INVESTIGATING THE YIELD POTENTIAL AND GENETIC RESILIENCE TO DROUGHT STRESS OF DIVERSE RICE GENOTYPES

Qurat ul Ain Sani[1], Rana Arsalan Javaid[1], Abid Majeed[1], Muhammad Shahzad Ahmed[1], Muhammad Jalal Hassan[2], Afia Gul[3], Faiza Siddique[1], Mashal Rehman[1], Shafiq Qalandrani[1], Muhammad Arshad[4]

[1]Rice Research Program, Crop Sciences Institute, National Agricultural Research Center, Islamabad

[2]National Rice Coordination, Pakistan Agriculture Research Council, Islamabad

[3]Department of Plant Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad

[4]Crop Sciences Institute, National Agricultural Research Center, Islamabad

Corresponding Author: Rana Arsalan Javaid

Abstract:

Rice (*Oryza sativa* L.) is a globally vital staple crop, feeding billions of people and serving as a cornerstone of many cultures and cuisines. Drought is one of the major abiotic stresses that delimit rice production and yield especially in rainfed ecosystems. To address this challenge and identify the promising drought tolerant rice cultivars, an effort was made in the present study to screen few potential rice genotypes aimed at investigating their genetic potential to withstand drought stress and ensure stable yields in water deficit conditions. Performance of 30 Rice genotypes were evaluated for yield on the bases of 8 morphological traits as days to flowering, days to maturity, plant height, number of tillers, panicle length, grains per panicle, thousand grain weight and grain yield.Assessment of genotypes to resist attack of stem borer and leaf folder was also carried out. Their genetic potential to withstand drought stress was assessed through SSR Marker Qdty1.1. 14 genotypes were screened out as drought tolerant genotypes.Study was conducted at Rice Research Program, CropSciences Institute, National Agricultural Research Center-Islamabad following Randomized Complete Block Design in three replications.After conducting an extensive assessment of yield under diverse conditions, three exceptional genotypes, NR 420, NR 429, and NR 421, emerged as the top performers in terms of yield. Furthermore, for prioritizing drought tolerance, NR 426, NR 415, and NR 414 stand out as promising choices, as they not only surpass the average yield but also exhibit genetic traits that make them resilient to drought stress.These efforts are crucial for food security in regions where rice is a staple crop and where drought events are increasingly common due to climate change.

Key words: *SSR Marker, Genetic Potential, Water Sensitive Crop, PCR,*

Introduction

Rice (*Oryza sativa* L.) which belongs to Poaceae family is a principal cereal crop, consumed as a staple diet all around the world. More than 50% of rice is being consumed and produced in Asia as it plays a significant role in attaining food safety in the region (FAO, 2015).Rice is included in *Oryza* genus which contains 24 species including 22 wild and 2 are cultivated species. The 2 well known cultivated species are *Oryza sativa* and *Oryza glaberrima*, originated mainly from US, Asia, Europe and African countries. Based on geographical adaptability *Oryza sativa* is most widely grown and is subdivided into *indica, japonica and javanica* cultivars. *Javanica* is rarely grown in hot and humid regions whereas *japonica* and *indica* are predominantly grown in temperate, tropical and sub-tropical climates (Hassan et al., 2023). Plant productivity is badly influenced by various abiotic stresses like drought, salinity, cold, heat, heavy metals and UV-B. However, drought stress remains to be the most challenging as it is the primary factor that leads to unstable and low yields potentially reducing the food production worldwide.It is estimated that almost 50% of rice produced in the world is affected by water shortage (Sajib et al. 2012).

Recently developed resistant cultivars face countless challenges in such a way that they may perform best in one dry year but may not be successful in another, or tolerant to terminal drought but may be non-tolerant to intermittent drought. Also enhancing drought endurance in crops is complex because of its interactions with coexisting stresses. Since the start of the 21st century, the world's drought affected land has doubled. In response to the worsening effects of global climate change, a central aim of the rice breeding initiative is the identification and development of high-yield rice varieties that can thrive under the stressors prevalent in rain-fed areas. In this context, it becomes imperative to identify sustainable germplasms(Mas-ud et al., 2022).

In most cases, rice productivity is estimated through the assessment of its agronomic traits, which encompass parameters like productive tiller count, grains per panicle, grain size, grain weight, 1000-grain weight, plant height, and panicle length. These agronomic traits are determined and controlled by several genetic mechanisms. The production potential of modern cultivars remains largely unchanged, primarily due to their susceptibility to abiotic stressors like drought, salt stress and submergence (Hassan et al., 2023).Developing genetically improved crop cultivars that can endure biotic and abiotic stressors is a primary objective for plant breeders (Gupta et al., 2019).

The impact of drought stress on rice is most pronounced during three critical growth stages: anthesis, early seedling and vegetative phase (Singh et al., 2012).Water shortage during the early seedling stage magnifies drought stress, resulting in an uneven and weak stand establishment (Vibhuti et al., 2015).Under drought stress, active seed germination was disrupted, resulting in osmotic imbalances, impaired membranes, reduced respiration rates, and decreased ATP synthesis (kadam et al., 2017).Restricted water availability during the vegetative phase results in postponed panicle initiation, subsequently leading to delayed maturation (Singh et al., 2012).

