

EFFECTS OF DROUGHT STRESS ON HYBRIDS OF *VIGNA RADIATA* AT GERMINATION STAGE

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Drought is one the critical abiotic factors that reduces the germination, growth and yield of crops. Therefore the present project was designed with the objective to screen the best drought tolerant hybrid of *Vigna radiata*. Genetic variations for drought tolerance among these hybrids were assessed by simple and efficient technique. Seven hybrids of *V. radiata* (9801, 7002, 9706, 08003, 07007, 97012 and 08007) were used for screening against three levels of drought stress (zero, control), 5% (–0.05 MPa) and 10% (–0.1 MPa) induced by Polyethyleneglycol (PEG₆₀₀₀). A higher proline content was observed in 07007 (6.10 µg/g fresh weight) as compared to all other hybrids. Treated seedlings of each hybrid were compared with their respective control to evaluate the differences in their growth under drought. Different parameters such as percentage of germination, germination stress tolerance index (GSI), shoot and root weights (both fresh and dry) and lengths, root length stress index (RLSI), dry matter stress index (DMSI) and plant height stress index (PHSI) showed considerable variations. Germination percentage, shoot weights, PHSI and DMSI decreased in all hybrids along with the increase of PEG induced drought stress (5% and 10% PEG). In contrary, root weights and RLSI were increased under drought. Overall 07007 showed a better performance, and can therefore be classified as a drought tolerant hybrid.

Keywords: Water stress – germination stress tolerance index – PEG – legumes – dry matter stress index

INTRODUCTION

In any region of the world, drought is among the most important stress factors that reduces crop production and has become a more severe problem during the last few decades [16, 24]. It reduces the average yield of crop plants by more than 50% [13]. The severity of damage caused by drought varies amongst different crops and at different growth stages [17]. In many crops, the seeds germination is one of the critical phases of plant life which is greatly influenced by drought [3, 22, 32], particularly water uptake by dry seeds. So, drought plays a crucial role in determining the rate of germination and the establishment of seedlings [6, 25]. Water stress reduces the rate of water uptake by germinating seeds as has been observed in a number of crop

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species such as in *Lycopersicon esculentum* [36], *Triticum aestivum* [4], Sorghum [12], *Helianthus annuus* [30], *Zea mays* [11] and *Pisum sativum* [28]. Furthermore, drought stress decreases both percentage and the rate of germination [15]. Several experiments have shown that plumule is more sensitive to water stress than any other stress [1].

The selection of different varieties/cultivars based on their drought tolerance has been considered to be a cost effective and efficient way of minimizing agricultural problems [5]. The tolerant cultivars have adaptations for the limited water usage [34]. It is very difficult to screen the water stress resistant varieties under field conditions. Hohl and Peter [18] reported that, *in vitro* screening approach is a potential economic method to screen large set of germplasm accurately in less time. Richards [33] also suggested that germination is a useful criterion for screening of water stress tolerance. The strategy of development to screen different varieties according to their tolerance to drought was based on the measurement of shoot and root moisture contents and dry matter.

Among the germination experiments, the most common is the germination of seeds under the PEG stress. It is a non-ionic polymer soluble in water and does not penetrate in the plant tissues quickly, therefore it is used commonly to create drought stress in higher plants [8, 40]. To maintain constant water potential, PEG has been used to simulate osmotic stress in germination trays of seeds throughout the experiment [23]. This water stressed environment created artificially, gave the opportunity to select tolerant genotypes out of a large population [29]. It was demonstrated that the varieties that were tolerant to water stress during the *in vitro* germination tests were equally tolerant in field conditions [2].

Leguminosae is the second most important family after Poaceae, as it provides about 27% of the world crops production [8]. Legumes provide approximately 30% of the daily life protein for human consumption, as well as carbohydrates and lipids. They are also used for production of oils and for forage and fodder for animals [37]. Besides their nutritional importance, legumes are also used successfully in the rotation of crops, that helps improve fertility status of soil through nitrogen fixation. In this relationship nitrogen-fixing bacteria (*Rhizobium*) and roots of higher plants reduce the atmospheric nitrogen to plant available ammonium [28]. To support the awareness about the importance of this, the United Nations declared 2016 as the International year of pulses [27].

