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Estimation of Variability and Correlation of Various Seed Vigour Traits in Rice (*Oryza sativa* L.) under In Vitro PEG-Induced Moisture Stress Condition

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ABSTRACT: Rice, which is regarded as a primary diet by half of the global population, is particularly susceptible to a variety of abiotic stresses. Drought is one of the most formidable challenges, owing to its detrimental effects on yield. Rice, a drought-sensitive cereal, demonstrates notable varietal variability in its drought tolerance, particularly during the germination and seedling stages. The assessment of drought tolerance in field conditions is a time-consuming and labour-intensive procedure. Consequently, an alternative approach involves the use of polyethylene glycol (PEG-6000) as an artificial inducer of drought stress, which enables the analysis of a diverse range of rice genotypes. The present study mainly examined the variability and correlation of various seed vigour-related physiological traits in 30 rice genotypes, specifically in response to moisture stress induced by 20% (w/v) solutions of polyethylene glycol (PEG)-6000 (osmotic potential of -0.49 Mpa). The results indicated that most rice genotypes exhibited a noticeable decrease in germination and seed vigour-related physiological parameters under moisture stress (MS) condition compared to the control (distilled water; 0 MPa). Among all the genotypes tested under MS condition, Karinellu was identified as the genotype with the highest germination potential (71.33%), while Magura exhibited the maximum seed vigour index I and II (774.17, 2.524). This study revealed significant genotypic variability among the seed vigour-related traits studied under MS condition, with the highest heritability exhibited by shoot length (99.8%), seedling length (99.6%), root length (99.3%), seed vigour index I (99.2%), seed vigour index II (98.5%), speed of germination (98.3%), germination (95.3%), and seedling dry weight (91.0%). Out of the 30 genotypes tested under MS condition, 9 genotypes exhibited the highest SVI-I, and 12 genotypes exhibited the highest SVI-II. The genotypes exhibited a significant amount of variation in every aspect of seed quality examined under MS condition. Principal component analysis (PCA) was performed to identify the most significant contributing variables for diversity, revealing the highest degree of variation by the first two principal components, that is, 82.67% and 12.44%, respectively. Subsequent correlation analysis revealed strong positive relationships among these traits under the imposed MS condition. Remarkably, seed vigour index I showcased strong and positive correlations with germination (0.946), shoot length (0.948), root length (0.963), seedling length (0.982), seedling dry weight (0.940) and seed vigour index II (0.975). The identification of tolerant genotypes that exhibit a higher degree of variability in the seed vigour-related traits, particularly those that are strongly correlated with drought resistance, can be a valuable source of donor parent candidates in future drought tolerance breeding programs. Variability and correlation analyses demonstrated a more effective selection of target traits for improving seed vigour under moisture-stress situations.

Keywords: Drought, Seed vigour index, Moisture stress, Polyethylene glycol, Variability, Correlation, Rice.

INTRODUCTION

Rice, scientifically known as *Oryza sativa* L., is the primary source of sustenance for about half of the global population. According to the FAO (2021), approximately 164.19 million acres of land globally yield approximately 755.45 million tonnes of rice annually. The total irrigation potential of the globe is 331 million ha (23%), with 1.417 billion hectares of arable cropland and 4.87 billion ha of agricultural land (FAO, 2017). Given that rice cultivation accounts for approximately 11.5% of the total arable cropland in the world, it is evident that rice is significant for global food security, nutrition, and economic considerations.

Rice crop plants are subjected to various biotic and abiotic stresses owing to unfavourable environmental conditions. Rice production is hindered by abiotic stresses, such as rising global temperature, droughts, floods, heavy winds, salinity, and nutrient deficiency. Among the abiotic stresses, drought is the most conspicuous stress that reduces crop productivity in a range of 40-70% (Lum *et al*., 2014). Erratic or below-average precipitation causes drought in rice production, characterized by limited plant productivity in an agricultural or natural setting (Dey and Upadhyaya 1996; Verslues *et al*., 2006; Deikman *et al*., 2012). This situation may persist in areas of low or intermittent water availability due to the unpredictable supply of water (Harb *et al*., 2010). The impact of water stress will continue to intensify in the future owing to increasing population load, changes in rainfall patterns, and the rise in global temperature as a result of global warming (Lesk *et al*., 2016).

Approximately 90% of the world's rice is produced and consumed in Asia, and its cultivation requires a semi-aquatic environment with high water demands, specifically 3000-5000 L of water per kg of rice (Ahmad *et al*., 2016; Kumar *et al*., 2022). Its cultivation is significantly dependent on rainy subsistence farming, which is becoming increasingly susceptible to droughts. Water stress in rice plants inhibits plant growth (Korres *et al*., 2017), induces the closure of stomata, reduces the photosynthetic process (Zia *et al*., 2021), and ultimately reduces yield. Rice plants are typically drought-susceptible, because they experience severe deleterious effects when subjected to water stress during critical growth stages, particularly during the reproductive stage, which includes panicle initiation, anthesis, and grain filling (Tao *et al*., 2006; Yang *et al*., 2008; Suriyan *et al*., 2010). In general, the seedling and flowering phases of rice are most susceptible to drought, adversely affecting the grain yield and quality of crops, resulting in a global food deficit (Isendahl and Schmidt, 2006; Panda *et al*., 2021).

Water is regarded as a fundamental necessity for the process of seed germination. Water stress or drought at the seedling stage has been reported to affect seed germination and seedling growth, which are potentially the most critical stages in rice (Ahmad *et al*., 2009). Drought stress has been shown to significantly hinder seed germination, seedling growth, and seed vigour, leading to seedling mortality and delayed seedling establishment (Sabouri, 2012; Swain *et al*., 2014; Vassilevska-Ivanova 2014; Vibhuti *et al*., 2015; Zaman 2018; Pessarakli, 1999).

Plants, on the other hand, respond to drought stress in complicated and multigenic ways, and the functions of many induced genes remain unknown (Ahmed *et al*., 2021). Because of this complexity, selecting and breeding drought-tolerant genotypes is extremely difficult (Tirado and Cotter 2010). Therefore, to easily select superior rice genotypes under drought stress, effective drought screening is required, which clearly distinguishes drought-susceptible genotypes from drought-tolerant genotypes (Swamy *et al.,* 2012; Xie *et al*., 2013). Seed germination and seedling growth are the critical stages for early plant establishment under stressful conditions (Guo *et al*., 2019; Mastinu *et al*., 2021). Selecting genotypes with rapid and uniform germination and seedling growth features under water-stress circumstances can help with early seedling establishment. Thus, the analysis of germination and seedling growth parameters linked to seed vigour, as well as their response to drought, can help in the effective selection of drought-tolerant rice genotypes. Seed vigour is therefore, considered an important trait for better plant population, establishment, and higher yield that can survive the negative effects of climate change, biotic obstacles, and drought effects on agricultural production (Daniel, 2017).

Modern rice cultivars tolerant to drought are very limited in number. Nevertheless, it is crucial to develop drought-resistant rice cultivars to meet the future food requirements of the country's growing population. The assessment of drought tolerance under field conditions is a time-consuming and labour-intensive process. Consequently, an alternative approach involves the use of polyethylene glycol (PEG-6000) as an artificial inducer of drought stress producing almost similar levels of hindering effects on plants, which ultimately helps to identify rice genotypes that have the potential to tolerate and resist drought.