While the reproductive growth stage seems to bear the outcome of drought stress's detrimental effects on grain yield, plants generally exhibit some degree of recovery during the vegetative growth phase. However, restoration from drought stress during the flowering phase presents greater complexity (Xangsayasane et al., 2014).A brief period of drought stress during the reproductive growth phase significantly reduces rice grain yield by causing shorter panicles, poor seed setting, a decrease in the number of kernels per panicle, and impaired spikelet development (Wei et al., 2017).Research findings indicate that drought stress experienced during the flowering stage negatively affects pollination, leading to decreased seed setting, smaller grains, and fewer grains per plant. In cases of severe drought, flower abortion occurs, resulting in a complete yield loss of 100% (Davatgar et al., 2009). It is firmly established that drought stress, whether mild or severe, during the reproductive growth phase reduces the overall grain production. This reduction is attributed to the disruption in the translocation of assimilates from leaves to reproductive organs such as the panicle and kernels. Rice cultivars that rebounded from temporary drought conditions showed more robust yield responses compared to drought-sensitive cultivars (Singh et al., 2012).

The cultivation of drought-resistant rice strains aimed at reducing water usage in rice farming stands as a prominent objective within agricultural research. Consequently, the pressing requirement for drought-resistant rice varieties is paramount to enduring food security and sustainable progress in Pakistan.To address these difficulties, numerous investigations have been conducted to investigate the molecular architecture and devising breeding techniques tailored for drought resistance. Markers linked to genes controlling specific traits of interest can be employed in the selection of genotypes within a breeding program. Therefore, the primary objective of the current study was to assess the performance of rice genotypes based on morphological attributes and potential to tolerate drought stress at molecular level (Manickavelu at al., 2006).

Material and Methods

Plant Material and Experimental Site:

Present study is conducted on 30 diverse rice genotypes collected from the Plant Genetic Resources Institute, National Agricultural Research Center (NARC). These accessions were chosen randomly and represented a mix of both the *indica* and *japonica* subspecies. The genotypes, encompassing basmati lines, GSR, coarse, and fine lines, underwent a rigorous screening process within the field and laboratory facilities of the National Agriculture Research Center in Islamabad during the calendar year spanning 2021 to 2022. Subsequently, these genotypes were cultivated, and seedlings aged approximately 30 days were transplanted into a rice field under controlled isolation conditions. Six plants were cultivated for each accession, with a uniform spacing of 20 centimeters between plants and rows. Data pertaining to various parameters were gathered from five leaves on each plant, and subsequently, the average values were computed.

Data Collection and Statistical Analysis:

Data for morphological parameters such as Days to Heading (DH), Days to Maturity (DM), Number of Tillers per Plant (TP), Panicle Length (PL), Grains per Panicle (GPP), 1000 Grain Weight (TGW), Grain Yield (GY), Insect/Pest resistance was recorded and evaluated. Analysis of Variance (ANOVA) and Least Significant Difference (LSD) tests were carried out, following the methodology detailed in Steel and Torrie's 1980 publication. This method was employed to evaluate morphological parameters for all genotypes, utilizing Statistix 8.1 software. The aim was to determine whether statistically significant variations exist in the means of different genotypes when exposed to the same conditions.

DNA Extraction:

DNA of all genotypes was extracted from fresh leaves (3 leaf stage) with the help of GeneJET Plant Genomic DNA Purification Mini Kit as per the standard protocol provided by Thermo Fisher Scientific Baltics UAB V.A. Graiciuno 8, LT-02241 Vilnius, Lithuania. DNA was also extracted through Cetyltrimethyl Ammonium Bromide (CTAB) based method following the protocol of Doyle and Doyle (1990) with slight modifications. Required reagents were prepared. 2-3 young rice leaves were ground into a fine powder in pestle and mortar using liquid nitrogen and transferred to a 1.5ml eppendorf tube. The powdered frozen material was gently mixed

several times with pre-heated 2XCTAB extraction buffer (0.1%v/v of β-mercaptoethanol added) and incubated at 65^0C for 45 minutes in water bath. The content was mixed 2-3 during incubation. After the process of incubation, 700µL of Chloroform-isoamylalcohol (24:1 v/v) was added into the tubes and all the content was mixed gently to form an emulsion. The tubes were transferred in refrigerated centrifuge machine (Hermle Labortechnik GmbH, Germany) for 10 minutes at 10000 rpm at room temperature and transferred the supernatant (aqueous layer) to a new eppendorf tube. In the supernatant an equal volume of chilled 2-propanol was added to precipitate the DNA. The tubes were centrifuged for 10 miutes at 10000 rpm at 4^0C. The supernatant was decanted and pellet was washed twice with 70% chilled ethanol. Pellet was dried thoroughly and dissolved in 500 µL of nuclease free water. After that the suspension was transferred to a new 1.5ml eppendorf tube and 5µl of RNAse A was added to digest the RNA. The tubes were incubated at 37^0C for an hour. 1 volume of chloroform isoamyl alcohol was added and centrifuged for 10 minutes at 13000rpm for phase separation. The supernatant was transferred to a new 1.5ml eppendorf tube and mixed gently by adding 0.1 volume of 3M NaCl. To the tubes 2 volumes of chilled ethanol were added in order to precipitate the DNA and centrifuged for 10 minutes at 13000rpm at 4^0C. The supernatant was discarded and pellet was washed with 70% chilled ethanol. Dried pellet was dissolved in 100µl of nuclease free water. DNA was quantified with the help of Qubit™ 4 Flourometer (Invitrogen by Thermo fisher Scientific) as per the protocol provided by the manufacturer.