In developing countries water availability to agriculture decreased from 87% to 73% during the last three decades [31]. In this situation, it is a race to adopt strategies to get maximum crop productions under stressful environment. Therefore, the present study was performed with the aim to determine the germination responses of *Vigna radiata* hybrids/breeding lines to water stress by using the PEG technique and to screen the best tolerant hybrids among different hybrids that can be grown successfully in drought prone areas.

MATERIALS AND METHODS

The experiment was conducted in a rain protected wire-house of Shaanxi Normal University, Xi'an, China. Seeds of seven cultivars of *Vigna radiata* (09801, 07002, 9706, 08003, 07007, 97012 and 08007) were obtained from NARC, Islamabad, Pakistan. Seeds of all hybrids were sterilized with 0.1% sodium hypochlorite before germination. Seeds of each hybrid were allowed to germinate in plastic trays (90 × 30 cm), tetra lined with filter paper supplemented with Rorrison nutrient solution with 0, 5% (−0.05 MPa) and 10% (−0.1 MPa) of PEG₆₀₀₀ (Polyethyleneglycol Fisher, England) to simulate drought stress and the maintenance of uniform osmotic potentials. Twenty seeds of each *Vigna* hybrid were planted in each of the filter paper lined labeled plastic trays. The experiment was carried out in a completely randomized design under laboratory conditions at (32 ± 3 °C). Each treatment was replicated thrice. Varying levels of water stress was maintained by adding 160 ml of different concentrations of PEG₆₀₀₀ in plastic trays, respectively. The number of germinated seeds of each cultivar was counted on a daily basis for a period of 10 days. When both radicle and plumule length were approximately 5 mm, the seeds were considered germinated. Germination rate (cumulative through time) was determined by the following [9] for each cultivar. At the end of the experiment, seedlings were harvested and separated into roots and shoots. Fresh weights of shoot and root were recorded and then oven dried at 70 °C for one week to measure dry weights after complete desiccation.

From the above seedlings the Promptness index (PI), root length stress index (RLSI), germination stress tolerance index (GSI), dry matter stress tolerance index (DMSI), plant height stress index (PHSI) were calculated by following [3].

$PI = nd_2 (1.00) + nd_4 (0.75) + nd_6 (0.5) + nd_8 (0.25)$ where n is the number of seeds germinated at day d

$$GSI = \frac{\text{P.I. of stressed seeds}}{\text{P.I control seeds}} \times 100,$$

$$PHSI = \frac{\text{Plant height of stressed plant}}{\text{Plant height of control plants}} \times 100,$$

$$PLSI = \frac{\text{Root length stressed plant}}{\text{Root length of control plants}} \times 100,$$

$$DMSI = \frac{\text{Dry matter of stressed plant}}{\text{Dry matter of control plants}} \times 100.$$

Proline contents were measured by the method described by Bates et al. [10] and expressed as $\mu\text{g/g}$ fresh weight.

Analysis of variance (ANOVA) was done with above data by using CoStat v 6.3 (CoHort, California, Berkely USA) statistical package. Means were also compared by the method of Snedecor and Cochran [38] and using the least significance difference.

RESULTS

Germination rate

Osmotic potentials affected significantly the germination percentage of hybrids of *V. radiata* and their interactions (Tables 1, 2). Germination of all hybrids started one day after sowing. Different hybrids reached its full germination at 0% PEG at different time intervals. Increased PEG concentrations considerably reduced the germination percentage of all hybrids relative to their controls (Fig. 1). 07007 and 08007 showed maximum germination (95%) at -0.05 MPa, while 07002 and 09706 showed minimum germination (50% and 60%, respectively). At the -0.1 MPa level of stress 07007 and 08003 hybrids had highest germination (90% and 70%, respectively) and 07002, and 97012 showed remarkably reduced and delayed germination percentage (20% and 50%, respectively).

