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INDIVIDUAL EFFECT OF DROUGHT AND HIGH TEMPERATURE ON SEED GERMINATION AND SEEDLING GROWTH OF GROUNDNUT (*ARACHIS HYPOGAEA* L.)

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ABSTRACT

Germination and seedling development in plants are crucial stages in their life cycle, strongly influenced by temperature and moisture conditions. This study aimed to investigate the germination and seedling development of Groundnut (*Arachis hypogaea* L.) seeds under various abiotic stresses.

Sixty groundnut genotypes were utilized in this study. Standardization was performed using PEG (Poly Ethylene Glycol) for inducing drought stress and TIR (Temperature Induction Response) for simulating high-temperature lethal conditions. The genotypes were evaluated for germination characteristics and seedling growth under drought and high-temperature stress.

Groundnut genotypes were screened for drought (-6MPa) and thermotolerance (52°C) under lab conditions. Genotypes VG 17046, VG 18005, VG 18096, VG 18102, VG 18111, VG 19572, VG 19620, VG 19681, VG 19688, and VG 19709 showed higher germination, vigor, and stress tolerance. Similarly, VG 17008, VG 17022, VG 18049, VG 18077, VG 18096, VG 18102, VG 18058, VG 19561, VG 19572, VG 19576, VG 19620, and VG 19709 exhibited survival rates over 50% and increased proline content under thermotolerance screening. Stress conditions adversely affected seedling growth, emphasizing genotype tolerance identification.

***Key words*:** *Arachis hypogaea* , drought, high temperature, seed germination.

Introduction

In rainfed Indian groundnut cultivation covering 65% of total land, drought stress exacerbated by high temperatures is common (Dai *et al*., 2015). Evaluating thermo-tolerance is vital for crop enhancement. Temperature Induction Response (TIR) method, assessing stress-responsive gene expression during peak stress (Kheir *et al*., 2012), is efficient for identifying thermo-tolerant germplasm. Seedling responses to drought and high temperature are crucial for survival and growth. Germination, pivotal in plant life cycles, establishes vigorous seedlings (Farooq *et al*., 2009). Drought or high-temperature stress at any stage can harm crop productivity. Soil moisture initiates embryo development, but water scarcity hampers cell growth (Kaya *et al*., 2006; Farooq *et al*., 2009). Polyethylene glycol mimics drought stress by maintaining soil drying osmotic potential (Larher *et al*., 1993). *In vitro* methods are cost-effective for germplasm evaluation (Manoj and Uday, 2007). Drought stress impacts pea, alfalfa, and rice seedling growth (Okcu *et al*., 2005; Manickavelu *et al*., 2006; Zeid and Shedeed, 2006). Increasing stress intensity decreases germination and growth (Ahmad and Paswan, 2008; Wafa'a *et al*., 2010). Osmotic stress from PEG hinders lentil and mung bean germination (Aniat-Ul-Haq and Agnihotri, 2010; Garg, 2010). Wheat seedlings under drought show reduced growth (Khayatnezhad and Gholamin, 2011). Higher root vigor indicates stress adaptability (Sadasivam *et al*., 2000). PEG-exposed tomato seedlings exhibit reduced growth rates (Aazami

et al., 2010). Groundnut cultivars vary in drought response (Singh *et al*., 2007). Gradual exposure to severe temperatures indicates thermotolerance in groundnut (Gangappa *et al*., 2006). Rapid acquired thermotolerance involves cellular acclimation (Hikosaka *et al*., 2006). High temperatures trigger acclimation mechanisms enhancing survival (Senthil Kumar *et al*., 2003). Heat shock proteins and amino acids reduce cellular damage (Kumar *et al*., 1998; Srikanthbabu *et al*., 2002). Rice seedlings under TIR show reduced MDA levels and increased proline content (Sung *et al*., 2003).