Polymerase Chain Reaction:

The polymerase chain reaction was performed on all the genotypes in Bio-Rad T100™ Thermal Cycler using primer pairs. PCR was performed on 25µl reaction mixture including, 2X Dream Master Mix 12.5µl, forward and reverse primers 1.5µl each, template DNA 3µl and ddH_2O 6.5µl. PCR procedure was adopted as 95^0C for 5 minutes, followed by 35 cycles included reaction at 95^0C for 30s, reaction at 53^0C-64^0C for 30s (depending on melting temperature of each primer) and initial extension reaction at 72^0C for 30s (depending on the size of target sequence); with subsequent last extension at 72^0C for 10 minute (Babar *et al*., 2022).The amplified products were preserved at -20°C for future utilization in gel electrophoresis and subsequent gel scanning.

Gel electrophoresis, Documentation and Allele Scoring:

Amplified PCR products were resolved based on their size in 2% agarose gel at 80V for 1.5 hours. For this purpose, a gel was prepared using 1xTAE (Tris base, acetic acid, ethylene diamine tetraacetic acid [EDTA]) buffer. This gel was employed to visualize distinct bands of each genotype, which were stained with ethidium bromide in the horizontal electrophoresis tank. The photograph was digitally documented in Gel Documentation system (Cleaver Scientific Ltd.). The size of the amplicon was measured in base pair by comparing with the ready to use Gene Ruler 100bp DNA Ladder (Thermofischer Scientific). Staining the agarose gels with ethidium bromide revealed distinct single bands. The size of the amplified band for the microsatellite marker was determined by comparing it to a 100 bp DNA ladder. Qualitative scoring was performed for the presence or absence of the marker allele in the amplified product from microsatellite analysis. The SSR band, generated using the primer, was treated as a discrete unit character, with a score of 1 indicating its presence and 0 indicating its absence or when an observation was missing for each genotype. In cases where an amplification product could not be detected, an accession was assigned a null allele for the respective microsatellite locus.

Designing SSR Primer Pair:

SSR primer pair was chosen for the evaluation of rice genotypes based on yield potential and drought tolerance, relying on the established framework map of rice microsatellites. Marker linked to drought resistance gene was selected on the basis of previous research conducted by Dhawan et al., 2021, Bhattarai et al., 2018 and Solis et al., 2018 . The qDTY1.1 linked simple sequence repeat (SSR) marker (F: tcctgcgaactgaagagttg; R: agagcaaaaccctggttcac) is present on Chromosome number 1 (~250bp).

Results and discussion

In the current research, ANOVA results showed highly significant variability among the different rice genotypes for all the traits as days to heading, days to maturity, plant height, panicle length, number of grains per panicle and yield except for number of tillers per plant and 1000 grain weight (Table 1).

Table 1: Mean Square Values, CV and LSD of Various Traits of Rice Genotypes from ANOVA result

Trait	Mean square	F value	P Value	CV	LSD
Days to Heading	51.5847 **	2.61	0.0009	4.36	7.27
Days to Maturity	24.1585*	2.17	0.0061	2.36	5.46
Plant Height	62.4459 **	2.42	0.0021	4.83	8.31
No. of Tillers	2.51265 ns	0.60	0.9328	19.85	3.35
Panicle Length	12.0402 *	2.37	0.0026	16.98	3.69
Grains per Panicle	766.484 *	2.34	0.0029	13.67	29.57
1000 Grain Weight	14.3270 ns	0.91	0.6044	16.31	6.50
Grain Yield	5.18025 **	7.65	0.0000	9.87	1.35

Highly significant difference (**) Significant difference (*) Non significant difference (ns)

Days to Heading:

The calculation of Days to Heading(DH) involves counting the days from the sowing date until the point when 50% of the plants in the plot display a fully open flower. The timing of flowering in rice is influenced by a combination of genetic and environmental factors. Various factors can impact the onset of flowering in rice, such as planting younger seedlings, reducing the spacing between plants, using conventional irrigation methods, and employing traditional weeding practices, all of which can lead to earlier flowering (Ranawake et al., 2014).

The average duration for days to flowering varied between 95 and 111 days across all genotypes, with a combined mean of 102.02 days. Among the genotypes, NR422 exhibited the longest time to flowering, with an average of 110 days, followed by NR421 and NR425, which had averages

of 108 days in all three replicates. In contrast, a shorter duration to flowering was observed in NR404, NR405, and NR406, taking only 95, 95, and 96 days, respectively (Fig 1).

Days to maturity:

Days to Maturity (DM) is determined by measuring the number of days from the sowing date until 50% of the panicles had transitioned from green to their mature color. It is a crucial trait in the cultivation of rice (Abdul et al., 2012).

The mean time required for achieving maturity ranged from 136.5 to 146.5 days among all genotypes, with an overall average of 141.44 days. Among these genotypes, NR421 and NR 422 displayed the lengthiest time to reach maturity, with an average of 146.5 days, while NR425 followed closely behind with average of 145.83 days across all three replicates. Conversely, a quicker maturation period was observed in NR404, NR405, and NR406, which only required 136.5, 137.0, and 137.08 days, respectively (Fig 1).

