PHYSIOLOGICAL AND BIOCHEMICAL ANALYSIS OF SOME BIPARENTAL LINES OF PEA (*Pisum sativum* L.) UNDER DROUGHT STRESS

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Abstract. Drought stress is one of the major environmental stresses that considerably decreased agricultural production worldwide, especially in arid and semi-arid regions. The present research was to study the response of fifteen pea genotypes at different levels of drought stress induced by five different concentrations of polyethylene glycol (0%, 5%, 10%, 20%, 30%) and three water deficit levels (100% Field Capacity, 50% Field Capacity, and 25% Field Capacity) to create different levels of drought stress and screening of drought tolerant pea genotypes in the Lab. plastic house. The fifteen pea genotypes in response to drought stress showed significant parameter variations. At the lowest dose of PEG (5%, 10%), all studied genotypes displayed excellent performance in all parameters. However, under the highest dose of PEG (30%), representing the most water-deficient condition, all genotypes experienced a reduction in germination percentage and mine germination rate, along with an increase in mean germination time. Exposure of plants to drought stress at plastic house significantly decreased seed yield by 52.29% in the case of 50% field capacity, whereas by 85.58% in the case of 25% field capacity compared to control. Plant crops developed different physiological, biochemical, and genetic mechanisms, including drought escape, drought avoidance, and tolerance to reduce the effect of water stress. The results of our study help to understand the possible role of physiological attributes, phytochemical investigation, and their contribution to enhancing pea's ability to withstand water deficits. Consequently, the scientific results were thoroughly evaluated and summarized in this study.
Keywords: *polyethylene glycol, phytochemical components, water deficit, yield, tolerance index*

Introduction

Climate change and variability are anticipated to further reduce water availability for agriculture in the near future. Water availability in many crop species is a major limitation of yield and quality during or at critical times of the growing (Dalila et al., 2018). Drought stress and water deficit are considered major factors constraining crop production. Drought is estimated to affect over 60-70% of the entire arable land (Radić et al., 2018). Reduced water availability in the soil is the primary and predominant factor contributing to drought (Trenberth and Zhang, 2019), Physiological, morphological, and biochemical, processes in plants can be modified due to the impact of drought stress, which results in changes in gene expression leading to alterations in protein production (Lobell et al., 2014).

Furthermore, drought exerts an impact on various biological processes such as photosynthesis, respiration, mineral nutrition, enzyme activities, oxidation/reduction (Redox) homeostasis, and chloroplast metabolism, as reported in studies by Maiti et al. (2014) and Szalonek et al. (2015). Analysis of proteomics data in plant leaves indicated

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that, during drought stress, a multitude of drought-responsive proteins responsible for osmotic regulation, modulation of cell structure, ROS scavenging, drought signal transduction, and carbohydrate metabolism were found to be upregulated (Wang et al., 2016). The impact of drought stress on many crops results in a decrease in both their quality and output. Additionally, extended periods of water deficits have been observed to negatively affect plant development and the efficiency of photosynthesis, triggering a series of responses aimed at sustaining plant survival.

In the wild zones of the world, the primary abiotic factor constraining the survival and yield of plants is limited water availability, and enhancing drought tolerance can solely be achieved through the enhancement of drought tolerance. Legumes generally have low water demands at the beginning of development, but they are very sensitive to water stress during flowering and pod filling due to the high evapotranspiration (Nemeskéri et al., 2015). The advancement of pea genotypes that are highly adapted to arid conditions has been a prominent objective in breeding programs. Developing new cultivars suited for dry conditions through conventional breeding necessitates thorough selection and testing of yield performance across diverse environments using various biometrical approaches. Numerous morphological and biochemical traits have demonstrated associations with drought resistance, and techniques based on physiological attributes can also contribute to the enhancement of superior varieties. In 2019, *Pisum sativum* L., held the distinction of being the second most significant plant in terms of production among grain legumes. Globally, it produced 21.8 million tons of vegetable peas and 14.2 million tons of dry peas (FAO, 2021). As well as being the primary legume crop in temperate climatic regions, serving as a staple for both human consumption and animal feed, it contains significant quantities of carbohydrates, proteins, fats, minerals, vitamins, as well as soluble fiber and antioxidants (Ashraf et al., 2011). Since field peas help in nitrogen fixation harboring root-nodule symbiotic Rhizobium bacteria, it is grown in rotation with cereals and other crops to conserve soil fertility worldwide (Mus et al., 2016).

The present study aimed to evaluate the physiological and biochemical modification of different pea genotypes under water deficit stress to identify some biparental lines' tolerance and susceptibility.

Materials and methods

Plant materials

The plant materials consisted of 15 genotypes (11 biparental lines of Pea with their four parents). Eleven biparental breeding lines OR-M01 (G1), OR-M02 (G2), OR-M03 (G3), OR-M04 (G4), OR-M05 (G5), OR-M06 (G6), OR-M07 (G7), OR-M08 (G8), OR-M09 (G9), OR-M10 (G10), and OR-M11 (G11) have been released by Oral M. Musa from the Agricultural Research Center of Sulaymaniyah. The Four parents consisted of Pea-26 (G12, wild type collected from Piramagrun mountain), Kaspa (G13), Pea-33 (G14, wild type collected from Sharazur) and Desire (G15) genotypes. The experiments were conducted at the University of Sulaimani's College of Agricultural Engineering Sciences.

Laboratory experiment

Polyethylene glycol (PEG) 6000, a biologically inert compound, is commonly utilized to simulate water stress conditions in vitro by creating osmotic potential. It acts as an osmotic agent and functions as a non-ionic polymer with a high molecular weight

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range, preventing penetration into plant cells and thereby averting potential toxic effects (Channaoui et al., 2019).

The experimental design was structured as a completely randomized factorial design with three replications and 10 Pea seeds per replicate. The two factors were the genotypes of *pisium sativum* and the PEG solutions.

Drought stress was created with the use of Polyethylene glycol treatment. Seeds were sterilized with hypochloride sodium (5%), then treated with PEG in concentrations (0, 5, 10, 20, and 30) %, and distilled water served as a control, each Petri dish, 10 mL of distilled water was added as a control treatment. Subsequently, 10 mL of the PEG solution were added to the Petri dishes for each treatment with each concentration of PEG solution. The seeds germinated in double-lined Petri dishes with filter paper, each moistened with the respective solutions, within a germination chamber set at 25 ± 1°C and relative humidity of 60-70% (ISTA, 2020).