Materials and Methods

Standardization of drought stress using Polyethylene glycol (PEG 6000)

Groundnut varieties VRI 2, VRI 6, VRI 7 and VRI 8 were used for the standardization of drought stress by using polyethylene glycol (PEG 6000). Healthy seeds of uniform size were surface sterilized with 0.1% Mercuric chloride ($HgCl_2$) for 2-3 min and then washed thoroughly with distilled water. Twelve sterilized seeds were sown in Petri dishes containing moistened blotting paper with various water potentials *viz*., 0.0 (control), -0.5, -0.6, -0.7 and -0.8 MPa of PEG 6000. Five replications were maintained for each treatment.

Seed germination percentage was determined by counting germinated seeds every alternate day from day 6 to day 12 after sowing, with the emergence of a 2mm radicle set as the criteria, while seedling growth parameters were recorded on the 8th day.

Promptness Index (PI) and Germination stress index (GSI) (Maiti *et al.*, 1994).

PI (%) = nd2 (1.00)+ nd4 (0.75)+ nd6 (0.5)+ nd8 (0.25)

PI is promptness index, nd is the number of seeds germinated on the day of observation which is mentioned next to 'nd' in the formula (George, 1967)

$$\text{GSI (\%)} = \frac{\text{PI of stressed seeds}}{\text{PI control seeds}} \times 100$$

Root length stress index (RLSI) and Seed vigour (SV) were measured using the formula.

$$\text{RLSI (\%)} = \frac{\text{Root length of stressed plant}}{\text{Root length of control plants}} \times 100$$

SV (%) = Germination percentage × Seedling length.

Screening of groundnut genotypes for drought stress tolerance at the seedling stage

Groundnut genotypes were evaluated for drought stress tolerance with -0.6 MPa PEG 6000 treatment inducing 50% mortality. Seeds were sown on moist blotting paper (0.0 MPa control, -0.6 MPa treatment). Three replications with 12 seeds each were maintained. Plumule and radicle lengths, seed vigor, germination stress index (GSI), and root length stress index (RLSI) were recorded on the 8th day in ten randomly selected seedlings per replication.

Standardization of high-temperature stress using Temperature induction response (TIR)

Temperature induction response (TIR) assesses seedling thermo-tolerance by subjecting germinated groundnut seedlings to induced high temperatures, then exposing them to lethal temperatures for a set period. After stress induction, seedlings recover at room temperature. Survival percentage post-recovery determines thermo-tolerant genotypes. (Gangappa *et al*., 2006).

Four groundnut varieties such as VRI 2, VRI 6, VRI 7, and VRI 8 were used for the standardization of OIT and lethal temperature.

Groundnut seeds were surface sterilized with 0.1% $HgCl_2$ for 2-3 min, washed with sterile distilled water and two seeds were sown in plastic juice cups filled with vermicompost, sand, and coir pith mixture. Three replications were maintained for each variety. Three-day-old seedlings were exposed to temperatures ranging from 49, 51, 53, 55 and 57°C with 60% RH in a controlled Plant Growth Chamber to determine survival percentages.

OIT was determined by exposing seedlings to gradual temperature increases temperature at the rate of 2°C per h (38-44, 44-50, 46-52 and 48-54°C) followed by 3-hour exposure to challenging temperatures. Seedlings recovered at 30°C, 60% humidity for 72 hours. Survival percentage was calculated at the end of the recovery period using a specific formula.

Screening of groundnut genotypes for high-temperature stress tolerance at seedling stage

After the standardization of OIT and challenging temperature, the seedlings of 60 groundnut genotypes were exposed to OIT of 46-52°C, @ 2°C rise per h and then the seedlings were exposed to a challenging temperature of 57°C for 3 h and were allowed to recover at 30°C for 72 h to identify the high-temperature tolerant groundnut genotypes by TIR technique (Temperature induction response).

$$\text{Survival of seedlings (\%)} = \frac{\text{No. of seedlings survival}}{\text{Total no. of seedlings}} \times 100$$

Proline content

Leaf samples were homogenized in 3% sulfosalicylic

acid and centrifuged at 11,500 rpm. The supernatant was mixed with acid ninhydrin, glacial acetic acid, and phosphoric acid, then incubated at 100°C for 1 hr. Toluene extraction yielded a pink colour upper layer for proline content determination at 520 nm in UV-VIS spectrophotometer (Eppendorf BioSpectrometer kinetic) at 520 nm (Bates *et al.*, 1973).