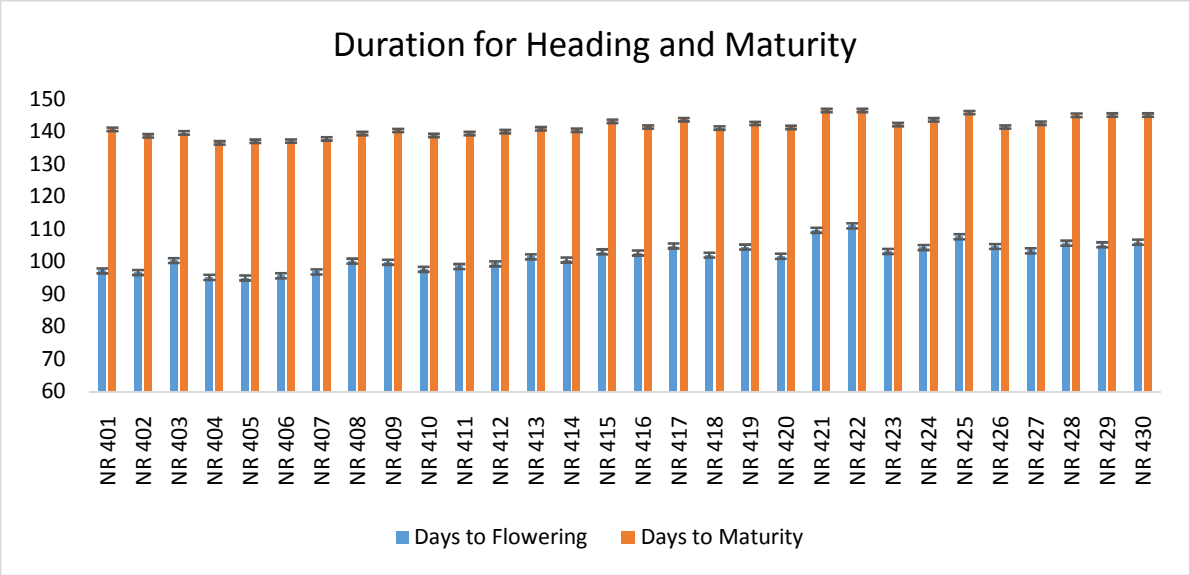

Figure 1: Performance of Rice Genotypes for Days to Heading and Days to Maturity

Plant height:

The average plant height spanned from 97.62 cm to 112.65 cm across all varieties, with an overall mean of 105.28 cm. The lowest plant height was recorded in NR 414, measuring 97.62 cm (Fig 2). In the LSD analysis, the critical value for genotype comparisons was 8.31 (Table 1). It's worth noting that rice plants exhibit a wide range of sizes, from dwarf mutants as short as 3

to 4 meters to floating varieties that can exceed 7 meters in height. Most commercial rice types typically fall within the range of 1 to 2 meters in height (Dhungana et al., 2022).

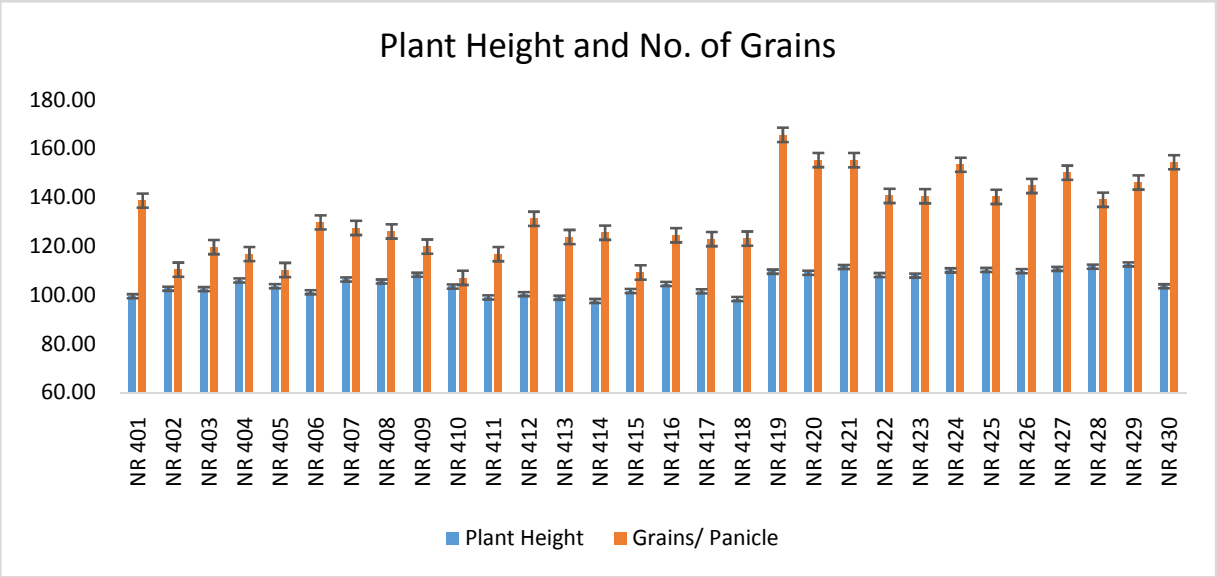

Figure 2: Variation in terms of Plant Height and No. of Grains per Panicle in diverse rice genotypes

Number of tillers per plant:

The formation of grain yield in rice is significantly influenced by the quantity of productive tillers per plant (Yan et al., 1998).Therefore, such traits can be regarded as selection criteria for achieving higher yields, given their mutual and direct association with crop productivity (Babu et al., 2012).