The seeds were deemed germinated once the emerging radicle extended to 2 mm or longer. The germination percentage will be assessed every 24 h over a period of 10 days. To assess the rate of germination, Mean Germination Time (MGT) was computed using the method described by Ellis and Roberts (1980) as follows:

$$MGT = (fx) / f$$

where 'f' represents the count of newly sprouted seeds on each given day, while 'x' signifies the specific day of counting. On the 10th day, measurements were taken for root length, and shoot length, as well as seedling fresh and dry weights. The dry weights were determined after subjecting samples to a 70°C drying process lasting 48 h in an oven (Ranal et al., 2006).

Plastic house experiment

All genotypes were cultivated in pots with 10 kg soil (5 plants per pot) in the plastic house (6m × 30m) and were treated with water deficit levels (100% Field Capacity, 50% Field Capacity, and 25% Field Capacity). Drought stress was initiated at the 3-leaf stage, approximately one month after sowing, by ceasing plant irrigation. Drought stress treatment was applied to the plants by withholding irrigation. After that, the pots were irrigated according to the treatments (100% F.C., 50% F.C., and 25% F.C.). The amount of water needed for each pot and each time was calculated as follows (Karim, 1999):

$$V = [(\Theta F.C. – \Theta \text{ Initial}) \times \text{depth root zone} \times \text{bulk density}] \times \text{area}$$

$$F.C = 13.28 + 0.397 \times (\text{clay}\%)$$

where V is the amount of water needed for each pot each time, Θ FC is the Field Capacity, and Θ Initial is the Initial Soil Moisture.

Field capacity was determined through a methodical process involving soil saturation and drainage. Initially, the soil within the pots was thoroughly saturated with water until excess water began to drain from the bottom, ensuring that the soil was completely saturated. Following this, the soil was left to drain naturally for a period of 1-2 days, allowing gravitational water to escape and the soil to reach a stable moisture level. After this drainage period, soil samples were collected from the pots for moisture content analysis. The wet weight of each sample was recorded before the samples were dried in

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an oven at 105°C for 24 h to remove all moisture. The dry weight of the samples was then measured. The moisture content, representing the field capacity, was calculated using the formula:

$$\text{Moisture Content} = \text{Wet Weight} - \text{Dry Weight} / \text{Dry Weight} \times 100$$

Physiological tests

Estimation of relative water content

In order to estimate the water content of leaves, the Relative Water Content (RWC) in leaves was assessed for both control and drought treatments using the formula outlined by Barrs and Weatherley (1962):

$$\text{RWC (\%)} = 100 \times (\text{Fresh Weight} - \text{Dry Weight}) / (\text{Saturated Weight} - \text{Dry Weight})$$

Leaf dry weight (DW) was determined by subjecting the leaves to oven drying at 75°C for 48 h, while saturated weight was measured after immersing the leaves in water for 24 h in a 25 ml tube at room temperature.

Physiological growth parameters assay

Following a 21-day drought treatment period, measurements were taken for fresh and dry weight (g). Shoot fresh weight was measured by directly weighing the harvested plant shoot tissues, whereas shoot dry weight was determined by weighing the plant's shoot tissues after subjecting them to an oven drying process at 75°C for 48 h.

Estimation of photosynthetic pigments

A quantity of 0.5 grammes of fresh leaves was meticulously homogenized using 80% acetone in a mortar and pestle under dark conditions at a temperature of 4°C. Following this, the resulting homogenates were centrifuged at 13,000 g for 10 min. After centrifugation, the supernatants were gathered, and a UV-visible spectrophotometer (Spectra Max Plus; Molecular Devices, USA) was used to detect the absorbances of the acetone extracts at 663 nm, 646 nm, and 470 nm. The content of Chlorophyll a, Chlorophyll b, and total carotenoids was computed using the equations formulated by Lichtenthaler and Wellburn (1983).

$$\text{Chl a (µg/ml)} = 12.21\ A663 - 2.81\ A646$$

$$\text{Chl b (µg/ml)} = 20.13\ A646 - 5.03\ A663$$

$$\text{Carotenoids (µg/ml)} = \frac{1000A470 - 3.27(Chl\ a) - 104(Chl\ b)}{227}$$

Yield and yield components

During the fully ripe stage, two plants were collected from each pot for the determination of yield structure characteristics. The number of pods (PN), pods weight per plant (PW), number of seeds per plant (SP), seed weight (SW), biological yield (BY) and seed yield (SY) were analyzed.

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Stress tolerance index

The stress tolerance index (STI) was calculated for each genotype according to the following equation (Fernandez, 1992):

$$\text{STI} = (Yp \times Ys) / Xp\ 2$$

where: Yp is the yield of the genotype under non-stress conditions, Ys is the yield of the genotype under stress conditions, Xp is the mean yield of all genotypes under non-stress conditions.

Biochemical tests

Proline content

The determination of proline content in seedling samples was carried out in accordance with the method described by Bates et al. (1973). A quantity of 0.1 g of fresh seedling powder was subjected to homogenization in 3 mL of a 3% (w/v) solution of sulphosalicylic acid, followed by centrifugation at 4000 rpm for 28 min at a temperature of 5°C. After centrifugation, proline was estimated spectrophotometrically at 520 nm using the ninhydrin method. Purified proline was used for standardization.

Soluble sugar content

A total of 0.1 g of freshly ground seedlings were submerged in 800 µL of deionized water. The solution mixture was heated to a boil at 100°C for 30 min, followed by cooling and subsequent centrifugation at 4000 rpm for 15 min at 4000 rpm. This residue used for the analysis of soluble sugar content (SSC). The SSC in each extract was assessed following the methods outlined in Yemm and Willis (1954) and Zheng et al. (2008).

Total phenolic content

Tissues under both control and stress conditions were pulverized in a mortar with a pestle, employing liquid nitrogen. Subsequently, 0.1 g of the resulting fresh powder was extracted with 80% methanol or ethanol and incubated at 10°C for 16 h. Following this incubation period, the mixture was subjected to centrifugation at 13,000 rpm for 20 min at 4°C. The resulting supernatant layer was used to evaluate the total phenolic content (TPC). The total phenolic compound content in each extracted sample was quantified according to Djeridane et al. (2006) and Tahir et al. (2022) using the Folin–Ciocalteu method with some modifications.