Table 1

Analysis of variance (ANOVA) of the data for germination days of seven cultivars of *Vigna radiata* under different concentrations of PEG for 10 days

Source	DF	MS of Germination
Days	2	3456.76***
PEG	2	3949.96***
Cultivar (Cvr)	6	132.34***
Days * PEG	4	484.06***
Days * Cvr	12	42.73**
PEG * Cvr	12	41.72**
Days * PEG * Cvr	24	20.11ns
Error	124	17.17

Table 2

Analysis of variance (ANOVA) of the data of mean square for germination days of seven cultivars of *Vigna radiata* under different concentrations of PEG for different days

Source	DF	Germination day (1)	Germination day (2)	Germination day (3)
PEG	2	3737.39***	771.30***	409.39***
Cultivar (Cvr)	6	170.32***	26.92ns	20.57 ns
PEG * Cvr	12	36.35ns	29.07ns	16.50 ns
Error	42	19.80	16<-	16.25

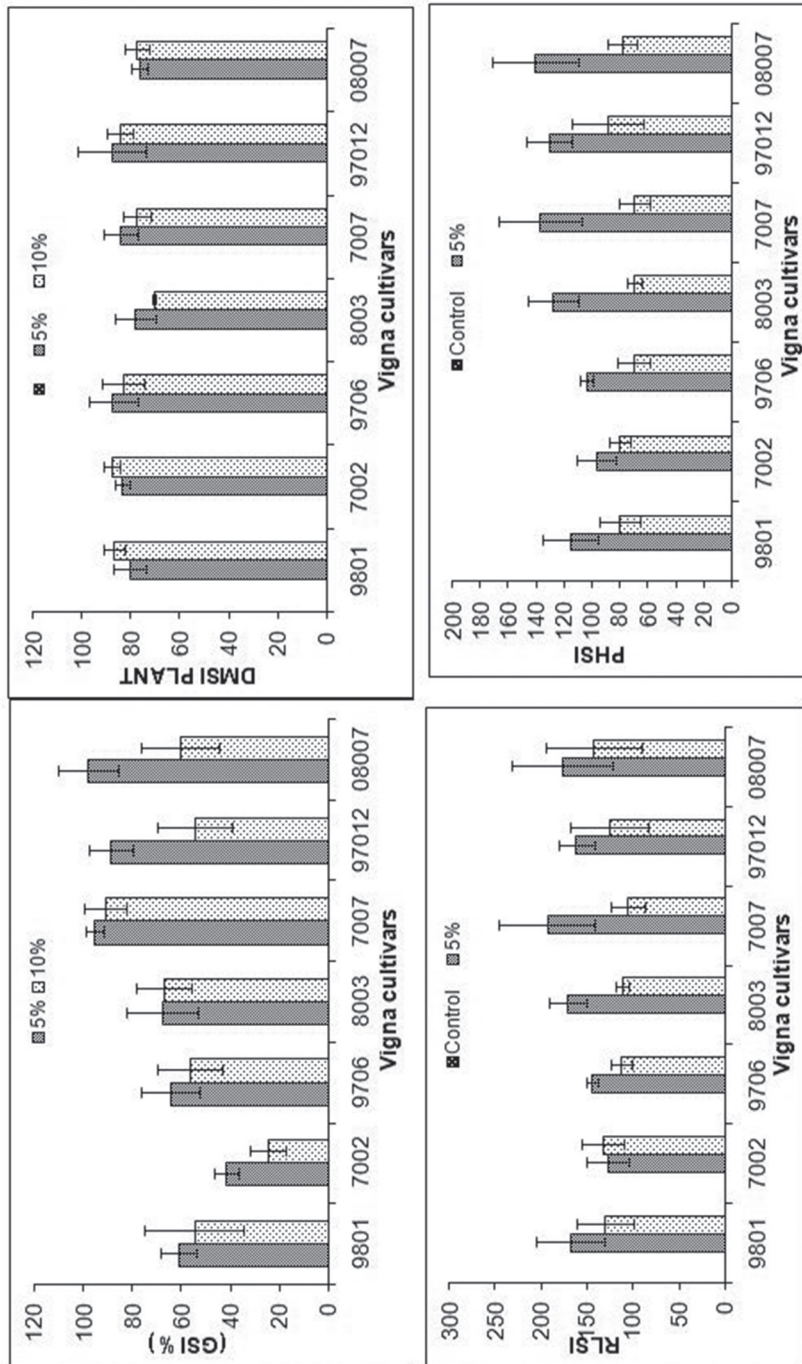


Fig. 1. Effect of different PEG concentrations on germination stress tolerance index (GSI), Dry matter stress index (DMSI), root length stress index (RLSI) and plant height stress index (PHSI) of seven cultivars of *Vigna radiata*

