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Estimation of drought resilience potential of rice genotypes: A PEG-based evaluation

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Abstract

Rice is a crucial global staple food grown in various climates. However, the increasing scarcity of freshwater resources poses a significant challenge to rice production. Drought, a consequence of climate change, is a main problem to rice yield, affecting its yield significantly. This study evaluated drought tolerance in 25 rice genotypes in a research trial AYT-3 using various concentrations of Polyethylene Glycol (PEG) as an osmotic stress inducer. Results indicated significant variations in drought tolerance index among the genotypes. Notably, AYT 3-9 exhibited the highest root length stress tolerance index (RLSTI), AYT 3-22 displayed the highest shoot length stress tolerance index (SLSTI), and AYT 3-24 showed the highest plant fresh weight stress tolerance index (PFWSTI). Root properties and growth were essential for drought resilience, with deep and extensive root systems contributing to tolerance. However, leaf growth was reduced due to restricted water potential under drought stress. While germination stress tolerance index (GSTI) did not vary significantly, the study highlighted the importance of early germination evaluation in drought tolerance assessment. Genotypes resilient to 15% PEG concentration may be suitable for breeding programs to develop drought-tolerant rice cultivars. This research emphasized the potential of early screening techniques to select genotypes with superior drought tolerance, essential for ensuring food security in regions vulnerable to water scarcity.

Keywords: Abiotic stresses, Drought, Evaluation, Polyethylene glycol, Tolerance

Abbreviations: PEG = Polyethylene glycol; RLSI = Root length stress tolerance index; SLSI = Shoot length stress tolerance index; PFWSI = Plant fresh weight salt tolerance index; PDWSI = Plant dry weight salt tolerance index; GSTI = Germination stress tolerance index (GSTI); AYT = Advance yield trial; ANOVA = Analysis of variance

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Introduction

The second-largest staple food in the world, rice (Oryza sativa L.), is usually cultivated in both tropical and temperate climates (Ibrahim et al., 2016; Kumar et al., 2017; Ali et al., 2019). Rice (Oryza sativa L.), a "semi-aquatic plant", is typically grown in water. The scarcity of freshwater resources has become a significant limiting factor in rice production because of the decline in the supply of fresh water and the rise in water use in agriculture (Xu, Q et al., 2020). Due to its unique qualities, including its long grain, aroma, and amylose quantity, aromatic rice is well-known throughout Asia, Europe, and the United States (Ahmad et al., 2005). Over 163.3 million hectares of rice are farmed in more than a hundred countries as a multi-product commodity (GRISP, 2013). According to Bufon et al. (2018), For over 50% of the global population, rice is a staple food, and Simova-Stoilova et al. (2008) found that water restrictions in nature and global environmental change have a substantial impact on the harvest yield of rice. In comparison to other crops, rice output requires a lot of water, and about 30.9% of the rice cultivated worldwide is produced using rain-fed agriculture (Dixit et al., 2014). The current and predicted global food shortages demand a significant increase in crop output in the less favorable rainfed regions. The main challenge to agriculture, particularly in establishing countries, is the change in climate, which affects the frequency and intensity of hydrological variations and results in a different abiotic stress for the plants (Turral et al., 2011).

Drought has a significant and substantial impact on the yield of rice crop in rainfed ecosystems among the abiotic variables that have impacted on the plant development (Nelson et al., 2014; Pandey & Shukla, 2015). A drought, as defined by Rollins et al. (2013), is a duration of low average precipitation, insufficient rain, or higher rates of evaporation that hinder crop growth and yield. The amount of soil moisture, evaporation, and rainfall frequency are just a few factors that determine how severe or intense drought happens to be. (Hao et al., 2018; Oladosu et al., 2019).

The main factors limiting crop output are abiotic factors, such as water scarcity, salinity, heat stress, metals stress, etc. (Du et al., 2013). According to Shiferaw et al. (2011), the single most important element in the world is water scarcity, which poses a severe danger to the security of the food supply. One of the main concerns to sustainable rice productivity under a climate change scenario is drought stress (Bellard et al., 2012). According to Jaleel et al. (2009) and Wilhite (2018), a period without much rainfall (a water scarcity) causes significant crop damage and a massive loss in yields. Drought is regarded as a natural occurrence. For example, Super basmati and IR-64 are both thought to be vulnerable to abiotic factors, mainly field-level drought stress and decreased yield (Sabar et al., 2019; Kumar et al., 2014).

