve as a useful tool for distinguishing drought tolerance among rice genotypes.

RM-232

RM232, an SSR marker, demonstrated polymorphism effectively distinguishing genotypes based on band positions, and similar results were found in positive check variety N-22 (Anisuzzaman *et al*., 2022). 14 genotypes (Nonabokra, Vehari, Pokkoli, Hossooli, Kalomonk, CSR-13, PK8892 Tol-19, Basmati 515 Tol-19, Shaheen Tol-19, Basmati 385, Super Basmati, Basmati 515, PK1121aromatic) had similar bands at 130 bp, while Chenab Basmati Tol-19 showed different band at 135 bp. RM232 can serve as a useful marker for identifying drought tolerant rice genotypes (Anisuzzaman *et al*., 2022).

RM-261

The SSR marker RM-261 is polymorphic, effectively distinguishing genotypes by their band positions. Results show similarity with that of Positive check variety (Andarini *et al*., 2022). Variety N-22 showed a band at 130 bp, indicating drought tolerance. Twelve genotypes (Pokkoli, Hossooli, Kalomonk, CSR-13, PK8892 Tol-19, Basmati 515 Tol-19, Basmati 385, Super Basmati, Chenab Basmati Tol-19, Basmati 515, and PK1121aromatic) have a similar band size at 130 bp, differing from Nonabokra Vehari and Shaheen Tol-19 with a band at 125 bp. RM 261 can be used as an indicator of drought tolerance in rice varieties (Andarini *et al*., 2022).

Conclusion

A total of 35 rice lines were screened for tolerance against drought stress in the Rice Research Institute Kala Shah Kaku. Fifteen genotypes were found to perform better in drought stress conditions, namely Nonabokra, Vehari, Pokkoli, Hossooli, Kalomonk, CSR-13, PK8892 Tol-19, Chenab Basmati Tol-19, Basmati 515 Tol-19, Shaheen Tol-19, Basmati 385, Super Basmati, Basmati 515, and PK1121-aromatic. Additionally, five SSR markers, RM-109, RM-22, RM-135, RM-232, and RM-261, were identified as potential markers linked to drought resistance and used to check resistance in these 15 varieties, which were consequently found to be resistant to drought stress, thus making the linked markers able to be used as a tool for screening genotypes against drought stress.

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