Statistical analysis for number of tillers per plant showed non-significant variation. In this study, the mean number of tiller per plant was observed to range between 8 and 13, encompassing all the different plant varieties. The overall average tillers per plant recorded was 10.317. NR 420 showed maximum tillers per plant (12.13) followed by NR 410 (11.97) and NR 407 (11.74). Minimum tillers per plant were observed in NR 403 (8.36) followed by NR 419 (8.90) and NR 409 (8.91) (Fig 3).

Panicle Length:

Panicle length is a component of panicle structure often assessed in relation to yield (Ahmed et al., 2016).The length of the panicle, along with factors such as spikelet number, spikelet density, seed setting rate, and grain plumpness, collectively influences the total grain count per panicle, consequently contributing to increased rice yields (Liu et al., 2016).

The average panicle length ranged from 10 cm to 16 cm. The combined mean for panicle length across all genotypes was 13.28cm. Among the tested varieties, the lowest panicle length were recorded for NR 403 at 10.54cm, followed by NR 409 at 10.68 and NR 415 at 10.73cm. Conversely, NR 420 exhibited the highest panicle length at 16.89cm, followed by NR 427 with 16.44cm and NR 425 with 16.11cm length of panicle (Fig 3). In the context of LSD analysis for genotype comparisons, the critical value was determined to be 3.68 (Table 1).

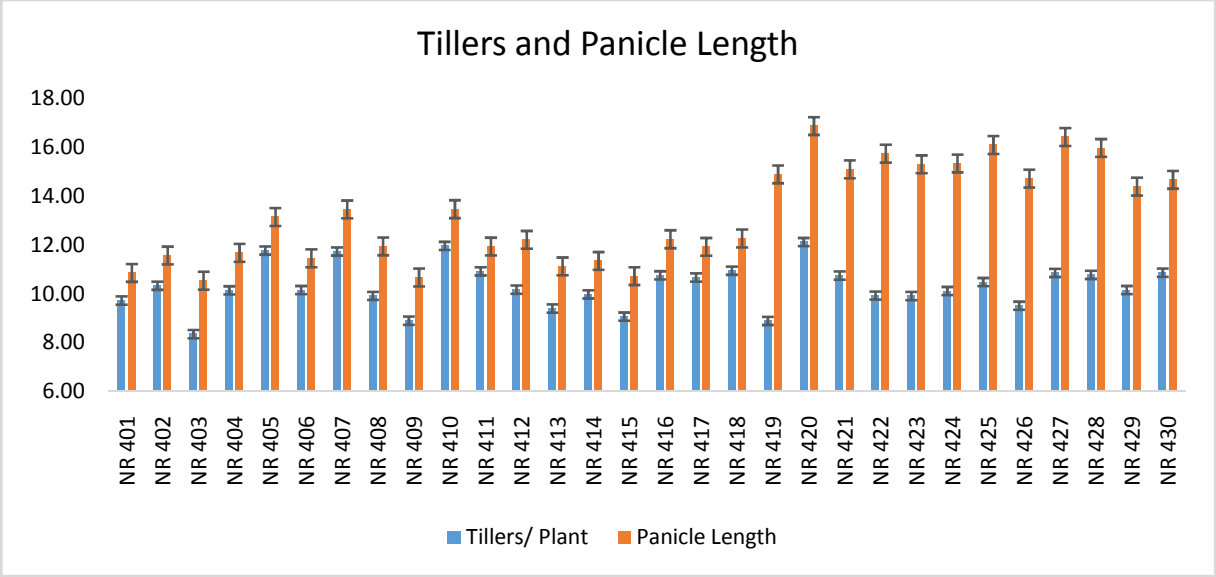

Figure 3: No. of Tillers per plant and panicle length of 30 Rice genotypes

Number of Grains:

The quantity of filled grains per panicle was identified as the most critical factor, exhibiting a robust positive impact on yield (Luzikihupi, 1998).

Statistical analysis of the number of grains per panicle revealed significant variability. Within this study, the mean number of grains per panicle ranged from 107 to 165 across all the diverse plant varieties. The overall average number of grains per panicle was calculated at 132.38. Notably, NR 410 displayed the lowestnumber of grains per panicle (107.13), followed closely by NR 415 (109.33) and NR 405 (110.37). Conversely, the highestnumber of grains per panicle were observed in NR 419 (165.74), NR 420 (155.41), and NR 421 (155.38) (Fig 2). In the LSD analysis conducted for genotype comparisons, the critical value was determined to be 29.56 (Table 1).

1000 grain weight (TGW):

The weight of grains constitutes a significant portion of the total yield (Luz et al., 2016).Statistical analysis for 1000 Grain Weight showed non-significant variation.The average 1000 grain weight spanned from 19.01g to 30.65g across all varieties, with an overall mean of 24.366g. The lowest TGW was recorded in NR 418 (19.01) followed by NR 423 and NR 427 weighing 20.58g and 21.82g respectively. While, highest TGW was observed in NR 424 (30.65) followed by NR 421 and NR 419 weighing 26.80g and 26.60g respectively (Fig 4).

Grain Yield (T/Ha):

The crop yield is determined by the interaction of genetic composition, physiological mechanisms, and agronomic characteristics, and any imbalance in these factors can hinder crop productivity. The paramount focus in rice breeding programs is the yield per hectare. However, yield is a multifaceted trait in terms of inheritance and often encompasses several interconnected components. Rice yield is a culmination of factors such as the number of panicles per unit area, the number of spikelets per panicle, the proportion of filled grains, and the weight of 1000 grains (Xing & Zhang, 2010).