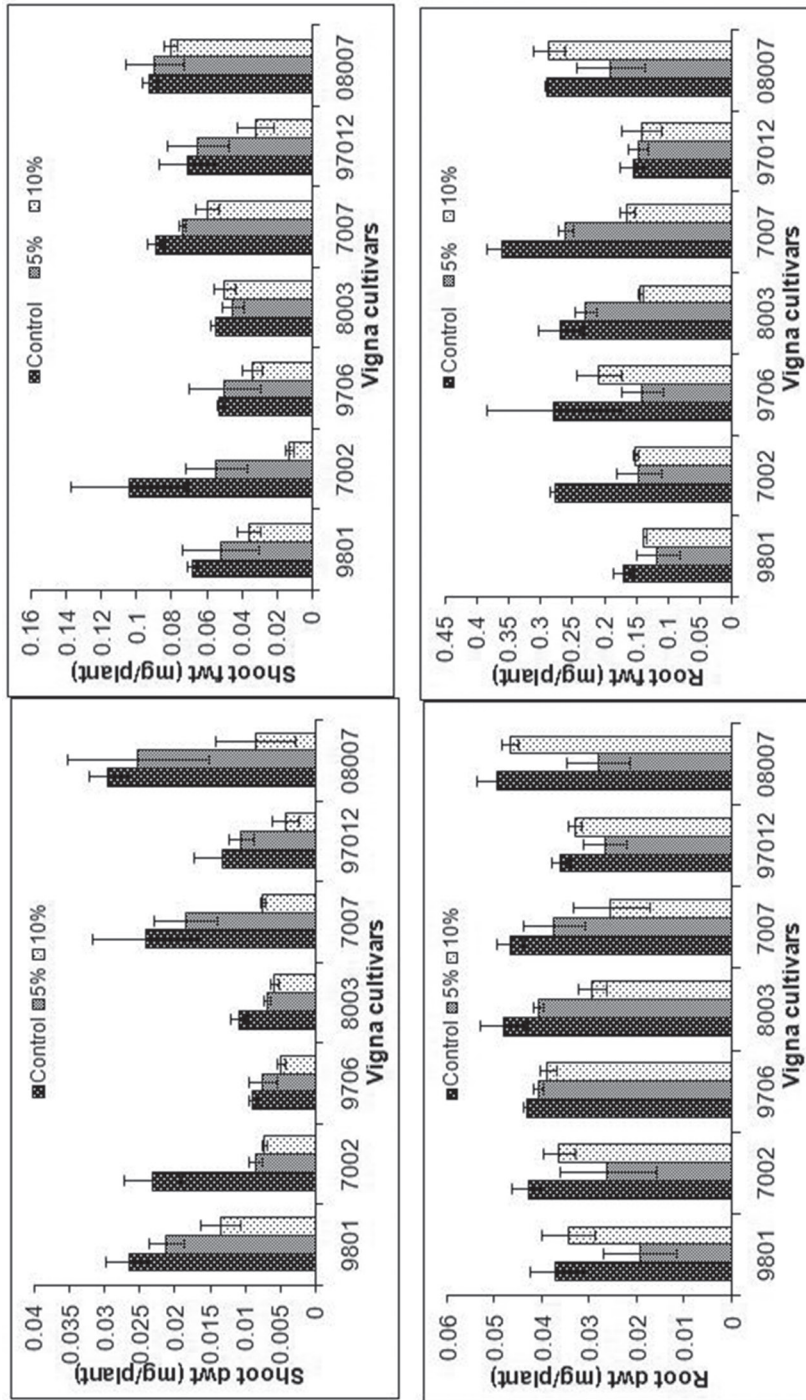


Fig. 2. Effect of different PEG concentrations on shoots and root lengths, shoots and roots fresh and dry weights of seven cultivars of *Vigna radiata*

Fresh and dry biomass

The analysis of variance revealed the significant effects of drought stress and the interaction of PEG concentration with hybrids on shoot weight (Table 3). At different concentration of PEG, the hybrids showed different shoot fresh and dry weights.

09801, 07007 and 08007 hybrids had higher weights, both fresh and dry, at -0.05 MPa stress, compared to the rest of hybrids that exhibited greater reduction in weights. However, the reduction in both fresh and dry weights was hybrid specific (Fig. 2). At higher concentration of PEG (10%), all hybrids showed a decline in weight but this reduction was more prominent in 07002, 09706, 08003 and 97012.

The effect of PEG stress was significant on root fresh and dry weights but the hybrids and their interaction were not significant according to the overall analysis of variance (Table 3).

Stress Indices