Polyethylene glycol 6000 (PEG 6000) is a suitable substrate material for recreating soil-like conditions in vitro, thus allowing for easy manipulation of osmotic potential (Cui *et al*., 2008; Rajeswar and Narasimhan 2021). High molecular weight PEG is advantageous because it does not penetrate the cell wall or get absorbed by plant cells. Instead, it accumulates in the roots and leaves, causing osmotic changes that result in water-deficit conditions or drought stress on the plants (Rauf *et al*., 2007; Cui *et al*., 2008; Bagher *et al*., 2012; Tang *et al*., 2019).

Polyethylene glycol (PEG)-induced water stress significantly lowers the seed germination potential (Pirdashti *et al*., 2003; Coleman *et al*., 2018), decreases root and shoot length resulting in a reduction in seedling growth (Murillo-Amador *et al*., 2002; Sokoto and Muhammad 2014) and reduced seedling dry weight (Mohammadkhani and Heidari 2008; Farooq *et al*., 2015; Sagar, 2017; Roy *et al*., 2018; Islam *et al*., 2018; Ashaduzzaman *et al*., 2020) in rice. Rice genotypes with enhanced drought tolerance have been identified using in-vitro approaches involving the use of polyethylene glycol (PEG) in a suitable nutrient medium (Huang *et al*., 2009; Chutia and Borah 2012).

Exploitation of the potential parent genotypes with a higher degree of drought tolerance may help provide insights into the genetic mechanisms underlying the ability to tolerate drought stress and may lead to the development of improved drought-tolerant rice varieties.

In this study, thirty different rice germplasm varieties were examined to evaluate their variability and investigate the correlation between various physiological traits, such as seed germination percentage, speed of germination, shoot length, root length, seedling length, seedling dry weight, and seed vigour index I and II under in vitro PEG-induced moisture stress condition. The basic objective of this experiment was to identify superior genotypes by studying the correlation and variability of seed vigour-related physiological traits under artificially induced moisture stress condition using polyethylene glycol (PEG)-6000 (osmotic potential of -0.49 Mpa) at the seedling stage in a variable rice natural population.

MATERIALS AND METHODS

In the current study, thirty distinct rice genotypes obtained from eight distinct states, including Assam, MP, Kerala, Karnataka, Tamil Nadu, Jeypore, Odisha, and Manipur were preserved and collected from the gene bank of the ICAR-National Rice Research Institute (ICAR-NRRI), Cuttack. The landraces from Odisha have been collected from the Jeypore tract of Odisha, which is acknowledged as the secondary centre of origin for rice and is known to have extensive variation in the genetic makeup of the landraces. All the germplasms were cultivated in the experimental field of ICAR-NRRI, Cuttack, Odisha, India, during the *Kharif* season of 2020. The freshly harvested seeds were used to estimate several seed vigour parameters in both normal and moisture-stress conditions after being treated with a dormancy-breaking chemical, that is, 2% KNO_3 solution.

A. Germination (%)

The seed germination test was conducted according to the methods described by Verslues *et al*. (2006) using the top-of-paper method of germination. Germination assays of 30 genotypes were performed by evenly distributing 100 seeds each in three replicates in a 10 cm diameter sterilized petri dish with two layers of Whatman No. 1 filter paper and subjected to a standard germination test under both MS and control conditions. To induce MS, each petri dish was moistened with 10 ml of a 20% (w/v) solution of polyethylene glycol (PEG)-6000 (with an osmotic potential of -0.49 MPa) as per Michel and Kaufmann (1973) while distilled water was used for the control treatment (with an osmotic potential of 0 MPa). The experiment was arranged in a completely randomized design (CRD) within a germinator set at 30±1°C and 80% relative humidity for 14 days. The final germination percentage and seed vigour traits were recorded on the 14th day for both the MS-treated and untreated seeds.

Germination percentage was calculated by the following formula

$$\text{Germination \%} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds}} \times 100$$

B. Speed of germination

The speed of germination was calculated using the following formula (Maguire, 1962).

$$\text{Speed of germination} = \sum \left(\frac{n_i}{d_i}\right)$$

Where,

n_i= Number of seeds germinating on the day 'd_i^{th}'

d_i = Number of days after putting seeds for germination

C. Shoot Length, Root Length, and Seedling Length (cm)

A total number of ten normal seedlings were randomly picked from each replication on the 14th day. The measurements of root length, shoot length, and total seedling length were taken, and the average value was expressed as centimetres per seedling.

D. Seedling Dry Weight (g)

The ten number of normal seedlings chosen for measuring the seedling length from each replication were also utilized for measuring the seedling dry weight. After removing the cotyledons, the seedlings were dried in an oven at 70°C for 48 hours. The dry weight was then measured and expressed in grams per seedling following the method described by Kleyer *et al*. (2008).

E. Seed vigour

Based on the observation data of mean seedling length and mean seedling dry weight, the seed vigour index (SVI-I and SVI-II) was calculated to evaluate seed vigour (Abdul-Baki and Anderson 1973).

SVI-I = Seed germination (%) × Mean seedling length (cm)

SVI-II = Seed germination (%) × Mean seedling dry weight (g)

The statistical software Crop stat 7.0 was used to estimate the analysis of variance (ANOVA), coefficient of variation (CV %), mean, and range. A correlation matrix heat map was constructed by analyzing Pearson's correlation coefficients and was used to assess the relationship between various physiological parameters. In this experiment, the mean estimates of the thirty rice genotypes for the eight physiological traits were classified into three broad groups: low, medium, and high value-containing germplasm lines.

RESULTS AND DISCUSSION

Drought stress is a major hindrance to the growth and development of crops. The main effect of drought is the suppression of seed germination, seedling growth, and overall plant growth, which hampers crop progress and yield (Hartmann *et al*., 2005; Van den Berg and Zeng 2006; Sathyabharathi *et al*., 2022). Evaluating the drought resistance of different rice varieties under field conditions is difficult because it is impossible to artificially produce controlled and consistent drought conditions in the field. By employing drought screening procedures under controlled conditions and using a standardized PEG solution, it is possible to create consistent environments for inducing water stress (Swain *et al*., 2020). According to existing literature, PEG is regarded as a superior chemical due to its inert, non-ionic, and essentially impermeable chains, which can induce water stress in crop plants (Kaur *et al*., 1998; Landjeva *et al*., 2008).

Among the 30 landraces, three germplasms studied under MS condition namely, Karinellu was observed with the highest germination (71.33%); Gondiachampeisiali was found with the highest speed of germination (4.03); Magura with the longest shoot length (3.27cm), longest root length (7.63 cm), longest seedling length (10.90 cm), highest seedling dry weight (0.036 g), highest SVI-I (774.17), and SVI-II (2.524), respectively. Magura genotype showed better results for more than two physiological traits under MS condition. Whereas Latamahu was observed with the lowest germination (45.33%); Kundadhan with the lowest speed of germination (2.00); Kabokphou with the shortest shoot length (0.81 cm), lowest seedling dry weight (0.010 g) and lowest SVI-II (0.500); Bilipandya was found with the shortest root length (2.74 cm), shortest seedling length (3.69 cm) and lowest SVI-I (188.85).