Different physiological functions are adversely affected by the drought stress, and plants react to it in order to adjust to unfavorable conditions. Before initiating a program for breeding, it is essential to optimize the physiological processes and factors for the increase of yield in drought conditions (Dash et al., 2018; Gupta et al., 2020; Barik et al., 2019). Water scarcity has a negative impact on many physiological traits of rice, including net rate of photosynthesis, transportation rate, stomatal conductivity, efficiency of water utilization, intracellular carbon dioxide level, the photosystem II (PSII) activity, comparative amount of water, and the stability of the membrane index. (Farooq et al., 2009; Dash et al., 2018; Mishra et al., 2018; Zhu et al., 2020).

According to Simova-Stoilova et al. (2009), drought stress often shortens the life cycle of plants by reducing photosynthesis and accelerating the senescence process. These traits can be utilized to choose verities for the drought stress tolerance because seed sproutng and the early seedling development are important for developing a crop stand against environmental stress (Rana et al., 2017). Due to excessive ROS production, drought stress has inhibitory effects on plants that are partially caused by oxidative damage (Noctor et al., 2014). H_2O_2, OH, and singlet oxygen ($1O_2$) are examples of reactive oxygen species (Choudhury et al., 2017; Foyer & Shigeoka, 2011; Gill & Tuteja, 2010). Because they cause significant damage to proteins, lipids, pigments, and nucleic acids, ROS are extremely reactive by nature and affect normal cellular metabolism (Sharma et al., 2012). To reduce ROS-induced oxidative damage to organelles and cell membranes, plants have evolved a prominent antioxidant system (Foyer & Shigeoka, 2011). To support rice cultivars resistant to drought and those with high yield output, it is crucial to identify the genetic potential for drought tolerance in rice germplasm (Sahebi et al., 2018). Plant breeders create genotypes that are resistant to drought and can identify those (Todaka et al., 2015). They proposed that genotypes with potential for drought tolerance are useful for farming in regions with conditions of water scarcity (Kausar et al., 2012). PEG is a common osmotica used to induce osmotic stress, which prevents seed sprouting (Zafar et al., 2015). PEG is utilized to change the water potential because it has a larger molecular weight, is inert, nonionic, and impermeable (Mendhulkar & Nisha, 2015). The study aimed to evaluate drought-tolerant rice genotypes using effective screening techniques under drought using PEG (polyethylene glycol) to identify the rice lines with greater resistance to varying degrees of drought stress and to determine the negative impacts of dry conditions on the rice plants.

Materials and Methods

The research was carried out at the National Agriculture Research Center (NARC), Islamabad, Pakistan's Rice Molecular Laboratory, Crop Sciences Institute in 2023. The trial was conducted during June and July in Rice research program, NARC. Rice Research Program provided the seeds of 25 genotypes of AYT-3 used in this experiment mentioned in Table 1.

Table 1 Genotype used in the present study

S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes
1	AYT 3-1	6	AYT 3-6	11	AYT 3-11	16	AYT 3-16	21	AYT 3-21
2	AYT 3-2	7	AYT 3-7	12	AYT 3-12	17	AYT 3-17	22	AYT 3-22
3	AYT 3-3	8	AYT 3-8	13	AYT 3-13	18	AYT 3-18	23	AYT 3-23
4	AYT 3-4	9	AYT 3-9	14	AYT 3-14	19	AYT 3-19	24	AYT 3-24
5	AYT 3-5	10	AYT 3-10	15	AYT 3-15	20	AYT 3-20	25	AYT 3-25

These lines were eliminated after being exposed to four different Polyethylene Glycol 6000 (PEG-6000) concentrations during the germination and seedling stages described in Table 2.