Results

Standardization of drought stress by using PEG 6000 (Fig. 1, 2, 3, 4, 5, 6 & 7)

A standardization experiment was set up with the groundnut varieties VRI 2, VRI 6, VRI 7 and VRI 8 to determine the stress level at which the groundnut varieties were screened. These varieties were germinated at different water potential levels *viz.*, 0 MPa, -0.5 MPa, -0.6 MPa, -0.7 MPa and -0.8 MPa. In general, the gradual reduction in germination percentage, promptness index, plumule and radicle length, seed vigour, germination stress index and root length stress index were observed with decreased water potential.

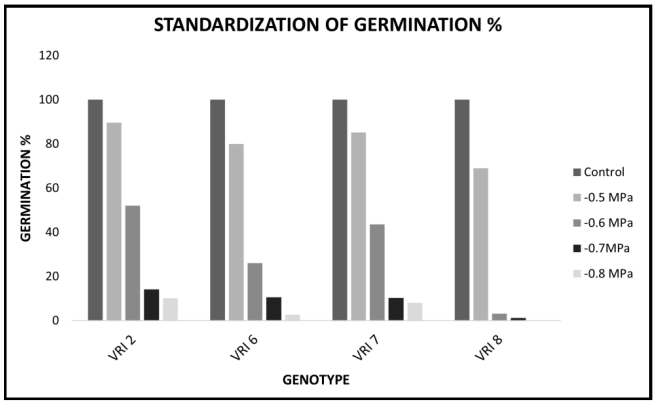

Fig. 1: Standardization of germination percentage (%) of groundnut varieties.

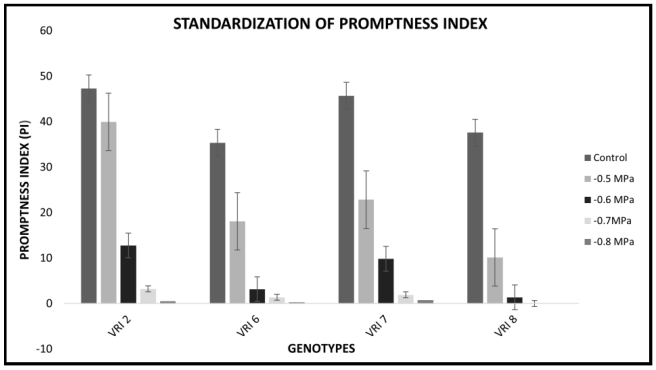

Fig. 2: Standardization of promptness index of groundnut varieties.