Similarly, under control condition the four germplasms Gondiachampeisiali observed with the highest germination (98.18%), longest root length (19.30 cm), longest seedling length (30.43 cm), and highest seed vigour index-I (2986.34); Magura was found with the highest speed of germination (18.01); Phoudum with the longest shoot length (12.84 cm); Karinellu with the highest seedling dry weight (0.079g), and highest SVI-II (7.731). The germplasms namely, Gondiachampeisiali and Karinellu showed better results for more than two physiological traits under the control condition. Whereas Kabokphou was observed with the lowest germination (87.00%), lowest speed of germination (11.88), shortest shoot length (8.60 cm); shortest root length (7.31 cm); shortest seedling length (15.91 cm), and lowest SVI-I (1383.84); Chingforechokua showed the lowest value for the seedling dry weight (0.051 g) and Chatuimuchi recorded the lowest SVI-II (4.710) followed by Landi (4.888). Apart from Kabokphou, Jayapadma recorded the lowest SVI-I (1522.84).

There was a considerable reduction in the mean values of various seed vigour-related physiological traits estimated under moisture stress condition in comparison to the control (Table 1 & 2). Under control condition, the germination percentage ranged from 87.00% (Kabokphou) to 98.18% (Gondiachampeisiali). However, under moisture stress condition, it decreased, ranging from 45.33% (Latamahu) to 71.33% (Karinellu). The speed of germination varied from 11.88 (Kabokphou) to 18.01 (Magura) in the control, and from 2.00 (Kundadhan) to 4.03 (Gondiachampeisiali) under MS condition. Shoot length in the control ranged between 8.60 cm (Kabokphou) and 12.84 cm (Phoudum), but after MS treatment, it reduced and ranged between 0.81 cm (Kabokphou) and 3.27 cm (Magura). Root length ranged from 7.31 cm (Kabokphou) to 19.30 cm (Gondiachampeisiali) in the control, and from 2.74 cm (Bilipandya) to 7.63 cm (Magura) in MS-treated seeds. Seedling length in the control ranged between 15.91 cm (Kabokphou) and 30.43 cm (Gondiachampeisiali) and decreased and ranged between 3.69 cm (Bilipandya) to 10.90 cm (Magura) after MS treatment. Seedling dry weight ranged from 0.051 g (Chingforechokua) to 0.079 g (Karinellu) in the control, and from 0.010 g (Kabokphou) to 0.036 g (Magura) under MS condition. The Seed vigour index-I varied from 1383.84 (Kabokphou) to 2986.34 (Gondiachampeisiali) in the control, and from 188.85 (Bilipandya) to 774.17 (Magura) under MS condition. The Seed vigour index-II ranged from 4.710 (Chatuimuchi) to 7.731 (Karinellu) in the control, and from 0.500 (Kabokphou) to 2.524 (Magura) under moisture stress condition.

Statistical analysis revealed substantial variations among the genotypes for all parameters evaluated under both MS and control conditions. The physiological traits examined under both the MS and control conditions also exhibited a wide range of variability (Table 1 & 2). The variability analysis included the landraces collected from different states where prior studies have identified significant genetic diversity in rice (Latha *et al*., 2013; Pandit *et al*., 2017; Pandit *et al*., 2020; Sahoo *et al*., 2020; Vanlalsanga and Singh 2019).

The findings of the present study indicate that the mean germination and various seed vigour-related physiological traits were significantly impaired under PEG-induced moisture stress condition, as compared to the control condition. Prior research has shown that the initiation of germination is delayed by PEG-induced osmotic stress, resulting in a decrease in the germination percentage (Pirdashti *et al*., 2003; Dhanda *et al*. 2004; Turk *et al*., 2004; Jajarmi 2009; Khakwani *et al*., 2011; Raza *et al*. 2012; Swain *et al*., 2014; Coleman *et al*., 2018). The purpose of PEG is to reduce the hydration rate of seeds, which is a determining factor in the inhibition of seed germination by reducing the water availability (Wang *et al*., 2002; Jiao *et al*., 2009). The metabolic process is activated by the physical process of water absorption by the seed, which is followed by hydration and breaking of the seed's dormancy. According to Islam *et al*. (2018); Yang *et al*. (2018), the decrease in germination that was observed with an increase in PEG level was linked to high seed nutrient imbalance, toxic ions, and diminished soluble osmotic potential. The control treatment had the highest germination percentage, indicating that germination proceeded smoothly owing to adequate water content.

Seedling length and dry weight are crucial traits that are significantly affected by moisture stress. In a study conducted by Zheng *et al*. (2016), drought stress significantly impacted the early growth of rice seedlings, leading to a substantial decrease in shoot and root length, as well as seedling fresh and dry weights. The reduction in seed germination and seedling growth due to water stress is a widely seen phenomenon, as shown by several researchers (Murillo-Amador *et al*., 2002; Sokoto and Muhammad 2014; Fahad *et al*., 2017; Kosar *et al*., 2018). This is because plants subjected to water stress exhibit a decrease in water potential and turgor pressure (Blackman, 2018), which increases the concentration of solutes in the cytosol and extracellular matrix (Saidur *et al*., 1985; Lisar *et al*., 2012), leading to a decline in cell enlargement. According to several studies (Mohammadkhani and Heidari 2008; Ji *et al*., 2012; Farooq *et al*., 2015; Sagar, 2017; Islam *et al*., 2018; Roy *et al*., 2018; Ashaduzzaman *et al*., 2020), reduced cell enlargement due to water stress impairs reproduction and thereby inhibits seedling growth, leading to reduced seedling fresh and dry weight of the paddy under stress treatment compared to paddy under control treatment.

Seed germination and seedling growth are the critical stages for early plant establishment under stressful conditions (Guo *et al*., 2019; Mastinu *et al*., 2021). Selecting genotypes with rapid and uniform germination and seedling growth features under water stress conditions can help ensure successful early seedling establishment. Hence, the investigation of the germination and seedling growth traits linked to seed vigour, as well as their response to drought, can help in the effective selection of drought-tolerant rice cultivars.

A. Estimation of Genetic Parameters

The GCV percentage of the various genotypes studied under MS condition varied from 13.41% to 48.79% (Table 1). Among the traits, GCV % was the highest for seed vigour index II (48.79%), shoot length (40.19%), seed vigour index I (38.31%), seedling length (27.43%), root length (24.47%), speed of germination (18.07%), seedling dry weight (15.24%), and germination (13.41%). The highest GCV was found for seed vigour index II and the lowest was recorded for germination. The PCV range of all the traits studied under MS condition varied from 13.74% to 49.16% (Table 1). Among the traits, PCV % recorded for seed vigour index II was highest (49.16%), followed by shoot length (40.23%), seed vigour index I (38.46%), seedling length (27.47%), root length (24.56%), speed of germination (18.23%), seedling dry weight (113.80%), and germination (13.74%). The highest PCV was found for seed vigour index II and the lowest was recorded for germination. The heritability of all traits studied under MS condition was more than 90% (Table 1). The heritability was highest for shoot length (99.8%), seedling length (99.6%), root length (99.3%), seed vigour index I (99.2%), seed vigour index II (98.5%), speed of germination (98.3%), germination (95.3%), and seedling dry weight (91.0%). The genetic advance for all the characters studied under MS condition varied from 26.97 to 99.74 (Table 1). The highest GA was recorded for seed vigour index II (99.74), followed by shoot length (82.71), seed vigour index I (78.59), seedling length (56.40), root length (50.23), speed of germination (36.91), seedling dry weight (27.21), and the lowest GA observed was for germination (26.97).