The grain yield varied significantly in the study, ranging from 5.35 T/Ha to 11.61 T/Ha across all varieties. On average, it was 8.33 T/Ha. The variety NR 409 had the lowest grain yield at 5.35 T/Ha, followed by NR 404 and NR 412 at 5.87 T/Ha and 6.43 T/Ha, respectively. Conversely, the highest grain yield was observed in NR 420 at 11.61 T/Ha, followed by NR 429 and NR 421 at 10.79 T/Ha and 10.34 T/Ha, respectively (Fig 4). The LSD analysis indicated a critical value of 1.34 for genotype comparisons, emphasizing the significant variation in grain yield based on statistical analysis (Table 1).

There was a remarkably wide range of variation observed in the examined traits, with a particularly pronounced difference in no. of tillers per plant and panicle length. This underlines the substantial diversity within the studied accessions, while the variation in the other parameters ranged from 2.36% to 16.31%.

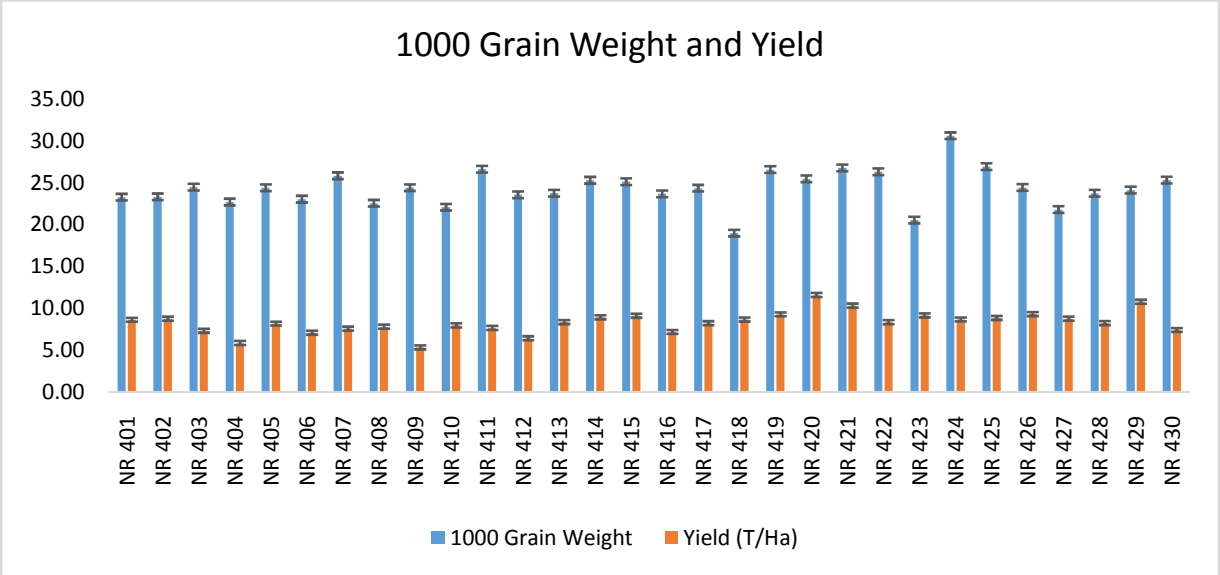

Figure 4: Evaluation of 3o Rice Genotypes for 1000 grain weight and grain yield

Insect/Pest resistance:

In rice crop insects and pests can cause significant damage, leading to reduced yields and economic losses for farmers. There are several insect pests that commonly affect rice plants, and the extent of damage they cause can vary depending on factors such as pest population, environmental conditions, and pest management practices(Buffon et al., 2018).

Stem borers are among the most damaging pests of rice. Larvae of various moth species bore into rice stems and feed on the inner tissues, weakening the plant and causing it to lodge (fall over). Common species include the Yellow Stem Borer (*Scirpophaga incertulas*) and the Striped Stem Borer (*Chilo suppressalis*) (Singh and Tiwari 2019).In the present study, all the genotypes were found to exhibit resistance response towards devastating stem borers. It has been found that yield has not been affected by the insect stress. Out of 30 rice genotypes screened for their resistance against stem borer, 13 genotypes were categorized as resistant while 17 genotypes showed moderate resistance (Table 2). This type of resistance indicates that genetically the genotypes are able to combat the stem borers if they attack the crop.

The rice leaf folder (RLF), known as *Cnaphalocrocis medinalis*, is considered one of the most destructive pests in Asian rice-growing regions. Initially regarded as a minor threat, the rice leaf folder's presence escalated notably in the late 1980s. As a consequence of this widespread epidemic of rice leaf folders, rice crop yields have suffered substantial losses, ranging from 30% to 80%(Bodlah et al., 2017).In our recent investigation, we observed that all the tested rice

genotypes displayed a robust resistance to the destructive leaf folder pest. Interestingly, it was noted that the presence of these leaf folders did not result in any discernible impact on crop yields. Among the 30 different rice genotypes subjected to screening for their resistance to leaf folders, a notable outcome emerged: nine genotypes were classified as highly resistant, while the remaining genotypes exhibited a level of moderate resistance (Table 2). This observed resistance suggests that, from a genetic standpoint, these rice genotypes possess the inherent ability to effectively counteract leaf folder infestations, should such infestations occur within the crop. This resilience in the face of a significant pest threat underscores the potential value of these genotypes in promoting rice crop health and productivity.