PEG significantly influenced the DMSI and GSI (Table 4). Increase in stress level decreased the DMSI of shoot in all hybrids, while DMSI of root increased with increase concentration of PEG (Fig. 3). PEG in all cultivars reduced germination significantly due to imposition of drought stress (Table 3). The minimum values of GSI were recorded at 10% PEG that were significantly lower than at 5% concentration. Among all the hybrids 08003 showed maximum GSI at both concentrations.

Table 3

Analysis of variance (ANOVA) of the data of mean square for fresh and dry biomass of shoots and roots of seven cultivars of *Vigna radiata* under different concentrations of PEG for 10 days

Source	DF	Shoot Fresh Wt	Shoot Dry Wt	Root Fresh Wt	Root Dry Wt
PEG	2	181199.3***	935.19***	1307.62***	20.07***
Cultivar (Cvr)	6	492.264ns	7.60ns	15.90ns	0.39ns
PEG * Cvr	12	519.08571*	12.63ns	9.45*	0.26ns
Error	42	250.59	6.82	4.87	0.22

Table 4

Analysis of variance (ANOVA) of the data of mean square for germination stress tolerance index (GSI), plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI) and dry matter stress tolerance index (DMSI) of seven hybrids of *Vigna radiata* under different concentrations of PEG for 10 days

Source	DF	GSI	PHSI	RLSI	DMSI
PEG	1	19543.28***	23679.22***	57550.55***	14971.4***
Cultivar (Cvr)	6	199.01ns	158.76***	133.91ns	170.70ns
PEG * Cvr	6	279.37ns	115.88**	404.05ns	93.82ns
Error	28	180.25	23.00	195.01	123.94