Similarly, the GCV percentage of the various genotypes studied under control condition varied from 3.42% to 21.64% (Table 2). Among the traits, GCV % was highest for root length (21.64%), SVI-I (17.19%), seedling dry weight (15.24%), seedling length (14.48%), SVI-II (13.70), shoot length (11.29%), speed of germination (11.11) and the lowest GCV% was for germination (3.42%). The PCV range of all traits studied under the control condition varied from4.67% to 21.78%. Among the traits, PCV% was highest for root length (21.78%), SVI-I (17.38%), seedling length (14.70%), SVI-II (14.36%), seedling dry weight (13.80%), shoot length (11.65%), speed of germination (11.32%), and lowest for germination (4.67%).The heritability of all the traits studied under the control condition varied from 53.5% to 98.7% (Table 2). The highest heritability was observed for root length (99.7%), followed by seed vigour index I (97.9%), seedling length (97.0%), speed of germination (96.3%), shoot length (93.8%), seedling dry weight (92.0%), seed vigour index II (91.0%), and germination (53.5%). The Genetic advance for all the characters studied under control condition varied from 5.15 to 44.28 (Table 2). The highest GA was recorded for root length (44.28), seed vigour index I (35.04), seedling length (29.37), seedling dry weight (27.21), seed vigour index II (26.91), shoot length (22.52), speed of germination (22.46), and the lowest GA was observed for germination (5.15).

The identification of a high PCV (phenotypic coefficient of variation) and GCV (genotypic coefficient of variation) for seed vigour-related physiological traits studied under both MS and control conditions might be valuable in breeding programs aimed at improving seed vigour under moisture-stress environments. In addition, seed vigour-related physiological parameters exhibited high heritability and genetic advance. Since heritability does not always indicate genetic gain, heritability coupled with genetic advance is more effective for selecting seed vigour traits in rice. Numerous researchers have previously documented high GCV, PCV, and genetic advances for different rice traits (Agrama *et al*., 2007; Zhao *et al*., 2011; Huang *et al*., 2012; Zhang *et al*., 2012; Zhao *et al*., 2013; Huang *et al*., 2015; Kumar *et al*., 2015; Pan *et al*., 2015; Anandan *et al*., 2016; Pradhan *et al*., 2016; Pandit *et al*., 2017; Swamy *et al*., 2017; Pradhan *et al*., 2019; Bastia *et al*., 2022). Rice crop has a great genetic and phenotypic diversity which enhances its adaptability as a species as reported by McNally *et al.* (2009). In addition, the findings indicated that seed vigour is a complex physiological parameter that is affected by both genetic and environmental factors, with significant variability observed in different seed vigour indices. Specifically, seed vigour exhibited the highest genotypic and phenotypic coefficients of variance, suggesting that genetic factors have a greater influence on the overall variation of this trait than environmental factors. Furthermore, Zhao *et al.* (2021) revealed that seed vigour is under strong genetic control and influenced by multiple genes.

B. Correlation Analysis

The association or correlation between traits is crucial in determining whether the selection of one characteristic might affect the selection of another. Table 3 and 4 presents the simple correlation coefficients calculated from the aggregated data. Using correlation coefficients computed across 30 rice genotypes for eight seedling vigour parameters examined under both MS and control condition, we discovered that most vigour-related traits were significantly correlated.

The data collected in the current study were subjected to a comprehensive analysis, which revealed substantial correlations among the various traits examined under moisture stress condition (Table 3 and Fig. 1). The germination % showcased a significant and positive correlation with seed vigour index I (0.946), seed vigour index II (0.929), seedling length (0.878), root length (0.870), seedling dry weight (0.859) and shoot length (0.830). Shoot length displayed a significant positive correlation with seedling length (0.950), seed vigour index I (0.948), seed vigour index II (0.896), root length (0.890), seedling dry weight (0.870) and germination (0.830). Furthermore, root length depicted a strong positive correlation with seedling length (0.988), seedling dry weight (0.969), seed vigour index II (0.964), seed vigour index I (0.963), shoot length (0.890), and germination (0.870). Notably, seedling length established a robust positive correlation with root length (0.988), seed vigour index I (0.982), seed vigour index II (0.965), seedling dry weight (0.960), shoot length (0.950), and germination (0.878). Moreover, a pronounced significant positive correlation for seedling dry weight with seed vigour index II (0.985), root length (0.969), seedling length (0.960), seed vigour index I (0.940), shoot length (0.870), and germination (0.859) was noticed. Remarkably, the seed vigour index I showcased strong and positive correlations with seedling length (0.982), seed vigour index II (0.975), root length (0.963), shoot length (0.948), germination (0.946), and seedling dry weight (0.940). The seed vigour index II demonstrated very high, strong, and positive correlations with seedling dry weight (0.985), seed vigour index I (0.975), seedling length (0.965), root length (0.964), germination (0.929), and shoot length (0.896). In a study conducted in MS condition, Mishra *et al.* (2019) observed that seedling vigour index, root length, shoot length and relative water content had a positive and significant correlation, and the shoot length had a significant positive correlation with fresh weight and dry weight. In a study by Nulit (2018), the germination percentage, fresh weight, dry weight, shoot length, and root length displayed a positive significant correlation in stress conditions. Similarly, Eslami *et al*. (2018) reported a significant positive correlation between root and shoot lengths at various PEG concentrations.

There was also the presence of significant correlations between different seed vigour-related physiological traits studied under the control condition (Table 4, Fig. 2). The germination % established a robust and positive correlation with the speed of germination (0.957), seed vigour index I (0.759), seedling length (0.640), root length (0.602), and shoot length (0.464). A significant positive correlation was recorded for speed of germination with germination (0.957), seed vigour index I (0.776), seedling length (0.677), root length (0.611), and shoot length (0.545). Additionally, shoot length showcased strong positive correlations with seedling length (0.726), seed vigour index I (0.705), speed of germination (0.545), and germination (0.464). Interestingly, root length established strong positive correlations with seedling length (0.941), seed vigour index I (0.932), speed of germination (0.611), germination (0.602), shoot length (0.450), and seed vigour index II (0.364). Furthermore, seedling length displayed significant positive correlations with SVI-I (0.985), followed by root length (0.941), shoot length (0.726), speed of germination (0.677), and germination (0.640). Moreover, a pronounced positive correlation between seedling dry weight and seed vigour index II was evident (0.960). Meanwhile, seed vigour index I demonstrated strong positive correlations with seedling length (0.985), root length (0.932), speed of germination (0.776), germination (0.759), and shoot length (0.705). Moreover, a pronounced significant and positive correlation of seed vigour index II with seedling dry weight (0.960) and root length (0.364) was evident. In the same manner, in a control experiment, Olawamide *et al*. (2018) reported that the seedling vigour index had a positive and substantial correlation with germination percentage, shoot length, and root length.