Table 2 Different levels of drought stress

S. No.	Treatments
1	T0 = Control
2	T1= 10%PEG
3	T2 = 15% PEG
4	T3 = 20% PEG

Polyethylene Glycol 6000 (PEG-6000) at four different concentrations 0, 10%, 15%, and 20%, was tested on the 25 rice genotypes of AYT-3. The first element was 25 rice genotypes, while the second was four levels of drought stress (0, 10, 15 and 20%). The seeds were immersed in a 5% NaOCl solution for 5 minutes, and then they were washed three times with distilled water. Five seeds from each rice line were put into petri plates that included filter paper and subjected to the appropriate treatments. In treatment 1, distilled water was used, whereas for the other treatments, 5 mL of PEG solution was used two times per day for the first two days, and then one time daily for the remaining days. Each entry received the appropriate treatment and was then given time for sprouting at 25 ˚C. Up until full sprouting, the germination data was recorded every day. The data was collected on the tenth day of the experiment.

Studied parameters

The parameters that were examined are as follows: Root length stress tolerance index (RLSI) Shoot length stress tolerance index (SLSI), Plant fresh and dry weight tolerance Index, (PFWSI & PDWSI). These parameters were determined by using the formula given by Fernandez (1992).

$$\mathrm{RLSI} = {}^{\text{Root length of stress seedling}}\!/_{\text{Root length of non} - \text{stress seedling}} \times 100$$

$$\mathrm{SLSI} = {}^{\text{Shoot length of stress seedling}}\!/_{\text{Shoot length of non} - \text{stress seedling}} \times 100$$

$$\mathrm{PFWSI} = {}^{\text{Plant fresh weight of stress seedlings}}\!/_{\text{Plant fresh weight of non} - \text{stress seedling}} \times 100$$

$$\mathrm{PFWSI} = {}^{\text{Plant dry weight of stress seedlings}}\!/_{\text{Plant dry weight of non} - \text{stress seedling}} \times 100$$

Height of seedling, fresh and dry weight

Height of seedling was examined in centimeters by using a measuring scale and a digital assessing balance was used to determine the plant's fresh weight in grams (g). The plant was then dried for 24 hours at 70ºC.

Germination stress tolerance index

The germination stress tolerance index (GSTI) was calculated as a percentage, and promptness index was necessary for GSTI calculation. The following formula was used to calculate the promptness index. (Ashraf et al., 2008).

$$PI = (\mathrm{nd1} \times 1.0) + ((\mathrm{nd2} \times 0.75) + (\mathrm{nd3} \times 0.50) + (\mathrm{nd4} \times 0.25)$$

In contrast, PI stands for the promptness index, and nd1; number of seeds that germinated on day 1, nd2; number of seeds that germinated on day 2, nd3; number of seeds that germinated on day 3 and nd4; number of seeds that germinated on day 4 correspondingly.

$$\mathrm{GSTI} = {}^{\text{PI of stressed}}\!/_{\text{PI of Control}}$$

Vigor index

Sagar et al., (2018) states that the following procedure was used to calculate the vigor index (VI):

$$\mathrm{VI} = {}^{SL}\!/_{GE}$$

Where SL = Seedling length
GE = Rate of germination

Statistical analysis

The data were examined using analysis of variance (ANOVA) using statistical software Statistix 8.1 (Steel et al., 1997).

Results and Discussion

Root lengths stress tolerance index (RLSTI)

Induced drought stress significantly affected the root lengths stress tolerance index of 25 rice lines. (Table 3) Of all rice lines, AYT 3-9 showed the highest rate of RLSTI (159.07) at concentration of RLSTI 15%. In contrast, AYT 3-24 had the lowest RLSTI (46.02) at RLSTI 10%. At 10% concentration, the highest RLSI (144.5) was shown by AYT 3-25 closely followed by AYT 3-3 (141.57) and AYT 3-22 (139.17), minimum RLSTI was found in AYT 3-24 (46.02). At 15% concentration, AYT 3-9 (154.07), AYT 3-8 (149.80), and AYT 3-10 (147.64) kept maximum RLSTI while the minimum score obtained in AYT 3-13 (50.87). The root length stress tolerance index of 25 genotypes were shown in Fig. 1. Genotypes including AYT 3-9, AYT 3-25, AYT 3-3, and AYT 3-22 offer hope for breeding initiatives focused at enhancing rice crops' ability to withstand drought. In contrast, AYT 3-24 and AYT 3-13 would need more research or might not be the best options for places that experience drought stress. Root length of drought tolerant lines was noticed increased in drought tolerant lines. When a plant suffers from stress from drought, its root attributes are crucial for improving yields. The way a rice crop responds to water stress depends on the composition and growth of its root system. The dry root mass and length can be utilized to predict rice yield in the presence of water stress. The characteristics of root growth exhibit a wide range of responses when there is water stress (Comas et al., 2013). Manivannan et al. (2007) observed that rice roots grew longer in response to drought stress because their abscisic acid content raised. Rice cultivars with multiple and deep roots are more drought tolerant (Mishra et al., 2019; Kim et al., 2020).