Total flavonoid content

Both the control and stressed tissue samples were pulverized in a mortar and pestle using liquid nitrogen. 0.1 gram of fresh powder was extracted with 80% methanol or ethanol and incubated at 10°C for 16 h. After centrifugation at 13,000 rpm and 4°C for 20 min, the supernatant layer (extract) was collected and retained for total flavonoid content (TFC) analysis. TFC was determined in each extract following the method described by Rigane et al. (2017) with some modifications.

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Radical scavenging activity by DPPH

Tissues under both control and stress conditions, were finely ground in a mortar with a pestle using liquid nitrogen. Subsequently, 0.1 g of this fresh powder were subjected to extraction using either 80% methanol or ethanol, followed by a 16-h incubation period at 10°C. Afterward, the resulting mixture was centrifuged for 20 min at 13,000 rpm at 4°C, yielding a supernatant (referred to as the extract), which was carefully collected and employed for the DPPH test. The assessment of the antioxidant potential of these extracts was conducted using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging method, as outlined by Shimada et al. (1992) and Tahir et al. (2019) with some modifications.

Statistical data analysis

One-way ANOVA with a Completely Randomized Design (CRD) was used for the lab experiment, and a Completely Randomized Block Design (CRBD) was applied for the field and greenhouse experiments to assess significant variations among pea genotypes. The significance level was set at $p < 0.01$ for the lab experiment and $p < 0.05$ for the field and greenhouse experiments. Duncan's new multiple range tests were conducted to identify specific differences between means. All analyses were performed using XLSTAT software version 2019. Additionally, cluster analysis and principal component analysis (PCA) were carried out using the same software.

Results

Effect of PEG on seed germination and growth of pea seedling

The analysis of variance revealed that germination percentage, seed Vigor, mean germination time and mean germination rate were significantly affected by the concentration of PEG solution (*Fig. 1*). The results obtained for all pea genotypes, as depicted in *Figure 1,* demonstrate a noteworthy decline in germination capacity due to the decrease in water potential. However, variations were observed among the various pea genotypes under investigation, it was observed that in all pea genotypes, there were decreasing in germination percentage, seed vigor, mean germination time and mean germination rate due to drought stress increment.

Based on the data illustrated in *Figure 1,* T0 exhibited the highest germination percentage (98.44%), while T30 displayed the lowest germination percentage (21.77%). The other values showed different status 96%, 94%, and 81% displayed beneath the influence of concentrations T5, T10, and T20, respectively. The germination percentage declined as the PEG concentration increased.

Seed vigor showed significant variation under different concentrations (0, 5, 10, 20, and 30%) of polyethylene glycol. The seed vigor declined as the concentration of PEG increased (*Fig. 1*). The highest seed vigor (956.67) was recorded with T0, while the lowest value (26.01) was recorded in T30, the second highest seed vigor (637.89) was found in T5 followed by T10 (518.22) and T20 (303.51), respectively. A significant difference was observed in the mean germination time of seedlings at petri dishes supplemented with different concentrations of PEG (*Fig. 1*). Mean germination time increased with the increasing level of PEG concentration. The lowest mean germination time (1.33) was recorded in T0, while the highest value (3.97) was recorded in T30. The

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second lowest mean germination time (1.71) was found in T5 followed by T10 (1.95) and T20 (3.46), respectively.

Significant variation was seen in the mean germination rate among all fifteen pea genotypes under differed levels of drought induced by different concentrations of PEG (*Fig. 1*). The highest mean germination rate (0.802) was recorded in T0, while the lowest value (0.207) was recorded in T30. The second highest mean germination rate (0.627) was found in T5 followed by t10 (0.550) and T20 (0.345), respectively.

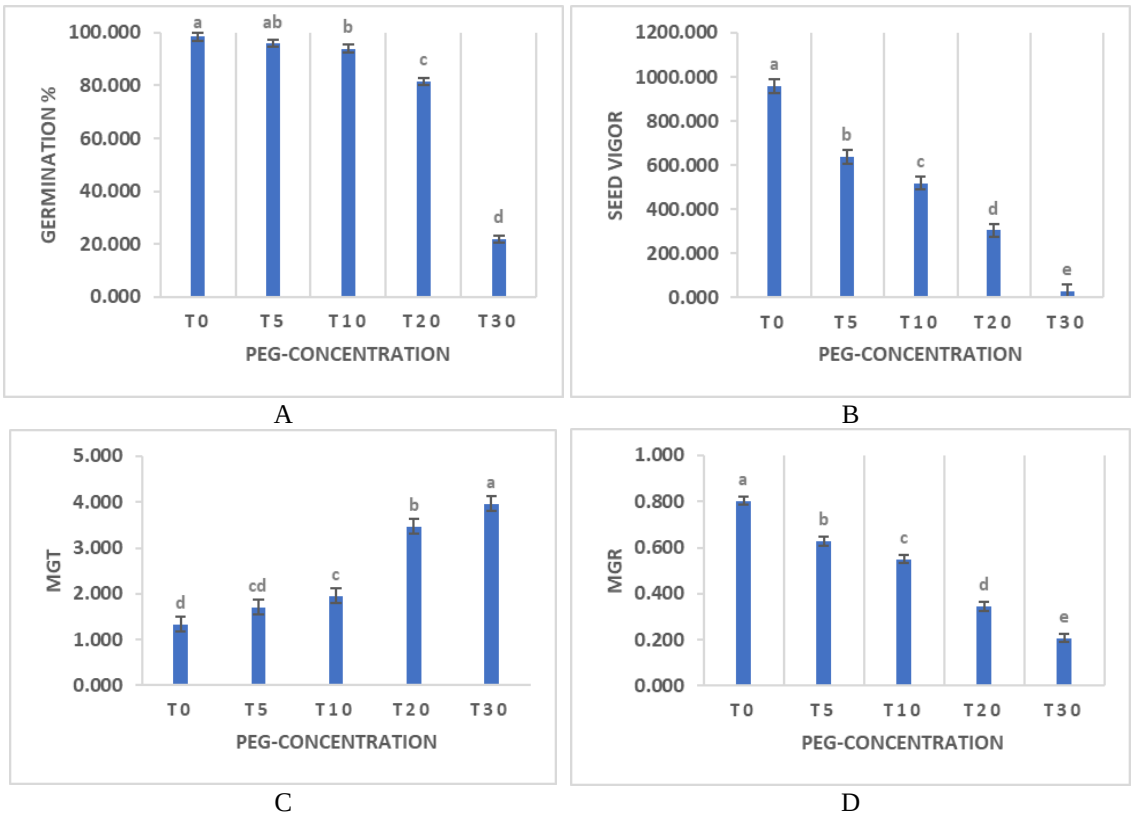

Figure 1. *Effect of different concentrations of polyethylene glycol (PEG) on (A) germination percentage, (B) seed vigor, (C) mean germination time and (D) mean germination rate. The values provided represent the mean values calculated from the three measurements obtained for both the control (T0) and various PEG concentrations (T5, T10, T20, and T30). Different Letters denote statistically significant differences between the mean values, determined using Duncan's Multiple-Range Test ($p \leq 0.01$)*