Seedling vigour is a critical trait for successful crop establishment in direct-seeded rice. Seed vigour, along with its associated traits, is inherited quantitatively (Wing *et al*., 1995; Panda *et al*., 2021b). Strong vigour is a characteristic of higher seed quality (Chowdhury and Singh 2000). The presence of a strong and significant correlation between several physiological traits and seed vigour in both the MS and control conditions could be useful for a more effective selection of seed vigour traits in rice and the simultaneous improvement of both traits concerned. The presence of correlations between various seed vigour-related physiological parameters has been documented in previous studies conducted by Cui *et al*. (2002); Sahoo *et al*. (2020); Sanghamitra *et al*. (2021); Barik *et al.* (2022); Sadhana *et al*. (2022). In addition, several correlation studies with different phenotypic traits in rice have been conducted by researchers such as Farooq *et al*. (2006); Namuco *et al*. (2009); Rajjou *et al.* (2012); Mithra *et al*. (2018); Panda *et al.* (2021a), and Anandan *et al.* (2022). Researchers have also documented Pearson's correlation coefficients among various seed vigour parameters in rice plants subjected to PEG-induced drought stress (Manonmani *et al*., 2020; Huang *et al*., 2022).

These correlations or associations that were observed offer valuable insights into the potential of drought-tolerant traits, which were the primary focus of our study. The importance of these correlations is that they have implications for rice cultivation, particularly in regions that are prone to drought. When traits associated with drought tolerance, including root length, shoot length, seedling length, seedling dry weight, germination percentage and seed vigour index exhibit robust and positive correlations, it implies that these traits are likely to co-occur or positively influence one another in response to drought conditions (Sabesan *et al*., 2016). It is feasible to incorporate critical traits or characteristics into other high-yielding varieties to optimize the number of populations under osmotic stress. As a result, they can be identified as the essential traits for the screening of rice genotypes under osmotic stress during germination and seedling establishment. This information is essential for agricultural scientists and plant breeders, as it enables the identification of vital traits that can be targeted for improvement in rice varieties.

C. Frequency distribution

The genotypes were categorized into three phenotypic groups: high, moderate, and low, based on the mean data of thirty genotypes for eight seed vigour-related physiological parameters investigated under both moisture stress and control conditions (Fig. 3 & 4). Out of the 30 genotypes examined for seed vigour traits under the moisture stress condition (Fig. 3), eight germplasms displayed low SVI-I, thirteen showed medium SVI-I, and nine had high SVI-I. Similarly, there were five germplasms with low SVI-II, thirteen with medium SVI-II, and twelve germplasms with high SVI-II.

Similarly, the genotypes were divided into three phenotypic groups based on the mean data of the thirty genotypes for seed vigour traits investigated under the control condition (Fig. 4). Out of the total number of 30 genotypes, four germplasms showed low SVI-I, sixteen had medium SVI-I, and ten had high SVI-I. Similarly, four germplasms exhibited low SVI-II, twelve genotypes displayed medium SVI-II, and fourteen genotypes exhibited high SVI-II. Sujay (2007) reported similar findings for various physiological parameters associated with seed vigour.

D. Genotype by trait biplot analysis of different seed vigour-related physiological quality traits

Drought tolerance is a complex quantitative trait that is controlled by numerous genes, each of which has a relatively small effect (Price *et al*., 2002). Principal component analysis (PCA) is an effective approach for handling complex data matrices with multiple parameters and measures the genetic differences between populations based on their attributes (Fuzy *et al*., 2019). The implementation of Principal Component Analysis (PCA) would enhance the ease with which biologists and plant breeders can investigate the number of plants to be evaluated and the specific traits that can be utilized to identify genotypes with drought resistance (Beena *et al*., 2021).

PCA is a technique that simplifies complex data by converting a set of correlated variables into a smaller set of variables known as principle components (PCs). The selection of genotypes from these two principal components will be beneficial (Ahmadizadeh *et al*., 2011; Bahrami *et al*., 2014). The current study utilized Principal Component Analysis (PCA) to identify the traits with the greatest variability that can be employed to screen the rice genotypes for enhanced drought tolerance (Bhattarai and Subudhi 2019).

The Eigen values in the Table 5 & 6 explain how the total variance is divided among each principal component and explain the variance percentage and cumulative proportion for all the components (Raza *et al*., 2017). For the number of principal components in Table 5 & 6, eigen values greater than 1 were taken into consideration.

A genotype by trait biplot analysis was conducted on a panel population consisting of 30 genotypes. The investigation focused on eight physiological traits under moisture stress condition (Table 5; Fig. 5). Out of the 8 principal components, PC1 had an eigen value of 6.614, which explained 82.672% of the variation in the population. PC2 exhibited an eigen value of 1.012, accounting for 12.439% of the total variation in the population, as seen in Table 5. Among the eight parameters in PC1, SVI-I had the highest contribution to the variation (14.952), followed by SVI-II (14.791), seedling length (14.787), root length (14.425), seedling dry weight (14.203), shoot length (13.362), and germination (13.089). In PC2, the highest recorded value was for speed of germination (97.600). The PCA analysis for traits studied under the MS condition involved the use of the first two principal components (PC) and the results are shown in Fig. 5. The scatter plot demonstrates that landraces exhibiting the highest values for the physiological parameters under investigation were situated in the upper right corner (1st quadrant) and lower right corner (2nd quadrant). The landraces displaying intermediate estimations were retained in the bottom left corner (3rd quadrant), whereas the majority of genotypes with low values were positioned in the top left quadrant (4th quadrant). However, the genotypes exhibiting the highest variation were located at the extreme end or are identified as outliers in each quadrant.

Furthermore, a Principal component analysis (PCA) was conducted on the panel population to analyze the traits studied under the control condition (Table 6; Fig. 6). Among the 8 principal components, PC1 had an eigen value greater than 1, namely 4.817, which accounted for 60.215% of the variation in the population. PC2 had an eigen value of 1.845, contributing 23.059% of the variation in the population (Table 6). Among the eight traits in PC1, SVI-I had the largest contribution of variation (19.812), followed by seedling length (18.378), root length (16.073), speed of germination (15.299), germination (14.544), and shoot length (9.995). In PC2, the seedling dry weight had the highest value of 49.822, followed by seed vigour index II (41.551). The PCA analysis for traits studied under the control condition involved the use of the first two principal components (PC), and the results are shown in Fig. 6. The scatter plot revealed that the landraces with the highest values for the physiological parameters under study occupied the top right corner of the 1st quadrant and the bottom right corner of the 2nd quadrant. The landraces with poor estimations were mostly present in the bottom left corner (3rd quadrant), whereas the top left quadrant (4th quadrant) accommodated the majority of genotypes with moderate estimates for the physiological traits. The performance of various genotypes during the vigour test, which was conducted under both MS and control conditions, indicated that the genotypes with low vigour will lose their viability at a quicker rate than those with high vigour when stored under moisture stress condition. Research by Mahadevappa and Nandisha (1987); Black and Halmer (2006); Richman *et al.* (2006); ISTA (2007) corroborates this conclusion. Specifically, seeds that exhibit high vigour generate uniform and robust stands, which offer young seedlings an increased ability to withstand extreme environmental conditions like drought. In contrast, seeds with low vigour generate weak seedlings that are susceptible to drought or moisture stress and are generally short-lived (IRRI, 2009).

Table 1: Estimation of variability of different seed vigour indices of 30 rice germplasms studied under moisture stress condition.