Table 3 Mean square analysis of measured traits in rice genotypes under drought stress

Source	DW	FW	RL	SL	PI	GR	VI
G	0.00117	0.00347	0.749	0.567	1.179	116.7	0.00005
T	0.10772	0.30332	155.855	157.416	163.548	54382.7	0.0176
Error	0.00035	0.00087	0.713	0.299	0.885	77.1	0.00003
CV	21.06	18.92	22.66	15.03	25.56	12.73	14.35

* Significant at *p*<0.05; ** highly significant at *p*<0.01; *** very high significant at *p*<0.001 Abbreviations: SOV= Source of variance; RLSTI= Root length stress tolerance indices; SLSTI= Shoot length stress tolerance indices; PFWSTI= Plant fresh weight stress tolerance indices; PDWSTI= Plant dry weight stress tolerance indices; GSTI= Germination Stress tolerance index

Shoot lengths stress tolerance index (SLSTI)

Drought-stressed rice plants demonstrated significant (p≤0.001) results in SLSTI. The genotypes of rice differed in SLSTI at different levels (Table 3). Maximum SLSTI was examined in AYT 3-22 (120.52), AYT 3-24 (107.50), and AYT 3-19 (106.61) at 10% concentration and closely followed by AYT 3-10 (94.74), AYT 3-9 (94.46), AYT 3-23 (94.02) and AYT 3-20 (92.5), while the minimum score was obtained in AYT 3-16 (65.97). Under 15% concentration, the highest SLSTI (99.58) was shown by AYT 3-19 closely followed by AYT 3-10 (98.41) and AYT 3-9 (92.74), while minimum SLSTI was found in AYT 3-7 (45.23) and AYT 3-11 (41.58) (Fig. 2). Leaf growth is reduced under drought stress because of the limited water potential (Zhu et al., 2020). When the flow of water from one cell to another is disturbed, crops react by having poor cell development and decreased leaf area, as well as by having lower turgor pressure due to water scarcity. (Hussain et al., 2018).