Table 2: Genetic potential of Diverse Rice genotypes against leaf folder and stem borer

Genotypes	Leaf Folder	Stem Borer	Genotypes	Leaf Folder	Stem Borer
NR 401	MR	MR	NR 416	MR	MR
NR 402	MR	R	NR 417	MR	R
NR 403	R	R	NR 418	R	MR
NR 404	MR	MR	NR 419	R	MR
NR 405	R	MR	NR 420	MR	MR
NR 406	MR	R	NR 421	MR	R
NR 407	MR	R	NR 422	R	MR
NR 408	MR	R	NR 423	MR	R
NR 409	R	MR	NR 424	MR	R
NR 410	MR	R	NR 425	R	MR
NR 411	MR	MR	NR 426	R	MR
NR 412	MR	MR	NR 427	MR	R
NR 413	MR	R	NR 428	R	MR
NR 414	MR	R	NR 429	MR	MR
NR 415	MR	MR	NR 430	MR	MR

Correlation Matrix:

In the light of correlation studies of the recorded data, days to flowering showed significant and positive correlation with days to maturity, grains per panicle, plant height and panicle length. But the magnitude of correlation was not strong for plant height. However, days to flowering are non-significantly correlated to thousand grain weight and grain yield. A negative and non-significant correlation was observed between days to flowering and number of tillers per plant.Days to maturity was found to be positively and significantly correlated to grains per panicle, plant height and panicle length. However, a less strong and significant correlation was

observed between days to maturity and grain yield. An insignificant correlation was found between days to maturity and thousand grain weight. Moreover, days to maturity were negatively and non-significantly correlated to number of tillers per plant (Table 3).

Grains per panicle showed positive and significant correlation toplant height and panicle length. A significant and positive correlation was found between grains per panicle and grain yield. Therefore, from the recorded data it can be inferred that a genotype with greater number of grains per panicle would have higher grain yield. However, grains per panicle were found to be non- significantly correlated to thousand grain weight. Also, grains per panicle were found to be negatively and non-significantly correlated to number of tillers per plant (Table 3).

Plant height was found to be only positively and significantly correlated to panicle length. It showed non-significant correlation to thousand grain weight, number of tillers per plant and grain yield. Panicle length was less significantly correlated to grain yield which indicates that more the length of panicle lesser will be its yield. However, panicle length was non-significantly correlated with thousand grain weight and number of tillers per plant.Thousand grain weight was found to be non-significantly correlated with number of tillers per plant and grain yield. Also, number of tillers per plant were non-significantly correlated to grain yield as observed in the recorded data (Table 3).

Table 3: Correlation Matrix for the morphological parameters of Rice Genotypes under observation

Correlations (Pearson)

	DTF	DTM	GPP	PH	PL	TGW	TP
DTM	0.9525						
GPP	0.6208	0.6182					
PH	0.5213	0.5013	0.6219				
PL	0.6083	0.5838	0.7397	0.7708			
TGW	0.3586	0.3577	0.3612	0.2957	0.2615		
TP	-0.1289	-0.1008	-0.0283	0.0545	0.3998	-0.0764	
Yld	0.4573	0.4750	0.5004	0.3469	0.5421	0.1670	0.1973

Cases Included 30 Missing Cases 0

Marker Assisted Selection of Drought Tolerance Potential:

Molecular markers serve as invaluable tool for enhancing drought stress tolerance across rice varieties.In this study Qdty1.1 SSR marker was used to assess the genetic potential of rice genotypes at seedling stage for drought stress tolerance. Out of 30 rice genotypes, drought resistant gene was amplified only in 14 lines (Table 4). In lines NR401-NR410, NR414, NR415, NR416 and NR426 the marker linked gene was amplified which indicates that these lines are potential genotypes against drought stress. The linked genes bands were found to be around 250 base pairs as 1Kb ladder is used for comparison (Fig 5).

Table 4: Identification of drought tolerant genotypes through MAS aided by Qdty1.1 SSR marker

Genotypes	Qdty1.1	Genotypes	Qdty1.1	Genotypes	Qdty1.1
NR 401	1	NR 411	0	NR 421	0
NR 402	1	NR 412	0	NR 422	0
NR 403	1	NR 413	0	NR 423	0
NR 404	1	NR 414	1	NR 424	0
NR 405	1	NR 415	1	NR 425	0
NR 406	1	NR 416	1	NR 426	1
NR 407	1	NR 417	0	NR 427	0
NR 408	1	NR 418	0	NR 428	0
NR 409	1	NR 419	0	NR 429	0
NR 410	1	NR 420	0	NR 430	0