Physiological and biochemical characterization of plant stress response

The severity of drought stress was estimated by the intensity of the plant stress response. For this, physiological and biochemical stress indicators were evaluated under water stress conditions of increasing severity. Drought stress has had a substantial effect on most of the plant's physiological and biochemical characteristics. Under control conditions, the total chlorophyll content (Chl a and Chl b) was significantly higher than other treatments (T50 and T25). On the other hand, carotenoid contents under the same conditions were significantly lower compared to other treatments (*Fig. 2A*). As shown in this figure, Chlorophyll contents in pea genotypes decreased under drought stress compared with the control, Chl a content decreased with the increasing level of water

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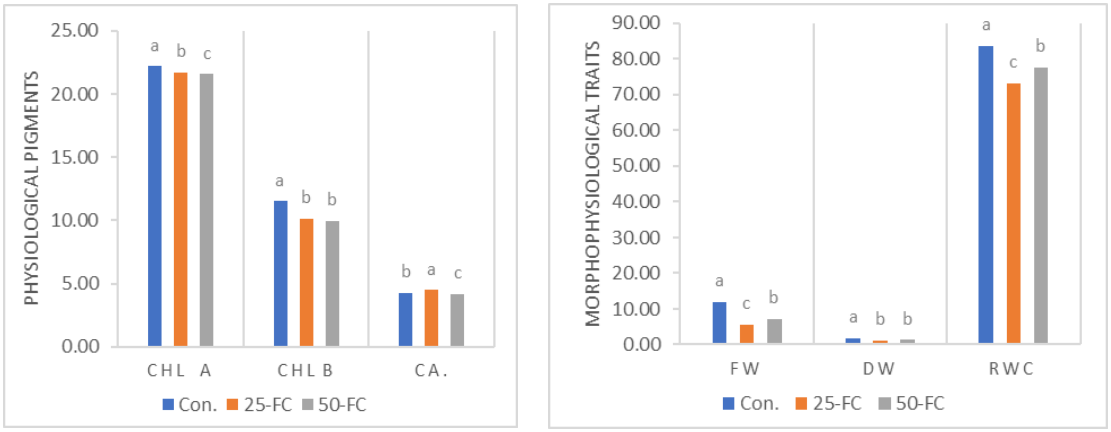
deficit. The highest Chl a content (22.19 mg/mL) was recorded in the control, while the lowest value (21.59 mg/mL) was recorded in T25. The highest Chl b content (11.53 mg/mL) was recorded in the control, and it recorded a significant value compared to other treatments, for both stress conditions T50 and T25 there was no significant variation between them.



A B

Figure 2. *Effect of different water deficit levels on (A) physiological pigments (Chl a, Chl b and carotenoids) and (B) fresh weight, dry weight and relative water content. The values provided represent the mean values calculated from the three measurements obtained from control (100% field Capacity), T50 (50% field Capacity) and T25 (25% field Capacity. Different Letters denote statistically significant variations between the mean values, determined using Duncan's Multiple-Range Test ($p \leq 0.05$)*

Analysis of biochemical stress markers revealed to decrease in total phenol content, while total flavonoid content, radical scavenging activity by DPPH, soluble sugar content and total proline content contents were increased with the plant stress response.

Under control conditions, the total phenol content was significantly higher than in stress conditions. The highest phenol content (620.24 µg GAE/gm FM) was recorded in control, followed by T25 (569.74 µg GAE/gm FM), while the lowest value (546.12 µg GAE/gm FM) was recorded in T50. Results presented here show no significant increase in total flavonoid content under different water-deficit levels compared to control (*Fig. 3B*)

The content of soluble sugar significantly increased with an increase in the severity of water stress (*Fig. 3C*). In control, the soluble sugar content was (605.16 µg/g FW), while the highest value (627.50 µg/g FW) was recorded in T25, and there was no significant difference between the control and T50.

As shown in *Figure 3*, radical scavenging activity by DPPH content and total proline content in pea plants increased under drought stress compared with control. Radical scavenging activity by DPPH showed significant variation under different concentrations of water levels. The lowest Radical scavenging activity by DPPH was (0.59 µg Trolox/gm FM) observed in the control treatment, which was statistically different from other values. While the highest value (0.64 µg Trolox/gm FM) was recorded in T25, the second highest value (0.61 µg Trolox/gm FM) was found in T50. (*Fig. 3D*). These results revealed that the high level of water stress positively influences the antioxidant potential (DPPH) in pea genotypes. On the other hand, the accumulation

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of proline was significantly greater under water stress conditions than under control (*Fig. 3E*). The highest increase of proline content was seen in T25 (2768.63 µg/g FW) and the lowest inT50 it was (981.56 µg/g FW) compared to unstressed conditions (632.12 µg/g FW).