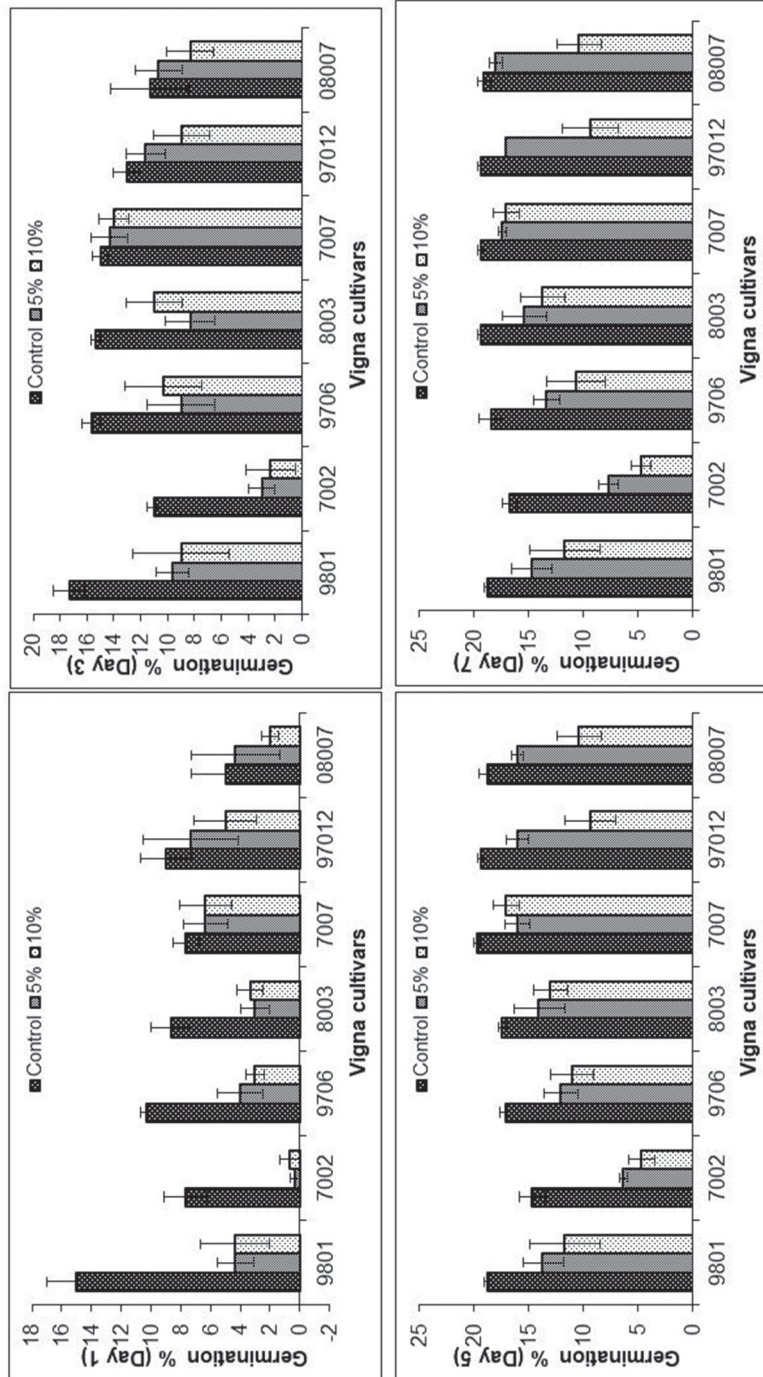


Fig. 3. Effect of different PEG concentrations on different days of germination of seven cultivars of *Vigna radiata*

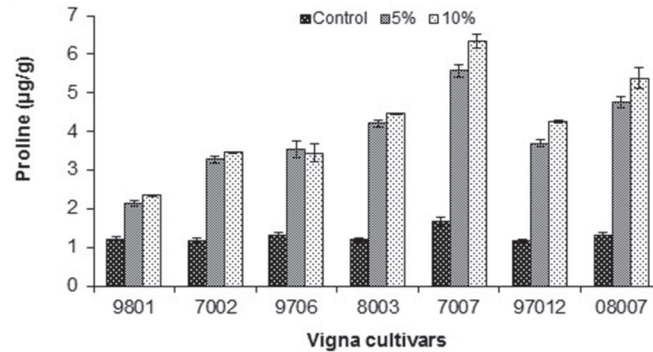


Fig. 4. Effect of different PEG concentrations on proline contents of seven cultivars of *Vigna radiata*

A significant effect of PEG, inter-hybrid variation and their interaction for plant height stress index was found (Table 4). All cultivars showed reduction in PHSI on imposition of stress however 07007 and 08007 hybrids showed maximum PHSI at 5% PEG concentration, mean while 09801, 07002 and 97012 had largest PHSI at 10% concentration. RLSI of all the cultivars was reduced significantly due to drought stress and this became more evident at 10% PEG concentration (Fig. 4). Although not significant but 07007 showed maximum RLSI than the rest of the hybrids.