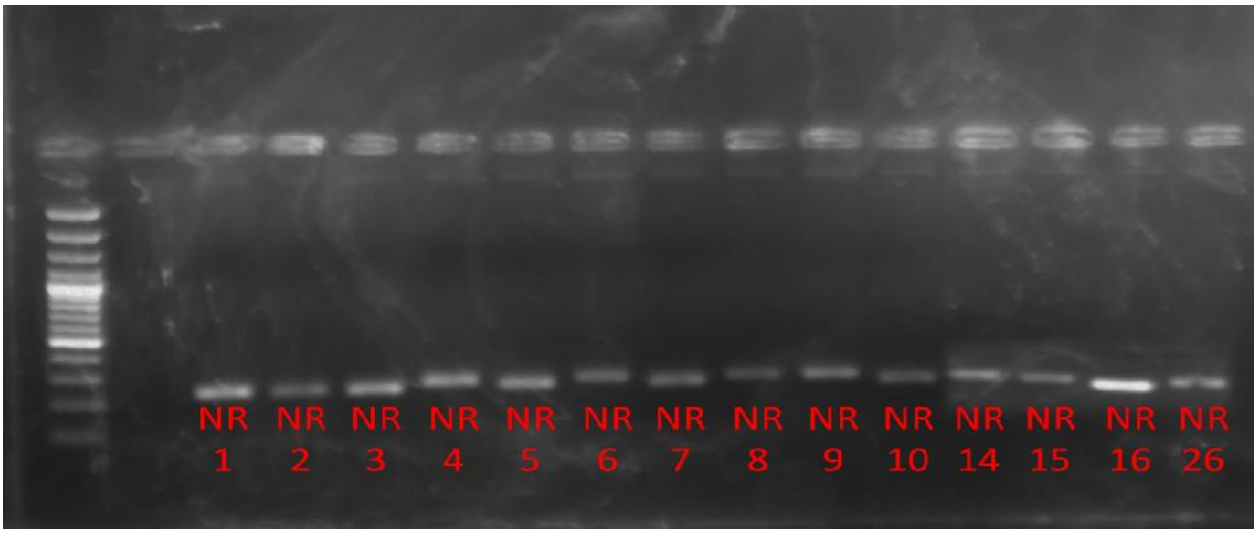

Figure 5: Genotypes found to have amplified genes for drought tolerance qDTY1.1

With the help of DNA based molecular marker screening, drought susceptible and drought tolerant genotypes have been revealed. Therefore, Qdty1.1 is considered as powerful tool for assessing genetic potential within and among different rice germplasm lines and this screening is devoid of any environmental influence. Utilizing diverse molecular markers, numerous investigations have identified QTLs that control drought tolerance in rice, primarily relying on grain yield under drought stress (DTY) as an indirect trait. qDTY1.1 is one of them mapped on Chromosome 1 and is shown to influence drought associated traits such as osmotic adjustment, biomass, root thickness, relative water content and root length (Dhawan et al., 2021).

Microsatellite or SSR markers are renowned for their higher diversity compared to other molecular markers when it comes to distinguishing between different rice genotypes. They produce distinctive SSR profiles in rice and find extensive application in various endeavors, including genome mapping, association mapping, chromosomal research, assessment of allelic diversity, genotype identification, phenotype mapping, evaluation of varietal diversity, and aiding marker-assistedselection(Ashfaqetal.,2014).

Comparable studies involving the screening and precise mapping of rice genotypes utilizing SSR markers were documented in the works of Lang and Buu (2008), Zhang et al. (2009), Kanagaraj et al. (2010), Gomez et al. (2010) and Ashfaq et al. (2014). Both molecular markers and phenotypic traits assume a crucial role in the assessment of rice genotypes under drought conditions. Breeding rice varieties with drought tolerance and other desirable root traits poses significant challenges. Firstly, this difficulty arises from the laborious and time-consuming process of screening numerous genotypes in field conditions, compounded by the lack of readily available and efficient screening techniques. Secondly, the non-uniform and inconsistent distribution of soil compaction across rice fields makes it challenging to evaluate root length and the ratio of dry roots accurately.

To address these challenges and develop new rice varieties with improved traits, molecular marker technology presents itself as a promising alternative strategy. Drought tolerance traits are inherently quantitative, necessitating the dissection of these complex characteristics into their underlying genetic components as a prerequisite for trait manipulation (Manickavelu et al., 2006). Enhancements in the management of drought screening and the selection process for grain yield under drought conditions have played a pivotal role in advancing drought breeding programs (Swamy and Kumar 2013).

Conclusion:

A study was conducted at Rice Research Program, NARC Islamabad, to assess 30 rice genotypes for yield contributing parameters and drought tolerance potential. The research followed a randomized complete block design with three replications. Data on various traits, including plant height, panicle length, effective tillers per plant, fertile grain number per panicle, and grain yield, were collected and found all showing significant differences except for no. of tillers and 1000 grains weight. Rice, a crucial staple crop, faces significant challenges in lowland rice ecosystems due to the adverse impact of drought. Examining the key traits and genomic architecture that play a role in enhancing drought tolerance and improving grain yield is essential. This research effort can provide valuable insights to breeders, allowing them to gain a deeper understanding of the genetic potential of rice genotypes to withstand drought stress, ultimately facilitating the development of drought-resistant rice varieties.Consequently, based on the comprehensive evaluation of yield across different conditions, three outstanding genotypes, NR 420, Nr 429 & NR 421 were identified as highest yield but was not having resistant gene for drought. For the selection in terms of drought tolerance NR 426, NR 415 & NR 414 can be picked as these are yielding above average and have genetic potential to withstand drought stress. These genotypes hold promise as valuable breeding materials suitable for suitable environments in Pakistan.

Authors Contribution

Qurat ul Ain Sani and Rana Arsalan Javaid conducted the research activity, performed the lab activities including DNA Extraction and PCR performance and drafted the manuscript.Abid Majeed and Muhammad Shahzad Ahmed organized the field and lab activities respectively, reviewed paper and provided the supportive environment. Muhammad Jalal Hassan and Afia Gul helped in Lab activities including DNA extraction and gel electrophoresis. Mahsal Rehman, Faiza Siddique and Shafiq Qalandraniparticipated in data recordingand field activities. Muhammad Arshad supervised the research and did proof reading of the paper.

Conflict of Interest

“The authors declare no conflict of interest”

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