Traits	Range	Mean	SD	$S.E_m$	CV (%)	GCV	PCV	h^2_{bs}	GA
Germination	45.33-71.33	60.88	8.25	1.51	2.985	13.41	13.74	0.953	26.97
Speed of germination	2.00-4.03	2.96	0.54	0.10	2.391	18.07	18.23	0.983	36.91
Shoot length	0.81-3.27	1.72	0.69	0.13	1.833	40.19	40.23	0.998	82.71
Root length	2.74-7.63	5.72	1.40	0.26	2.069	24.47	24.56	0.993	50.23
Seedling length	3.69-10.90	7.45	2.04	0.37	1.645	27.43	27.47	0.996	56.40
Seedling dry weight	0.010-0.036	0.025	0.010	0.002	0.62	15.24	13.80	0.910	27.21
Seed Vigour Index-I	188.85-774.17	467.53	179.34	32.74	3.473	38.31	38.46	0.992	78.59
Seed Vigour Index-II	0.500-2.524	1.575	0.770	0.141	6.022	48.79	49.16	0.985	99.74

SD- Standard Deviation, $S.E_m$- Standard error of mean, CV- Coefficient of variation, GCV- Genotypic Covariance, PCV- Phenotypic Covariance, h^2_{bs} – Broad sense heritability, GA- Genetic Advance

Table 2: Estimation of variability of different seed vigour indices of 30 rice germplasms studied under control condition.

Traits	Range	Mean	SD	$S.E_m$	CV (%)	GCV	PCV	h^2_{bs}	GA
Germination	87.00- 98.18	93.61	3.66	0.67	3.19	3.42	4.67	0.535	5.15
Speed of germination	11.88-18.01	15.85	1.77	0.32	2.18	11.11	11.32	0.963	22.46
Shoot length	8.60-12.84	10.88	1.24	0.23	2.89	11.29	11.65	0.938	22.52
Root length	7.31-19.30	11.65	2.53	0.46	2.49	21.64	21.78	0.997	44.28
Seedling length	15.91-30.43	22.53	3.28	0.60	2.54	14.48	14.70	0.970	29.37
Seedling dry weight	0.051-0.079	0.065	0.020	0.030	0.71	15.24	13.80	0.920	27.21
Seed Vigour Index-I	1383.84-2986.34	2115.70	365.07	66.65	2.55	17.19	17.38	0.979	35.04
Seed Vigour Index-II	4.710-7.731	6.045	0.841	0.154	0.139	13.70	14.36	0.910	26.91

SD- Standard Deviation, $S.E_m$- Standard error of mean, CV- Coefficient of variation, GCV- Genotypic Covariance, PCV- Phenotypic Covariance, h^2_{bs} – Broad sense heritability, GA- Genetic Advance

Table 3: Correlation matrix of different seed vigour contributing traits among the 30 rice germplasms studied under moisture stress condition.

	Germination	Speed of germination	Shoot length	Root length	Seedling length	Seedling dry weight	Seed Vigour Index-I	Seed Vigour Index-II
Germination	1	0.208	0.830***	0.870***	0.878***	0.859***	0.946***	0.929***
Speed of germination	0.208	1	0.148	0.063	0.093	0.122	0.146	0.156
Shoot length	0.830***	0.148	1	0.890***	0.950***	0.870***	0.948***	0.896***
Root length	0.870***	0.063	0.890***	1	0.988***	0.969***	0.963***	0.964***
Seedling length	0.878***	0.093	0.950***	0.988***	1	0.960***	0.982***	0.965***
Seedling dry weight	0.859***	0.122	0.870***	0.969***	0.960***	1	0.940***	0.985***
Seed Vigour Index-I	0.946***	0.146	0.948***	0.963***	0.982***	0.940***	1	0.975***
Seed Vigour Index-II	0.929***	0.156	0.896***	0.964***	0.965***	0.985***	0.975***	1

*** Correlation is significant at 0.001 level (two tailed) ; ** Correlation is significant at 0.01 level (two tailed); * Correlation is significant at 0.05 level (two tailed)

Table 4: Correlation matrix of different seed vigour contributing traits among the 30 rice germplasms studied under control condition.

	Germination	Speed of germination	Shoot length	Root length	Seedling length	Seedling dry weight	Seed Vigour Index-I	Seed Vigour Index-II
Germination	1	0.957***	0.464**	0.602***	0.640***	0.067	0.759***	0.342
Speed of germination	0.957***	1	0.545**	0.611***	0.677***	0.056	0.776***	0.321
Shoot length	0.464**	0.545**	1	0.450*	0.726***	-0.050	0.705***	0.080
Root length	0.602***	0.611***	0.450*	1	0.941***	0.201	0.932***	0.364*
Seedling length	0.640***	0.677***	0.726***	0.941***	1	0.136	0.985***	0.311
Seedling dry weight	0.067	0.056	-0.050	0.201	0.136	1	0.130	0.960***
Seed Vigour Index-I	0.759***	0.776***	0.705***	0.932***	0.985***	0.130	1	0.337
Seed Vigour Index-II	0.342	0.321	0.080	0.364*	0.311	0.960***	0.337	1

*** Correlation is significant at 0.001 level (two tailed) ; ** Correlation is significant at 0.01 level (two tailed); * Correlation is significant at 0.05 level (two tailed)

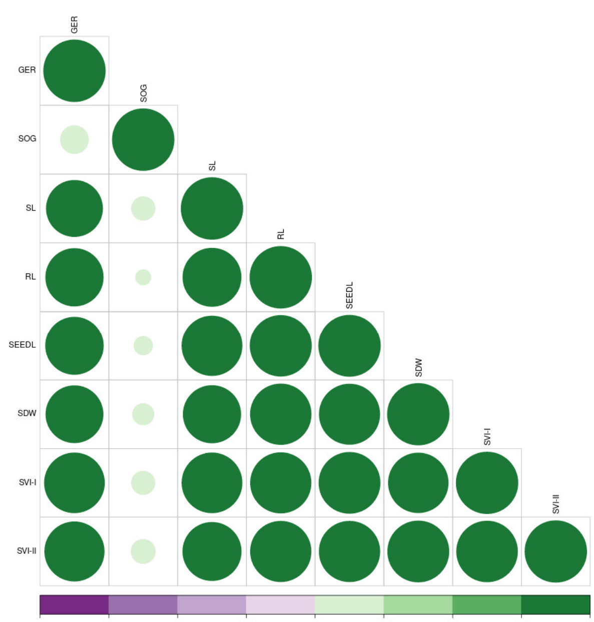

Fig. 1. Heat map showing correlation coefficients of different seed vigour contributing traits among the 30 rice germplasms under moisture stress condition.