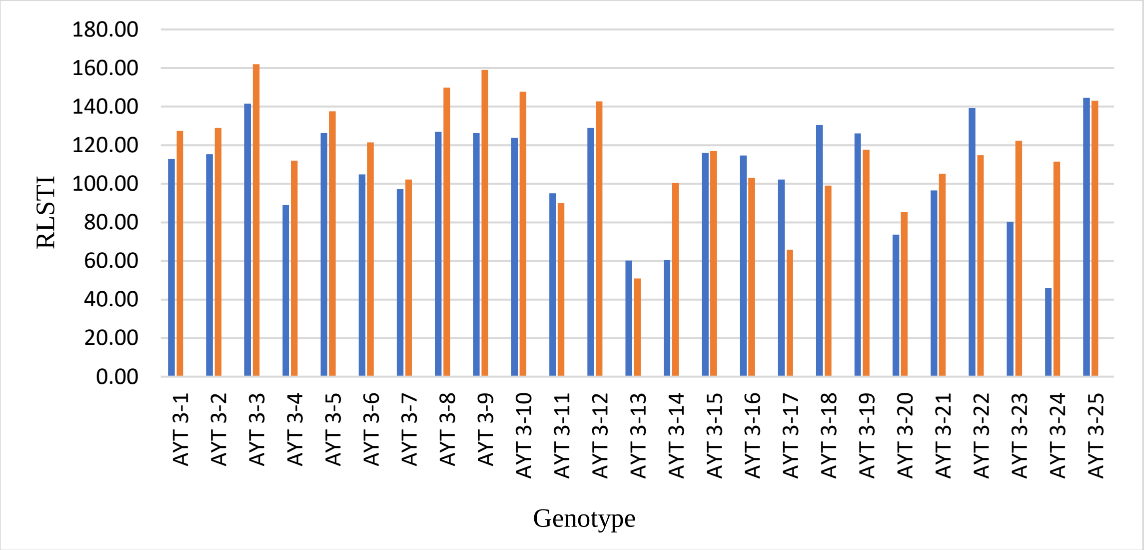

Fig. 1 Root lengths (cm) stress tolerance index (RLSTI) in 25 genotypes of rice

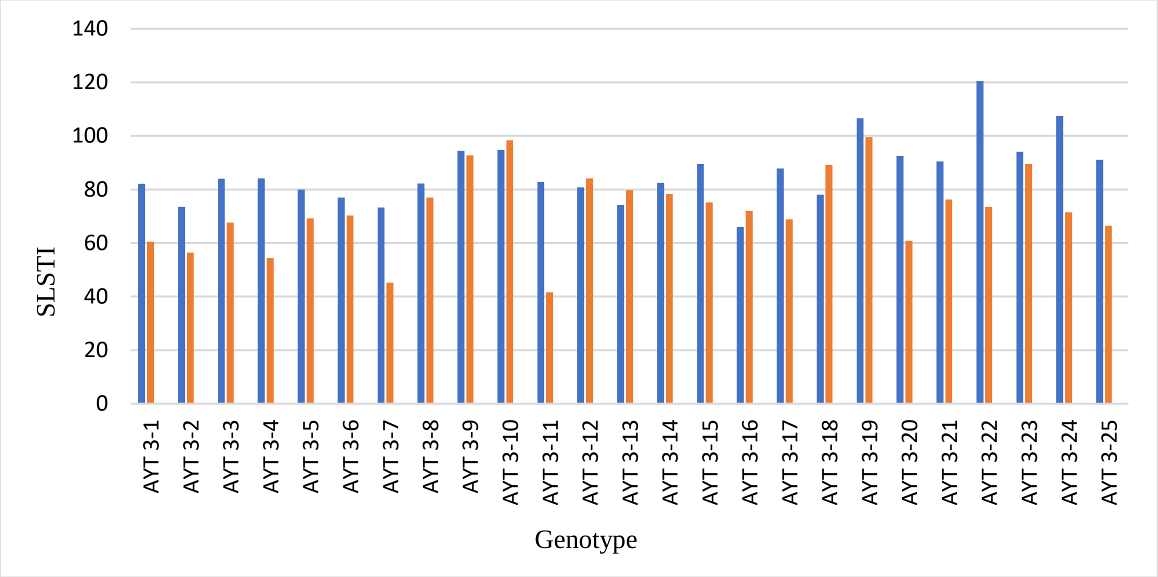

Fig. 2 Shoot lengths (cm) stress tolerance index (SLSTI) in 25 genotypes of rice

Plant fresh and dry weight stress tolerance index (PDWSTI and PFWSTI)

Significant results (p≤0.001) in PFWSTI were evident in rice plants raised under drought stress (Table 3). Under 10% concentration, AYT 3-24 (100), AYT 3-11 (95.45) and AYT 3-20 (92.59) maintained the highest PFWSTI, while minimum in AYT 3-21 (76), AYT 3-18 (68) and AYT 3-2 (66.67) and lowest values of PFWSI for AYT 3-9 (61.76) and AYT 3-8 (54.54) were measured. At 15% concentration, maximum value of PFWSTI was recorded for AYT 3-22 (92) and AYT 3-12 (85) and AYT 3-20 (81.48), while minimum in AYT 3-5 (50), AYT 3-2 (48.14) and AYT 3-4 (46.15) and lowest values of PFWSTI for AYT 3-1 (43.47) and AYT 3-6 (36.84) were measured (Fig. 3). In AYT3 22 and AYT3-12 observed a good response to the PEG. Significant results (p≤0.001) in PFWSTI were evident in rice plants raised under drought stress (Table 1). Under 10% concentration, AYT 3-6 (110), AYT 3-22 (107.7), AYT 3-24 (100), AYT 3-25 (100) and AYT 3-2 (100) maintained the maximum PDWSTI, while minimum in AYT 3-17 (64.3), AYT 3-14 (64.3) and AYT 3-9 (61.9) and lowest values of PDWSTI for AYT 3-15 (61.1) and AYT 3-8 (55) were measured. At 15% concentration, the highest value of PDWSTI was recorded for AYT 3-5 (87.5) and AYT 3-2 (85.7) and AYT 3-22 (76.9), while minimum in AYT 3-3 (46.7), AYT 3-9 (42.9) and AYT 3-18 (38.5) and lowest values of PDWSTI for AYT 3-14 (14.3) and AYT 3-6 (30) were measured (Fig. 4). Plant fresh and dry weight directly related to the PEG concentration (Farooq et al., 2009; Gómez-Luciano et al., 2012).