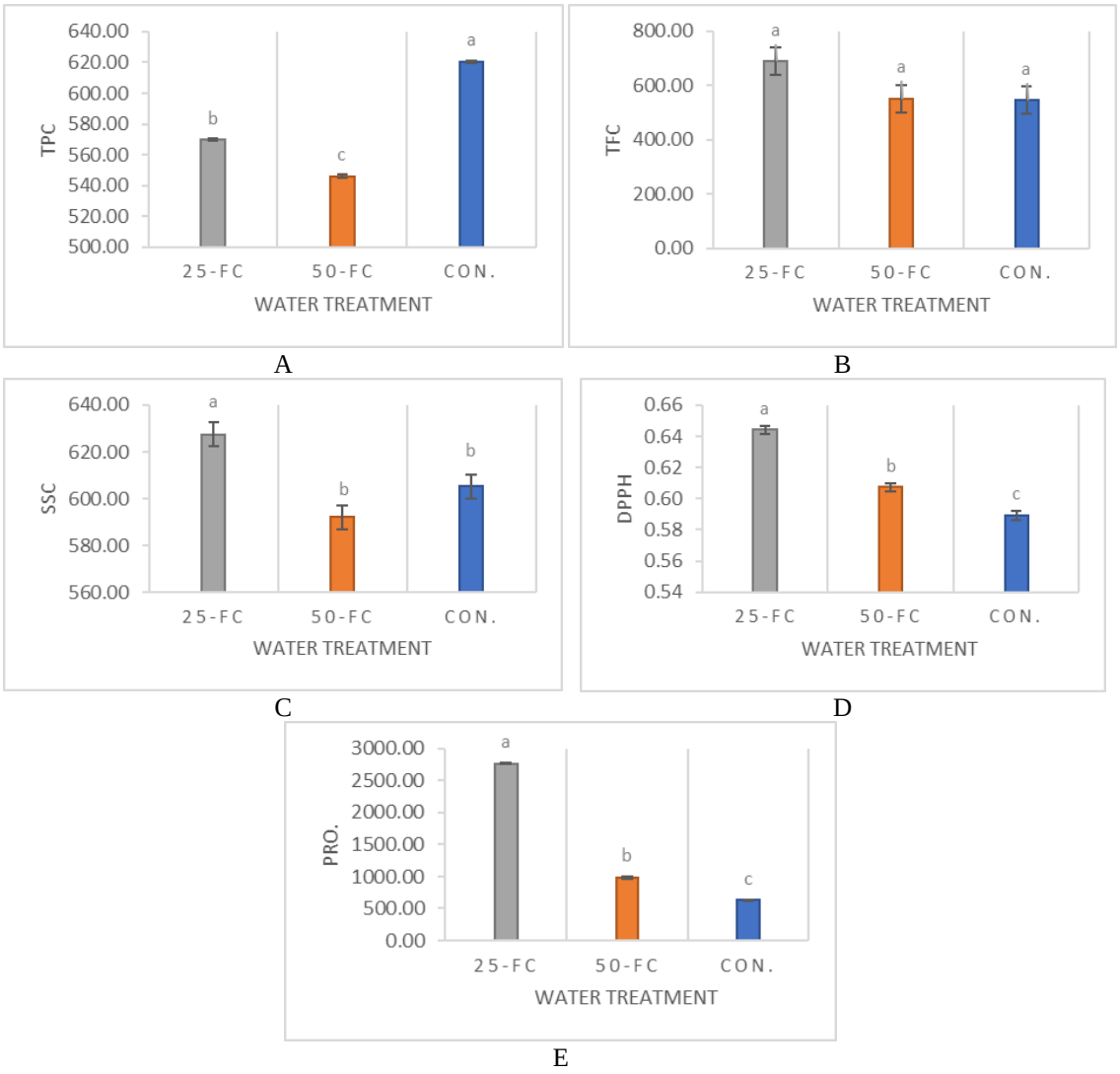

Figure 3. *Effect of different water deficit levels on (A) Total Phenol content, (B) total flavonoid content, (C) radical scavenging activity by DPPH, (D) soluble sugar content and (E) total proline content. Mean germination rate. The values provided represent the mean values calculated from the three measurements obtained from control (100% field Capacity), T50 (50% field Capacity) and T25 (25% field Capacity). Various letters are used to represent statistically significant variations between the mean values, as indicated by Duncan's Multiple-Range Test ($p \leq 0.05$)*

Cluster analysis of 15 pea genotypes based on the chemical proximity and phytochemical investigation of 15 pea genotypes have been stated among different genotypes (*Fig. 4*). As expected, cluster analysis classifies the 15 genotypes into four distinctive clades. It was found that the fourth clade includes the highest number of accessions, containing 6 genotypes, while the first clade records the minimum number

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of accessions containing only one genotype. It was observed that both accessions, G6 and G12 created a tightly associated sub-cluster within the third cluster (Euclidean distance of 29.192), whereas G5 appeared to have the highest content in proline, which had been located in an isolated class.

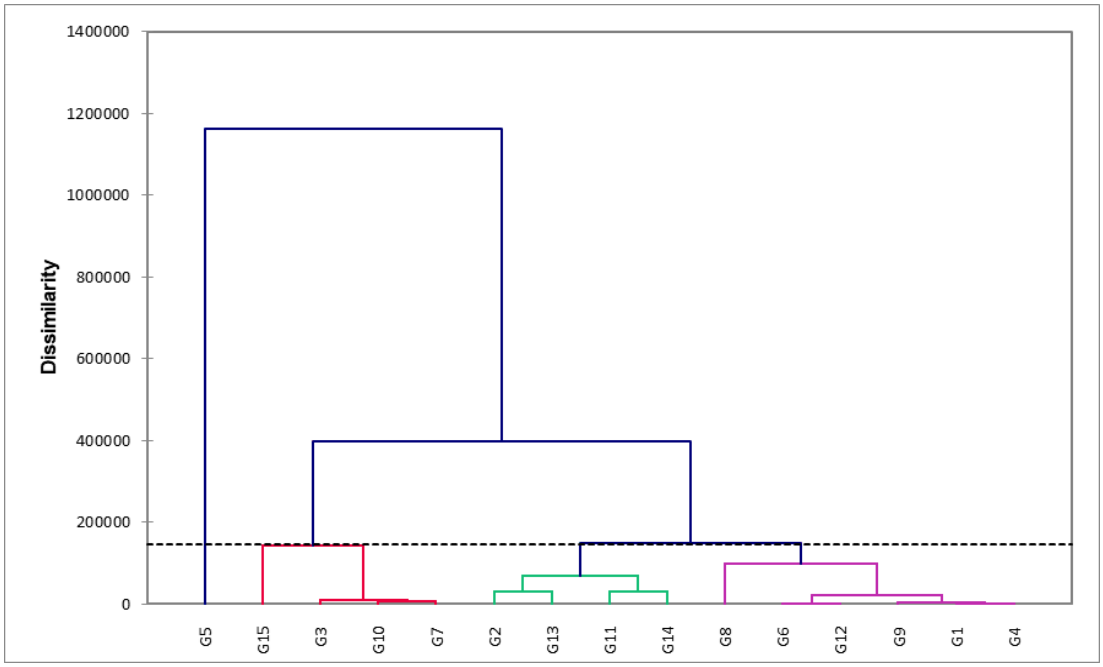

Figure 4. *Cluster analysis of 15 pea genotypes based on information on the phytochemical elements using Euclidean distance and UPGMA methods*