Higher proline contents were also observed in 07007 as shown in Table 5.

Table 5

Analysis of variance (ANOVA) of the data of mean square for Proline contents of seven hybrids of *Vigna radiata* under different concentrations of PEG for 10 days

Source	DF	Proline
PEG	1	27341.15***
Cultivar (Cvr)	6	201.01*
PEG * Cvr	6	305.42*
Error	28	179.03

DISCUSSION

In order to create uniform osmotic stress, polyethylene glycol (PEG) could be used as a strong water stress simulator as it reflects the type of stress created by a drying soil [14]. PEG-6000 was also used to create the same drought stress in the present investigation as most of the scientists [19] used it for the creation of water deficit environment under laboratory conditions. In a stressful environment, development of a crop mainly depends on the germination of seeds and the establishment of seedlings. The germination process consists of two stages; first comes the enzymatic hydrolysis of stored food material and the second step is the formation of new tissues

by hydrolysis [17]. Water shortage affects enzymatic activity and, in turn the percentage of germination, cell division and embryo formation, and radicle and plumule elongation also decreases a more negative osmotic potential [7]. Therefore, it is necessary to develop effective screening criteria at early growth stages to get the maximum yield [9].

In the present study, hybrids showed impressive variation in their tolerance to drought stress produced by PEG-6000, regarding germination rate, shoot and root weights, GSI, PHSI and DMSI. All these parameters decreased remarkably when applying increasing PEG-6000 concentrations. Many other researchers have also described similar results, revealing that increased concentrations of PEG severely reduced seeds germination and seedling establishment, as it hampered the water availability [36]. RLSI increased at -0.05 MPa in all cultivars, although different hybrids showed different performance under stress environment [22]. The reason of the increase in root length was that roots are the first place to encounter water stress and as an adaptive strategy in water stress tolerance plants reduced shoot growth and promoted the root growth [12]. In addition, longer roots might be supported through the accumulation of biomass by absorbing water from the medium under limited water conditions [5]. According to Rehman et al. [31], the length of roots at the seedling stage can be used as to estimate their growth under field conditions. Ahmad et al. [3] suggested GSI as a powerful drought tolerance measurement parameter to screen large germplasm. The variations in the hybrid performance assessed by growth of seedlings (i.e. dry biomass of shoot a root, root length and plant height) showed that seedling growth is an efficient and a reliable information when screening large number of hybrids for water stress tolerance. The more the degree of drought (i.e. 10%) the more reduction in plant biomass and plant height is found. Several studies are consistent with the present results, indicating that water stress significantly decreased the biomass and growth of higher plants [35]. Certain varieties of the same species have the genetic potential to keep the higher germination rate and growth under drought, hence are considered as drought tolerant. Growth tolerance indices and germination percentages can aid in finding this genetic potential in diverse germplasm. The results of this study are similar to those of other experiments done on different plants, i.e. such as in chickpea [20], wheat [39], [21] sunflower and *Zea mays* [11, 22]. The proline content was higher in an intolerant hybrid which has the ability to withstand the drought tolerance. Naidu et al. [26] also observed an increase in the proline content of *Vigna radiata* when exposed to drought stress.

By measuring seedling traits contributing to drought tolerance screening, it was found that 07007 is the most resistant and 07002 is the most sensitive hybrid. Based on the present investigations, it can be concluded that the screening of hybrids can be performed at germination stages by using correlated attributes of germination, plant biomass and various stress indices under drought conditions. Selection of these criteria would be more economical, would need less time and less laboratory work to screen the large germplasm at early growth stage. Our present findings may help *Vigna* breeders by providing a guideline for the cultivation of more tolerant hybrids in water stress areas.

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