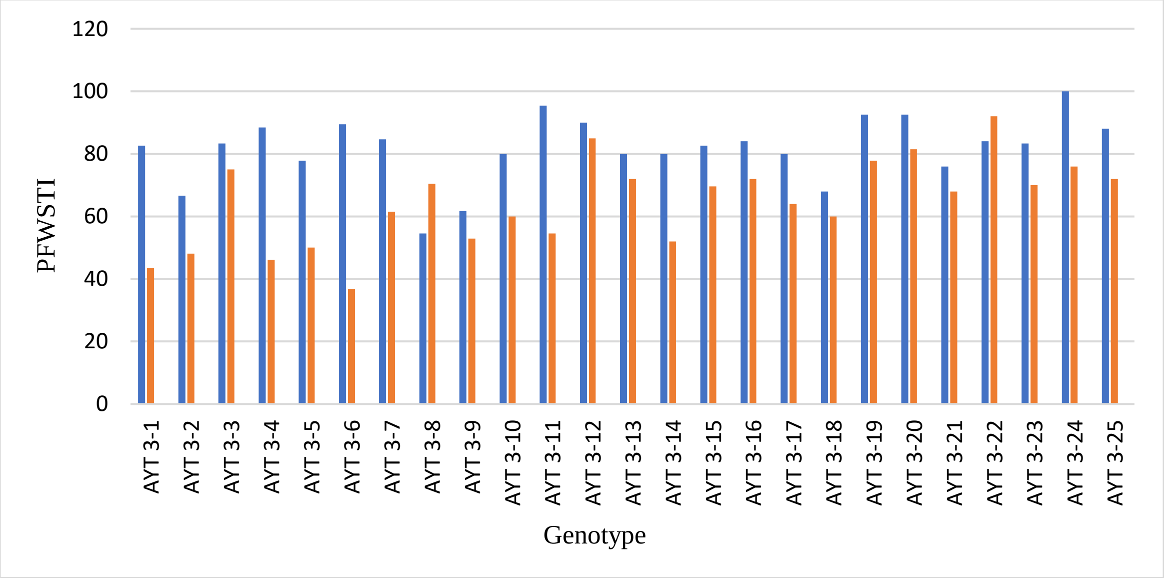

Fig. 3 Plant fresh weight stress tolerance index (PFWSTI) in 25 genotypes of rice

Germination stress tolerance index (GSTI)

Under drought stress, rice plants showed non-significant GSTI values (Table 3). Under 10% concentration, AYT 3-19, AYT 3-25 and AYT 3-23 maintained the maximum GSTI, while minimum in AYT 3-10 (0.47) were measured. At 15% concentration, the highest value of GSTI was recorded for AYT 3-13 (1.2), while minimum in AYT 3-20 (0.3) were recorded (Fig. 5). Germination is highly impacted by the drought stress. Germination percentage decreased with increased percentage of PEG. Germination percentage was 100% in all genotypes at 0% PEG. As PEG concentrations increase, less oxygen is present in the solution, causing the water potential to drop, for the seed to grow and begin to sprout (Purbajanti et al., 2019; Sagar et al., 2020).