Morphological characterization of plant stress response

Shoot fresh weight showed significant variation under different water deficit levels at 21 days after drought treatment (*Fig. 5A*). The highest value (12.05 cm) observed in the control treatment was statistically different from other values (T50.T25). Shoot fresh weight decreased with the increasing water deficit levels. The second highest fresh weight (7.15 g) was found in T50, while the lowest value (5.60 g) was recorded in T25 (*Fig. 5B*)

A significant difference was not observed in the shoot dry weight of the plant after 21 days of drought treatment. However, a different value was observed between treatments, the highest shoot dry weight (1.87 g) was recorded CO., while the lowest value (1.14 g) was recorded in T25 and (1.31 g) was found in T50.

The relative water content exhibited significant variation across various levels of drought stress induced by different water deficit. RWC exhibited significant variation at different levels of drought stress induced by different water deficit levels (CO., T50, and T25) on fifteen pea genotypes (*Fig. 2B*). The highest RWC (83.59%) was recorded in control (100% field capacity), while the lowest value (73.22%) was recorded in (T25). The second highest relative water content (77.73%) was found in T50**.** RWC significantly declined by 10.37% related to non-stressed plants, and it was estimated after one month from drought treatment.

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Plant growth and harvesting

The yield attributes were significantly influenced by the water-deficient conditions as shown in *Figure 5*. Drought stress decreased the pods number, pods weight, seeds number, seed weight, plant height and biological weight compared to the control treatment.

Pods number, Pods weight decreased significantly with drought stress, under non-stress conditions (control) produced the highest pods number (4.09 per plant), pods weight (4.27 g per plant), followed by T50 (2.73 per plant), (2.08 g per plant) respectively. The lowest yield was recorded in T25 pods number (1.49 per plant), pods weight (0.57 g per plant).

Due to drought stress imposition, a significant decrease was observed in seed number and seed weight of the pea genotypes in treated conditions than in control. The highest seeds number (15.20 per plant) and seed weight (3.40 g per plant) were recorded by control, followed by T50 (8.04 per plant) (1.69 g per plant), respectively. The lowest yield was recorded in T25 seeds number (2.64 per plant), seeds weight (0.49 g per plant).

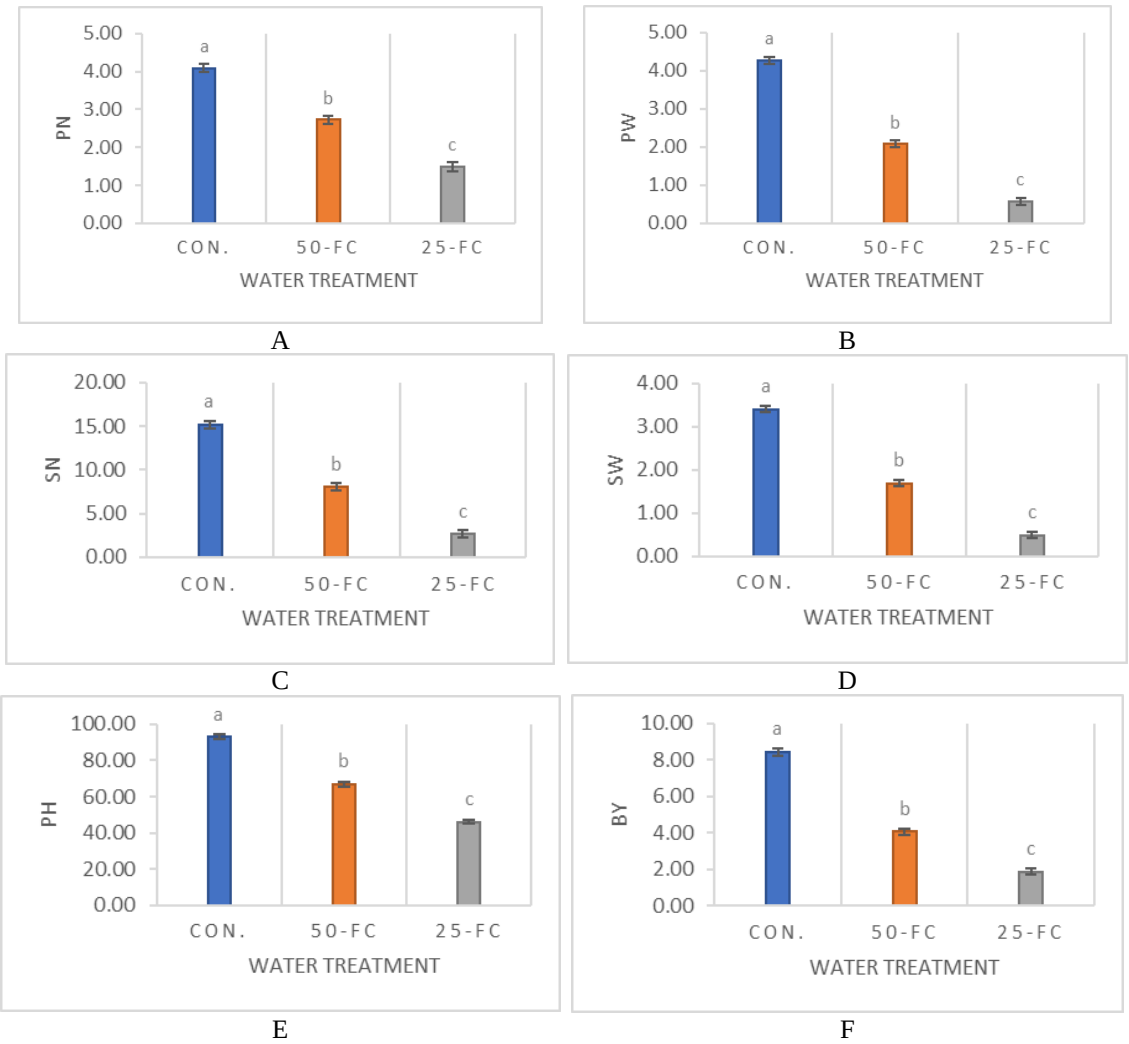

Figure 5. *Effect of different water deficit levels on (A) pods number, (B) pods weight, (C) seeds number, (D) seed weight, (E) plant height and (F) biological weight. The values provided represent the mean values calculated from the three measurements obtained form control (100% field Capacity), T50 (50% field Capacity) and T25 (25% field Capacity). Various letters are used to represent statistically significant variations between the mean values, as indicated by Duncan's Multiple-Range Test ($p \leq 0.05$)*

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Principal component analysis (PCA) was carried out for the 15 pea genotypes based on the yield component data under normal environmental and stress conditions (*Fig. 6*). A total of 4 phenotypic traits (Pods number, Pods weight, Seeds number, and Seed weight) were used to construct a two-dimensional scatter plot.