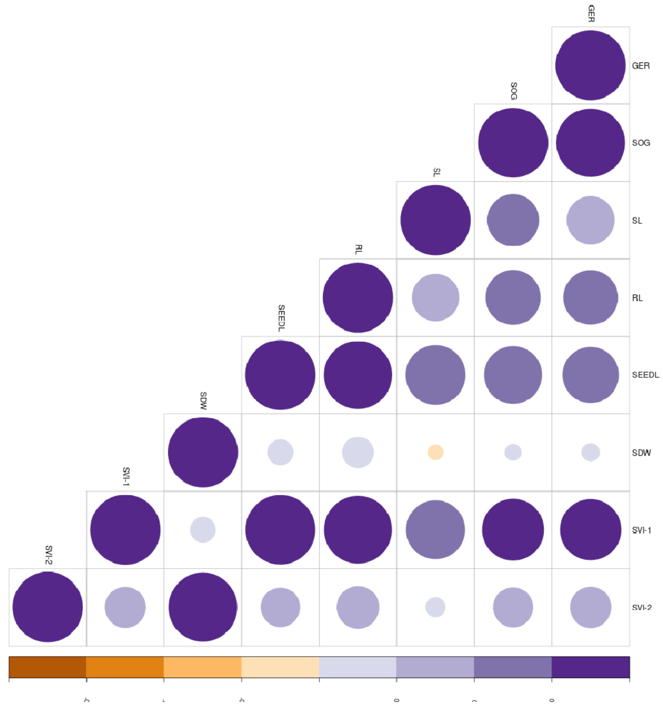

Fig. 2. Heat map showing correlation coefficients of different seed vigour contributing traits among the 30 rice germplasms under control condition.

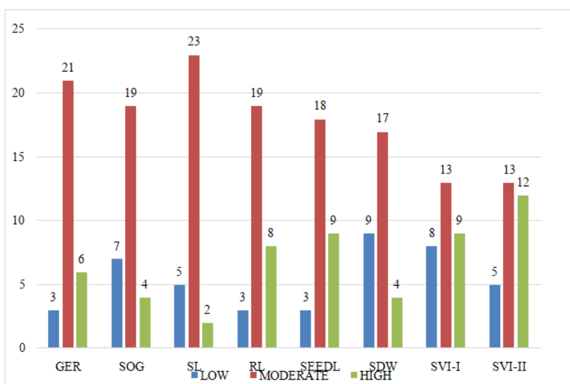

Fig. 3. Frequency distribution of 30 rice germplasm studied under moisture stress condition.

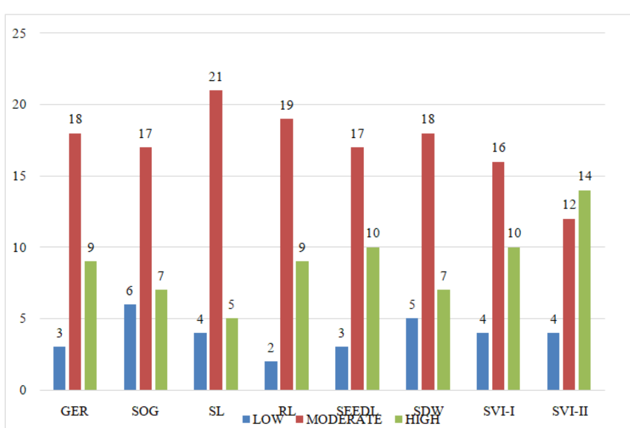

Fig. 4. Frequency distribution of 30 rice germplasm studied under control condition.

Table 5: Eigen value of different principal components and percentage contribution of different seed vigour contributing traits on PCs under moisture stress condition in rice.

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Germination	13.089	0.599	56.404	14.501	0.105	14.315	0.986	0
Speed of germination	0.391	97.600	0.810	0.727	0.470	0.001	0	0
Shoot length	13.362	0.002	32.294	32.699	8.654	3.729	1.816	7.444
Root length	14.425	1.078	0.759	10.644	39.921	0.361	2.800	30.011
Seedling length	14.787	0.537	6.422	0.086	11.053	1.078	3.537	62.499
Seedling dry weight	14.203	0.173	0.142	30.822	20.339	18.140	16.172	0.009
Seed Vigour Index-I	14.952	0.010	0.302	5.408	1.982	29.265	48.061	0.020
Seed Vigour Index-II	14.791	0.001	2.866	5.112	17.475	33.111	26.628	0.016
Principal components	**Eigen value**		**Percentage of variance**		**Cumulative percentage of variance**			
PC1	6.614		82.672		82.672			
PC2	1.012		12.439		95.111			

Table 6: Eigen value of different principal components and percentage contribution of different seed vigour traits on PCs under control condition in rice.

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Germination	14.544	0.519	35.886	0.051	40.647	8.265	0.088	0.001
Speed of germination	15.299	0.937	28.997	0.632	53.903	0.071	0.162	0
Shoot length	9.995	5.556	9.405	64.323	0.266	0.877	1.274	8.303
Root length	16.073	0	9.213	28.999	0.787	4.997	5.553	34.379
Seedling length	18.378	0.805	12.226	1.233	0.230	4.914	4.906	57.307
Seedling dry weight	1.293	49.822	0.964	2.001	0.635	21.905	23.377	0.004
Seed Vigour Index-I	19.812	0.809	2.807	1.437	2.831	33.937	38.365	0.002
Seed Vigour Index-II	4.607	41.551	0.501	1.324	0.702	25.034	26.276	0.004
Principal components	**Eigenvalue**		**Percentage of variance**		**Cumulative percentage of variance**			
PC1	4.817		60.215		60.215			
PC2	1.845		23.059		83.274			

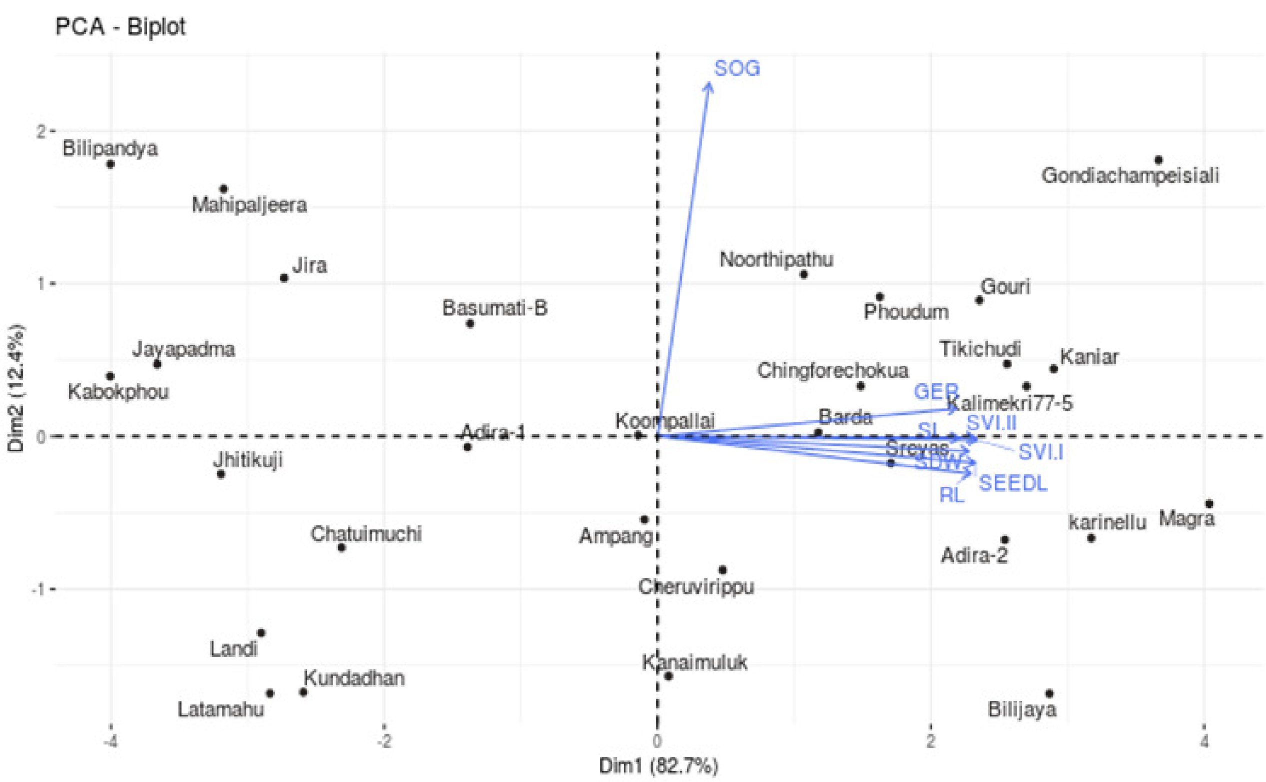

Fig. 5. Genotype by trait bi-plot of different seed vigour traits studied under moisture stress condition in 30 rice germplasm.