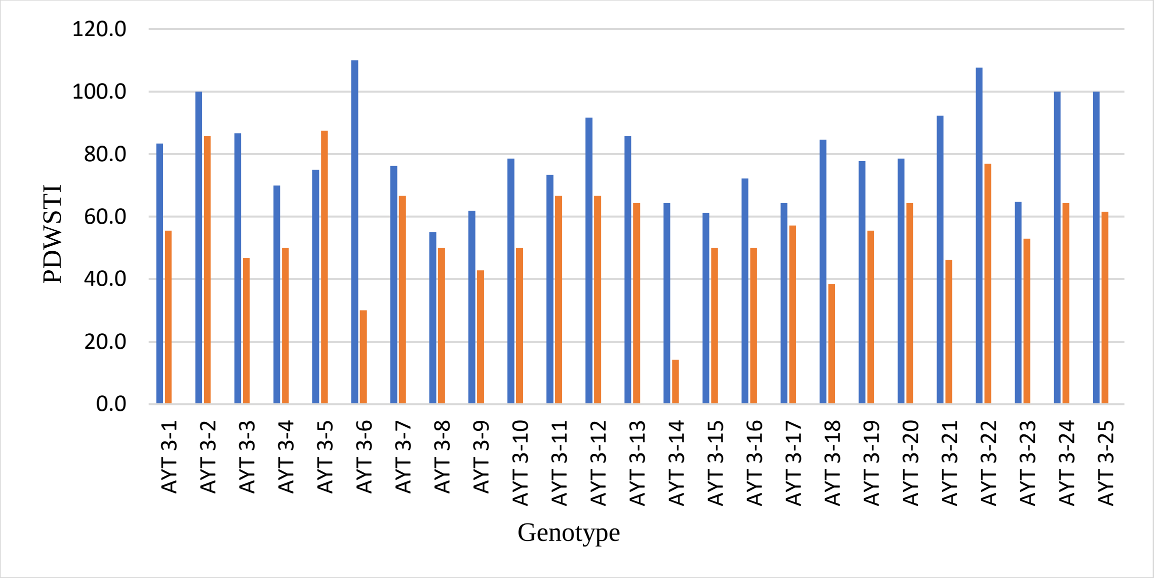

Fig. 4 Plant dry weight stress tolerance index (PDWSTI) in 25 genotypes of rice

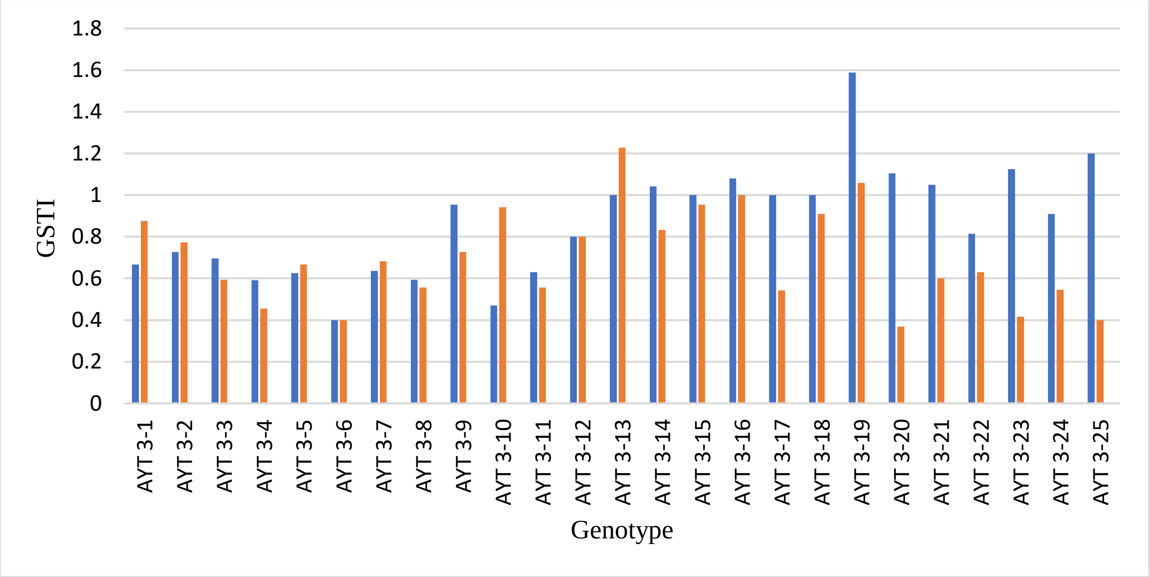

Fig. 5 Germination stress tolerance index (GSTI) in 25 genotypes of rice

Vigor index

Rice plants grown in drought stress showed notable improvements in the vigor index. In control, AYT 3-15 (0.068), AYT 3-16 (0.067) and AYT 3-18 (0.066) maintained the maximum vigor index, while minimum in AYT 3-23 (0.0402), AYT 3-20 (0.05) and AYT 3-9 (0.054) were measured. Under 10% concentration, AYT 3-15 (0.061), AYT 3-17 (0.061) and AYT 3-22 (0.064) maintained the highest vigor index, while minimum in AYT 3-23 (0.0378), AYT 3-16