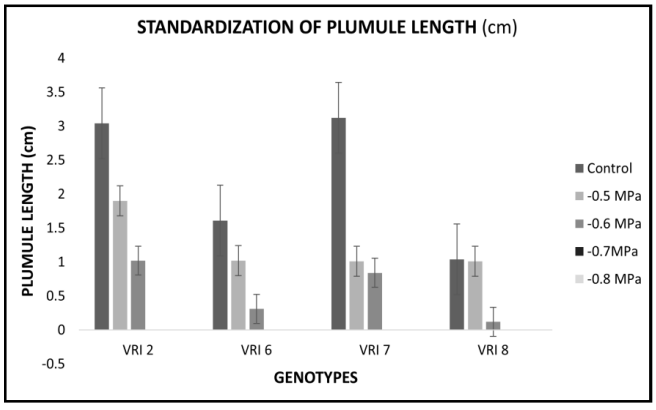

Fig. 3: Standardization of plumule length (cm) of groundnut varieties

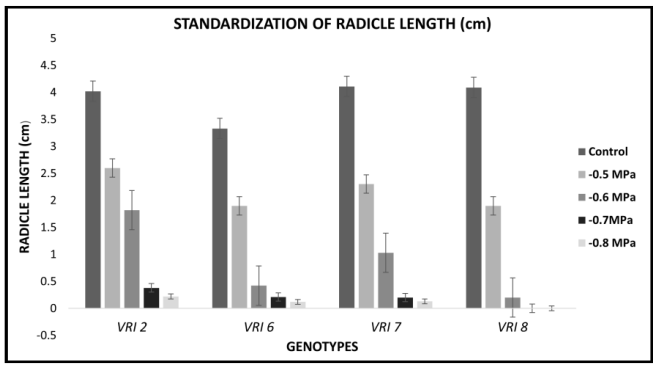

Fig. 4: Standardization of radicle length (cm) of groundnut varieties.

A significant reduction in seedling growth characters was observed at -0.6 MPa, where the germination percentage was higher in VRI 2 (52.12%) and it was lower in VRI 8 (1.21 %). The Promptness index was also higher in VRI 2 (12.76) and lower in VRI 8 (1.31) at -0.6 MPa.

Screening of groundnut genotypes for drought stress

Groundnut genotypes were screened for drought tolerance using a water potential of 0.6 MPa as a higher tolerance level to drought. Parameters including germination percentage, promptness index, plumule and radicle length, seed vigor, germination stress index, and root length stress index along with control (distilled water) were recorded on 10th days after sowing.

Impact of drought stress on germination percentage (%) in groundnut genotypes during seedling stage

The germination percentage of groundnut genotypes

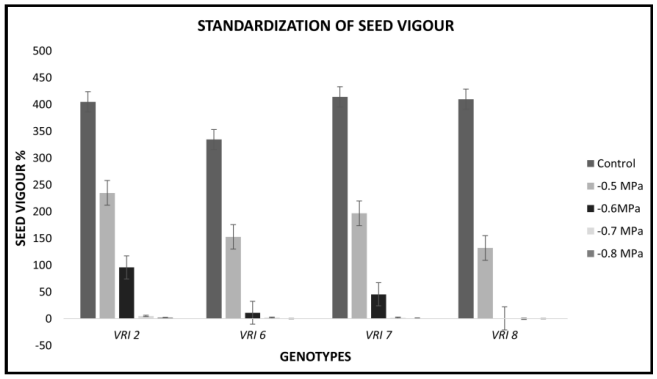

Fig. 5: Standardization of seed vigour of groundnut varieties.

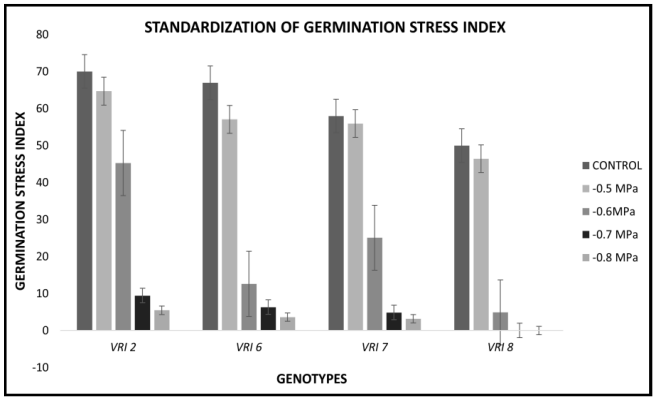

Fig. 6: Standardization of germination index of groundnut varieties.

was observed at -0.6 MPa. The results revealed that the groundnut genotypes, VG 18005 (63.56 %), VG 18077 (60.61%), VRI 2 (59.23 %), VG 18103 (58.37 %), VG 19709 (58.22%), VG 19572 (58.12 %), VG 18096 (57.49 %), VG 19681 (56.94 %), VG 18076 (53.89 %), VG 17046 (52.53 %), VG 19620 (52.33%), VG 18111 (50.82 %), VG 18097 (50.66 %), VG 19732 (50.43 %), VG 18102 (50.35 %) and VG 19688 (50.19 %) were recorded the higher germination percentage respectively. No germination was observed in VG 19719, VG 19654, VG 19548, VG 19539, VG 17017, VG 17019, VG 18081, VG 18090, and VG 18100 at -0.6 MPa. However, the rate of germination varied among the genotypes from -0.5 to 0.6 MPa.