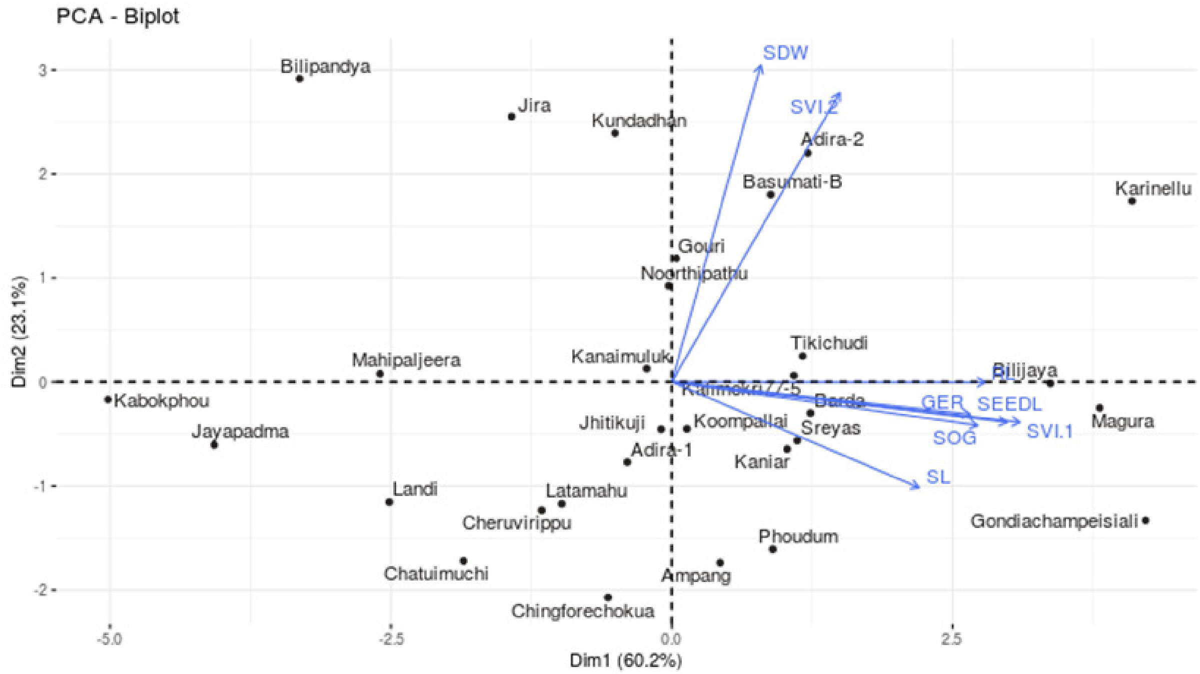

Fig. 6. Genotype by trait bi-plot of different seed vigour traits studied under control condition in 30 rice germplasm.

CONCLUSIONS

Drought is the foremost abiotic constraint on rice (*Oryza sativa* L.) production in India. Rice, a drought-sensitive crop, exhibits notable varietal variability concerning its drought tolerance, especially during the germination and seedling stages. The assessment of drought tolerance in field conditions is a time-consuming and labour-intensive procedure. In-vitro screening using polyethylene glycol is easy, as it facilitates more germplasm to be screened at once under a natural drought stress environment for further reliable and accurate results. Polyethylene glycol (PEG-6000) can be used as an alternative approach to artificially induce drought stress and enable the analysis of a diverse range of rice genotypes for the identification of drought tolerance in rice. The present study mainly examined the variability and correlation of various seed vigour-related physiological traits in thirty rice genotypes, specifically in response to moisture stress induced by 20% (w/v) solutions of polyethylene glycol (PEG)-6000 (osmotic potential of -0.49 Mpa). The results indicated that most rice genotypes exhibited a noticeable decrease in germination and seed vigour-related physiological parameters under moisture stress (MS) condition compared to the control (distilled water; 0 MPa). Among all the genotypes tested under MS condition, Karinellu was identified as the genotype with the highest germination potential (71.33%), while Magura exhibited the maximum seed vigour index I and II (774.17, 2.524). This study revealed significant genotypic variability among the seed vigour-related traits studied under MS condition, with the highest heritability exhibited by shoot length (99.8%), followed by seedling length (99.6%), root length (99.3%), seed vigour index I (99.2%), seed vigour index II (98.5%), speed of germination (98.3%), germination (95.3%), and seedling dry weight (91.0%). Out of the 30 genotypes tested under MS condition, 9 genotypes displayed the highest SVI-I, and 12 genotypes exhibited the highest SVI-II. The genotypes exhibited a significant amount of variation in every aspect of seed quality examined under MS condition. Principal component analysis (PCA) was performed to identify the most significant contributing variables for diversity, revealing the highest degree of variation by the first two principal components, that is, 82.67% and 12.44%, respectively. Subsequent correlation analysis illuminated strong positive relationships among these traits under the imposed MS condition. Remarkably, seed vigour index I showcased strong and positive correlations with germination (0.946), shoot length (0.948), root length (0.963), seedling length (0.982), seedling dry weight (0.940), and seed vigour index II (0.975). The identification of tolerant genotypes that exhibit a higher degree of variability in the seed vigour-related traits, particularly those that are strongly correlated with drought resistance, can be a valuable source of donor parent candidates in future drought tolerance breeding programs. Variability and correlation analyses demonstrated a more effective selection of target traits for improving seed vigour under moisture-stress situations.

FUTURE SCOPE

The study aims to identify the most superior genotype with high seed vigour under moisture stress condition.

Exploiting the potential of parent genotypes with a higher degree of tolerance may lead to the development of improved drought-tolerant rice varieties for future drought-tolerance breeding programs. Using polyethylene glycol (PEG) as a stress inducer in this experiment provides an opportunity to screen more germplasm and assess the ability of rice plants to survive under limited water availability, an essential trait for rice cultivation in regions with scarce water resources. By identifying genotypes with higher PEG tolerance, the study can offer insights into the genetic mechanisms underlying drought stress tolerance. Additionally, variability and correlation estimates could be useful in the better selection and improvement of seed vigour-related physiological traits under moisture stress condition.

Novelty Statement. In-vitro screening of rice germplasm for induced drought stress using polyethylene glycol (PEG) is a unique and rapid technique. This approach creates artificial drought conditions, which enables the rapid screening of a large quantity of rice germplasm for drought stress in a limited amount of time and with minimal laboratory space.

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