The graph based on PCA1 and PCA2 illustrates the relationships among various parameters within the rank correlation matrix. The first two principal components explained approximately 84.76% of the total morphological variation among the examined genotypes. The cosine of the angle between vectors representing distinct characteristics can provide an approximation of their associations. Seed number was strongly associated with pod number per plant, while seed number and pod number had a strong negative association with seed weight and pods weight. According to our results, the genotypes' performance for different traits indicates that G5, G8, and G9 performs better than other genotypes in terms of yield. However, a group of five genotypes, G3, G6, G10, G13, and G14 located on the right side of the plot, was in strong and positive association with pods number and seeds number per plant. While a group of seven genotypes concentrated on the plot's left side, having a clear boundary with the two other groups.

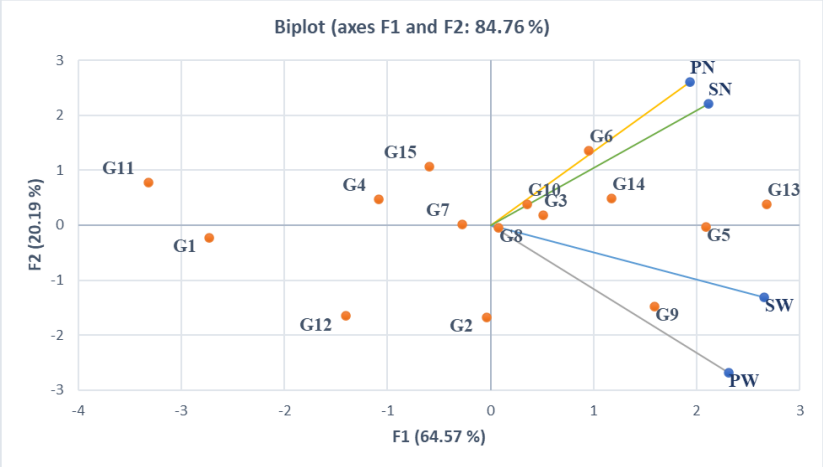

Figure 6. *Two-dimensional PCA scatter plot of the 15 pea genotypes baes on the yield component traits*

Stress tolerance index

Drought stress affected the stress tolerance index (STI) of all genotypes of pea, as shown in *Figure 7*. Significantly, the highest value of STI was recorded from the genotypes G14 (treated with 50% field capacity) and G15 (treated with 25% field capacity) and the lowest value of STI was from genotypes G7 (treated with 50% field capacity) and G11 (treated with 25% field capacity). The stress tolerance index decreased with increasing drought stress.

Discussion

Plant response to drought stress includes different morphological, physiological, Biochemical and metabolic changes. It can occur at any plant developmental stage, but certain growth phases are particularly sensitive to soil moisture status, which can significantly hamper the overall crop yield (Toscano et al., 2016).

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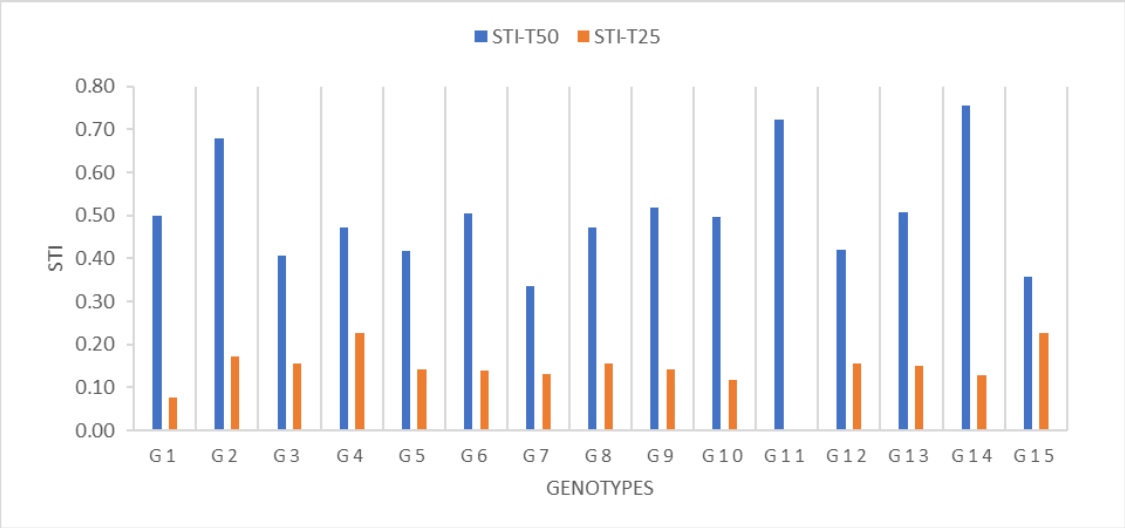

Figure 7. *Plant stress tolerance index (STI) of fifteen pea genotypes under water stress conditions*

Pertaining to the laboratory experiment, part of the current study demonstrated natural variations between the studied genotypes, which displayed varying growth characteristics under both control and stress conditions among the fifteen pea genotypes, and significant declines were observed as the PEG concentration increased from 0% to 30%. The results of this part of our study align with previous research and further support the utility of PEG as a means to simulate drought conditions in vitro. These results are consistent with earlier studies, underscoring that the response to drought is contingent upon both genotypes and levels of stress (Cai et al., 2020).