Impact of drought stress on promptness index (PI), radicle length (cm), plumule length (cm) and seed vigour in groundnut genotypes during seedling stage

Promptness index was higher in VG 17046 (28.67), VG 18103 (26.92), VG 19688 (24.25), VG 18005 (23.75), VG 19732 (22.33), VG 19709 (21.00), VG 17013 (21.00), VG 18055 (20.42), VG 18111 (19.83) and VG 18102 (19.17) respectively at -0.6 MPa. The radicle length and seed vigour were recorded. The highest radicle length was observed in VG 19681 (1.90 cm) followed by VG 18102 (1.89 cm) and VG 17003 (1.83 cm). The highest

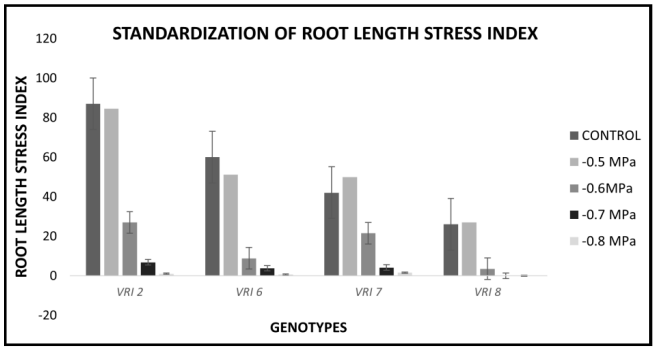

Fig. 7: Standardization of root length stress index of groundnut varieties.

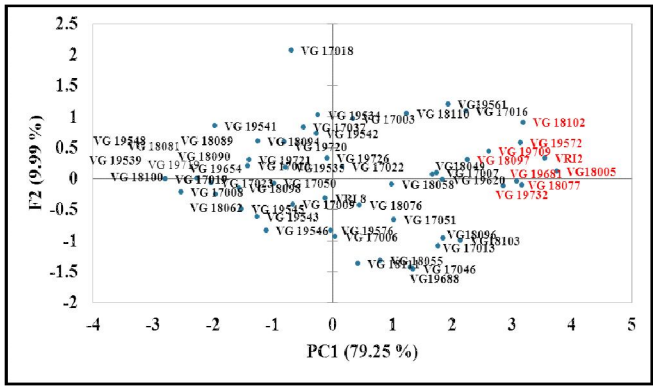

Fig. 8: PCA for the seedling character during drought stress.

plumule length was recorded in VG 18110 (1.92 cm) followed by VG 19561 (1.90 cm).

Seed vigour was decreased significantly in all the genotypes at -0.6 MPa, however, the genotypes VG 18077 (114.51) and VG 18005 (111.59) showed higher seed vigour as compared to other genotypes.

Impact of drought stress on germination stress index (GSI) and root length stress index (RLSI) in groundnut genotypes during seedling stage (Fig. 8)

The germination stress index and root length stress index were calculated for different groundnut genotypes at -0.6 MPa. The GSI was higher in VG18103 (58.10), VG 17046 (58.01), VRI 2 (56.18), VG18005 (52.49) and VG 19688 (51.05) and the genotypes which indicated a higher level of tolerance to drought stress. The root length stress index was also higher in VG 18096 (49.35) and VG 19542 (46.42).