(0.0442) and AYT 3-4 (0.0446) were measured. At 15% concentration, the maximum value of vigor index was recorded for AYT 3-6 (0.064) and AYT 3-17 (0.064), AYT 3-10 (0.062) and AYT 3-9 (0.062), while minimum in AYT 3-22 (0.039), AYT 3-11 (0.040) and AYT 3-25 (0.043) were measured (Fig. 6). According to Ashraf et al. (2002), seedlings, sprouting, development, and growth of seeds are all seriously threatened by drought stress (Almaghrabi & T.S. Abdelomoneim, 2012). According to Dhanda et al. (2004) seed vigor and seedling development are extremely vulnerable to lack of water condition. In this study, vigor index decreased with the increased in PEG concentration.

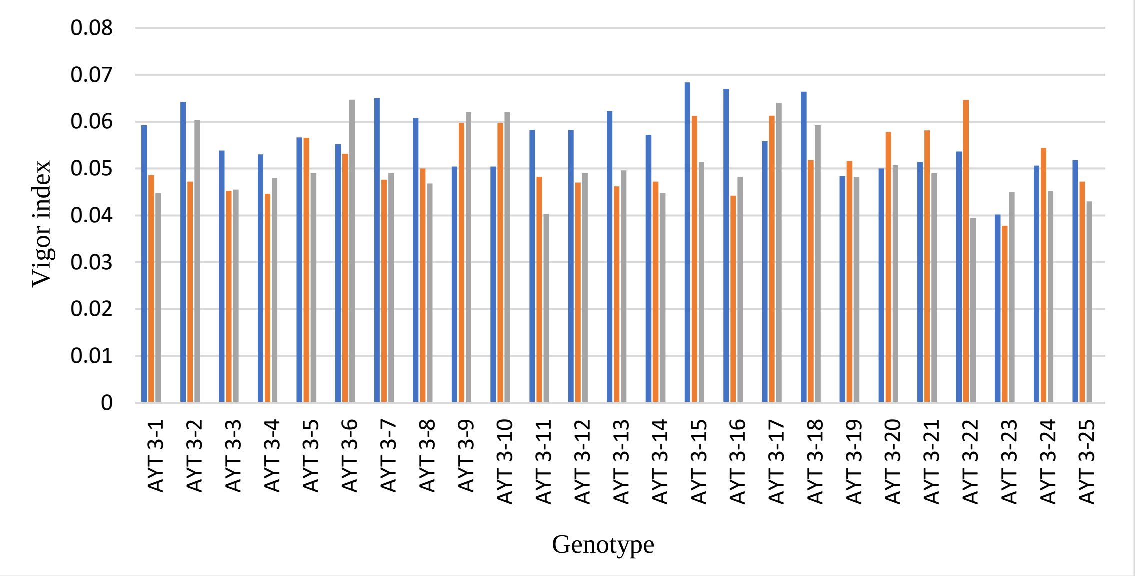

Fig. 6 Vigor index in 25 genotypes of rice

Conclusion

The results of this study suggest that lack of moisture has a particular effect on both seed germination and development of seedlings because it decreases the proportion of seeds that sprout when PEG concentration increases. During the early stages of growth, decisions may be taken depending on these characteristics to protect massive populations from the effects of drought. It would be more efficient, labor- and cost-intensive, and more productive, to screen the germplasm early in the growth process. The study also shown that variation in the germination stress tolerance index (GSTI) between genotypes was a reliable predictor of rice's ability to withstand drought. Germination was not seen at 20% PEG concentration; all lines survived at 10% PEG; resistant lines do, however, survive at 15% PEG under drought stress. These resistant lines may be used in breeding initiatives to create cultivars that are drought tolerant.

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