In general, the results indicating that PEG reduced the shoot vigor of pea genotypes are consistent with findings reported in vitro drought screening of other plant species as documented by Sakthivelu et al. (2008) and Bidabadi et al. (2012). With increasing concentrations of PEG, shoot vigor also declined with the highest reduction in 30% PEG. This might result from the hindered absorption of water and nutrients caused by a decrease in the medium's water potential or a significant suppression of cell elongation due to low turgor pressure (Jaleel et al., 2009).

Our results showed that increasing water stress caused by PEG treatment reduced the germination capacity of the pea genotypes. A reduction in the germination percentage by 352%, with the highest reduction in 30% PEG, concomitant with the decrease in the mean germination rate by 74.1%. Similar results were reported by Pereira et al. (2020) and Muscolo et al. (2014) in lentils. The decrease in germination capacity may be due to reduced water permeability through the seed coat and reduced water absorption by seeds during drought stress conditions (Bahrami et al., 2012; Channaoui et al., 2019).

Furthermore, there was a notable rise in the mean germination time among all the genotypes examined in this study under drought stress conditions. These findings result align with Khodarahmpour's research (2011), who found that the mean germination time increased with an increase in the concentration of PEG solution. Similar results were obtained in the studies on maize (Channaoui et al., 2019).

Pertaining to the Plastic house part of our study, it also showed variations between the studied genotypes, which exhibited varied growth traits in both the control and stress conditions among pea genotypes. The present study revealed that water deficit

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treatment caused a decrease in the contents of both Chl a and Chl b. The decrease in chlorophyll content as a result of pigment photo-oxidation and degradation during drought stress is a common indicator of oxidative stress. The reduction in chlorophyll contents, resulting from pigment photo-oxidation and degradation during drought stress, is a common indication of oxidative stress (Farooq et al., 2009). A reduction in chlorophyll levels during drought stress has been observed in numerous species, with the extent varying based on the duration and severity of the drought. Both chlorophyll a and chlorophyll b are susceptible to soil dehydration, leading to their decrease during such stressful conditions (Farooq et al., 2009).

Drought stress results in the loss of turgor, a reduction in both fresh and dry weight, and diminished biomass accumulation (Zhang et al., 2011; Wahab et al., 2022). In the current investigation, the water deficit treatment led to decreased shoot fresh weight, dry weight, and relative water content. In the present study, water deficit treatment reduced shoot fresh weight, dry weight and relative water content. The reduction in FW, DW, and RWC initially leads to stomatal closure, this closure, in turn, reduces the supply of carbon dioxide (CO2) to the mesophyll cells, resulting in a subsequent decline in photosynthesis rates (Zhang et al., 2011).

The accumulation of diverse chemical compounds represents one of the most crucial responses of plants to drought stress, potentially playing a role in the adaptation process, Proline is a widely accumulated amino acid in plants in response to various abiotic stresses (Marcińska et al., 2013). In our study, total proline content was boosted by 1.55-fold and 4.37-fold under T50 and T25 treatments compared to control. In drought-stressed plants, the amino acid proline accumulates in greater quantities than other amino acids (Kravić et al., 2013). In response to water deficit, proline predominantly accumulates within the cytosol, where it plays a crucial role in cytoplasmic osmotic adjustment (Cvikrová et al., 2013; Javadi et al., 2006). Furthermore, the accumulation of soluble sugars can decrease the leaf's osmotic potential, assisting in the maintenance of turgor pressure under water stress conditions (Hernandez et al. 2021). Soluble sugars can function as signals that not only sense and regulate photosynthetic activity but also play a role in sensing and controlling the balance of reactive oxygen species (ROS) (Couée et al., 2006). In our results, concentrations of total soluble sugars increased slightly by about 0.17%. While the accumulation of either proline or soluble sugars is common in many species in response to abiotic or biotic stresses, the simultaneous and substantial accumulation of both osmoprotectants has been rarely observed in other species.

During the reproductive periods of plants, which are the most sensitive stages to water deficiency, monitoring the changes in physiological responses can serve as a valuable approach to screen and assess the water stress tolerance of different genotypes. During this time, the water supply determines the yield production, according to the results of our study, Seed yield was reduced by 2.01-fold and 6.93-fold under T50 and T25 treatments, compared to control. This result agreed with previous results for wheat (Bogale et al., 2011), lentils and grass peas (Talukdar and Medicine, 2013), and *Zea mays* (Ali et al., 2016). Many yield-determining processes in plants exhibit responses to water stress. Yield integrates many of these processes in a complex way; grain yield results from the expression and association of several plant growth components. Water deficiency significantly decreases crop plant yield traits, likely due to the disruption of leaf gas exchange properties. This disruption not only restricts the size of source and sinks tissues but also hampers crucial processes such as phloem loading, assimilate translocation, and dry matter partitioning.

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As a consequence, the overall productivity and yield of crop plants are severely impacted under water-deficient conditions (Farooq et al., 2009). Drought stress predominantly inhibits dry matter production by exerting inhibitory effects on leaf expansion and leaf development, reducing light interception (Bhattacharya, 2021). Drought during the flowering stage often leads to crop sterility. One significant contributing factor, although not the sole one, is the reduction in assimilate flow to the developing ear, dropping below a critical threshold necessary to support optimal grain growth (Blum et al., 2011).

Conclusion

Drought tolerance in pea genotypes involves complex physiological, biochemical, and genetic processes. This study evaluated fifteen pea genotypes under various concentrations of polyethylene glycol (PEG) and water deficit levels to simulate drought conditions. Lower PEG concentrations allowed for better performance, while higher concentrations significantly reduced germination rates and seedling vigor. Water deficit conditions further decreased yield by nearly 50% and 85% under 50% and 25% field capacity, respectively.

Physiological and biochemical analyses indicated that the genotypes utilized strategies such as osmolyte accumulation and antioxidant activity to mitigate drought stress. Certain genotypes showed superior tolerance, with minimal reductions in growth and physiological traits, while others were more susceptible.

Overall, germination and seedling traits are effective for rapid screening of drought-tolerant pea genotypes. The study's findings provide insights into the mechanisms of drought tolerance, suggesting that future research should explore metabolic and proteomic profiles. Additionally, field evaluations are necessary to confirm the correlation between in vitro and field responses, aiding in the development of drought-resilient pea varieties.

Authors' contribution. All authors contributed equally.

Conflict of interest: There is no conflict of interest between the authors.

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