Screening of groundnut genotypes under high temperature by using temperature induction response studies (TIR)

Standardization of optimum induction temperature (OIT) and lethal temperature

Based on the recovery growth of groundnut seedlings, the OIT was identified between 46-52°C with a rise of 2°C per hr for 5 hrs. Nearly 50% of mortality was

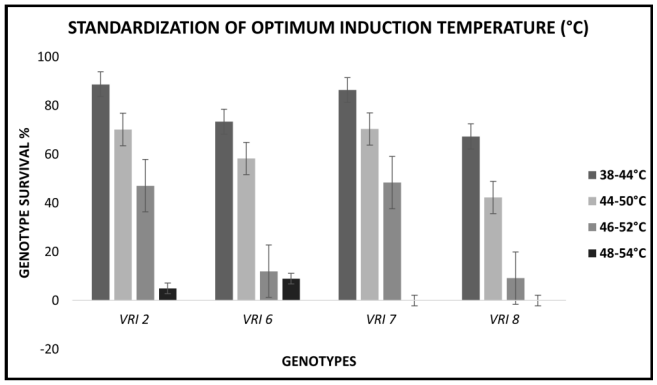

Fig. 9: Standardization of optimum induction temperature (°C) of groundnut varieties.

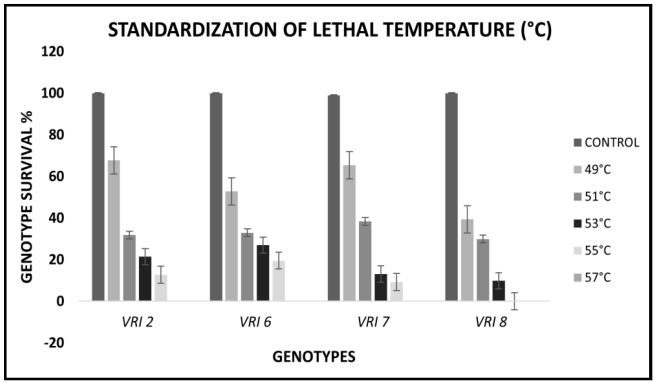

Fig. 10: Standardization of lethal temperature (°C) of groundnut varieties.

observed at this temperature (46-52°C). The survival percentage was higher in VRI 7 (48.43%), VRI 2 (47.13 %) and lower in VRI 8 (9.20%). About 90 % mortality was observed at 57°C in all groundnut varieties had been fixed as a lethal temperature.

Impact of high-temperature stress on survival percentage (%) in groundnut genotypes during the seedling stage

The sixty groundnut genotypes were exposed to OIT and lethal temperature to screen and identify the temperature-tolerant genotypes based on the standardization.

The genotypes VG 17008, VG 17010, VG 17019, VG 17050, VG 17051, VG 18090, VG 18081, VG19535, VG 19654, VG 19548, and VG 19541 had shown, zero percent survival were identified as highly susceptible to OIT and lethal temperature. The groundnut genotypes *viz*., VG 18102 (59.51), VG 19620 (58.33), VG 19561 (58.00), VG 18005 (57.67), VG 18077 (56.67), VG 18096 (54.56), VG 19576 (53.61), VRI 2 (53.12), VG 18058 (51.61), VG 19572 (51.02), VG 17022 (50.62), VG 19709 (50.33), VG 18049 (50.00) and VG 17009 (50.00) were showed the higher survival percentage (>50%) among the screened genotypes.

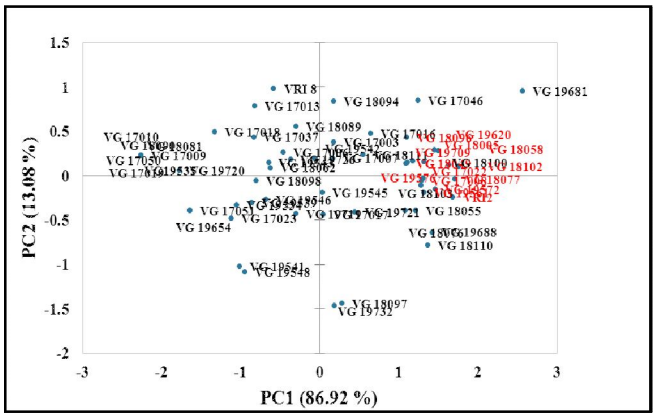

Fig. 11: PCA for the seedling character during high-temperature.

Impact of high-temperature stress on proline content in groundnut genotypes during seedling stage (Fig. 11)

Proline content was analyzed in all the seedlings exposed to OIT and lethal temperature. Significant differences in proline content were observed among the groundnut genotypes. The following genotypes recorded higher proline content VG 18102 (4.47 µM/g FW), VG 19561 (3.87 µM/g FW), VG 19688 (3.81 µM/g FW), VG 18058 (3.73 µM/g FW), VRI 2 (3.70 µM/g FW), VG 18077 (3.55 µM/g FW), VG 19732 (3.47 µM/g FW),VG 19572 (3.44 µM/g FW), VG 19681 (3.44 µM/g FW), VG 19576 (3.43 µM/g FW), VG 19561 (3.35 µM/g FW), VG 18103 (3.25 µM/g FW), VG 17022 (3.21 µM/g FW), VG 19620 (3.11 µM/g FW) and VG 18005 (3.05 µM/g FW). The genotype VG 18076 (3.32 µM/g FW) also recorded the higher proline content however, the survival percentage was lower when compared to other genotypes.

From this observation in laboratory studies 20 groundnut genotypes were identified as drought and high-temperature tolerant genotypes through experiments I and II during the seedling stage.

Discussion

Groundnut genotypes screening for drought stress at the seedling stage

Drought stress is a major constraint for crop growth and development. The reduction in plant growth is the primary effect of drought stress (Sapeta *et al.*, 2013). Groundnut genotypes were screened for drought stress using PEG 6000. The lower water potential during germination inhibits seed germination and suppresses the growth and development of seedlings (Kaur *et al.*, 2017).

The study confirmed that drought stress during the seedling stage impacts groundnut genotype germination rates, as noted by Redona and Mackill (1996). Reduced water potential led to decreased germination percentages, consistent with Steiner *et al*., (2019). Higher germination stress index (GSI) indicated faster seedling development, aligning with Nikale *et al*., (2022). Moisture stress affected plumule length more than radicle length, echoing Dutta and Bera (2008). Drought stress, as highlighted by Kaur *et al*., (2017), influences plant growth and development during the seedling stage. Principal component analysis of groundnut genotype seedling traits under drought stress, including germination rate, promptness index, radicle and plumule length, seed vigour, GSI, and root length stress index, was conducted, aiding in genotype identification for drought tolerance.

Groundnut genotypes screening for high-temperature stress at the seedling stage

The screened seedlings of groundnut genotypes under the temperature induction response technique expressed that there was severe mortality was observed above 42°C and the survival percentage gradually decreased with increased temperature, Similar observations were reported by (Kumar *et al.*, 2012). This mortality is due to the loss of water content in the cell gradually increasing the cell membrane rupture causing a reduction in the survival percentage of the seedlings (Piramila *et al.*, 2012). Further a stress indicator, proline increased in the tolerant genotypes up to 42°C in the stress-tolerant groundnut genotypes by osmotic adjustment (Gill and Tuteja, 2010). Similarly, the present investigation proline content was hiked to the level of 3-fold times in the tolerant genotypes. Based on the observation of the seedling characteristics such as survival percentage and proline, a principal compound analysis for high-temperature stress was analyzed. PC 1 expressed high variations.

Conclusion

The study evaluated groundnut genotypes' responses to drought and high-temperature stress during the seedling stage, revealing insights into stress tolerance Valuable insights were gained on the stress responses of different groundnut genotypes. Drought stress poses a significant challenge to crop growth. Groundnut genotypes were subjected to reduced water potential using PEG 6000 as a mimic of drought stress. Some genotypes exhibited higher germination stress index (GSI), indicating drought tolerance. Genotypes VG 18005, VG 18077, VRI 2, VG 18103, VG 19709, VG 19572, VG 18096, VG 19681, VG 18076, and VG 19542 showed higher drought tolerance. Promptness index, radicle and plumule length, and seed vigor indicated stress response. Some genotypes maintained better growth under limited water, vital for regions with erratic rainfall. High temperatures above 42°C caused severe mortality, with tolerant genotypes showing increased proline content, indicating stress tolerance. Certain genotypes displayed higher survival and proline content, highlighting their ability to withstand high-temperature stress.

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