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Calcium chloride enhances growth and physio-biochemical performance of barley (*Hordeum vulgare* L.) under drought-induced stress regimes: a future perspective of climate change in the region

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ABSTRACT

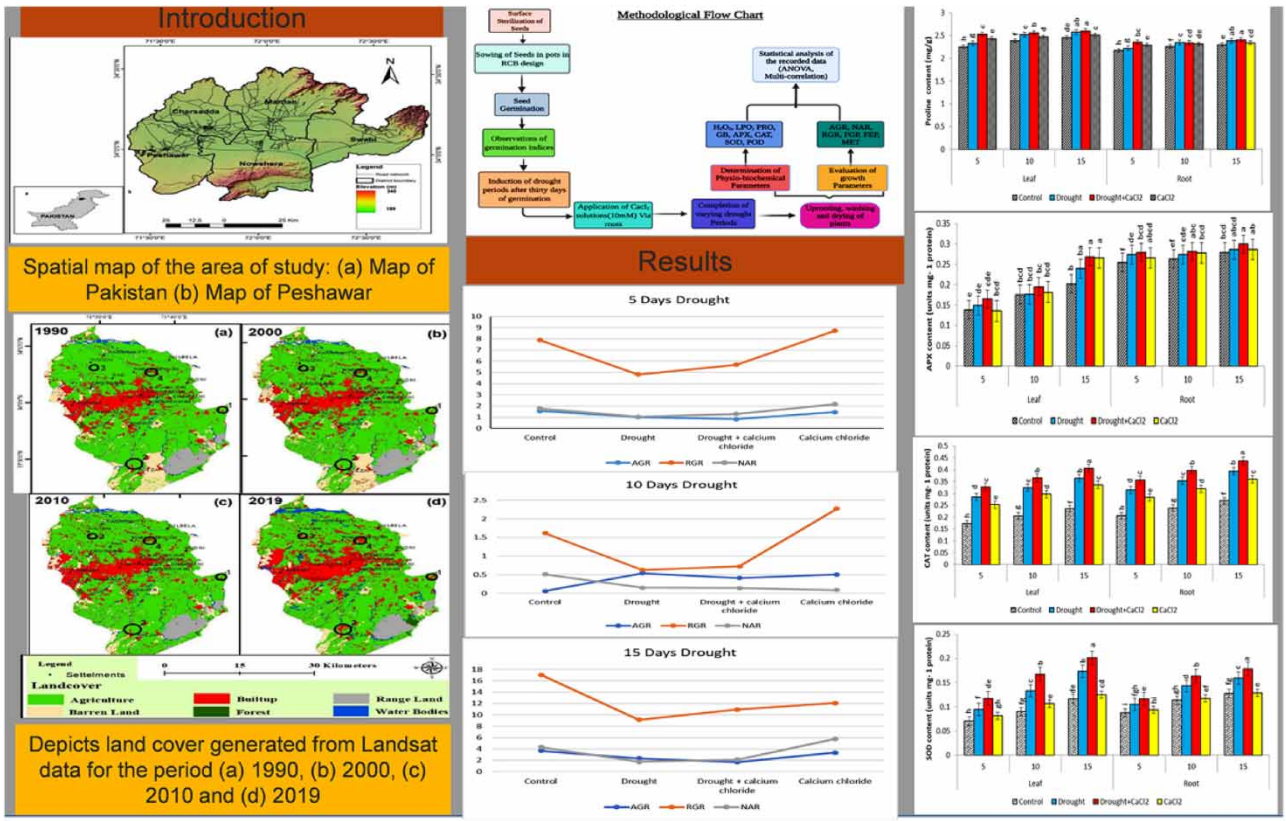
Water is the fundamental part of living systems and it plays a key role in supporting life on earth; however, fluctuations in climatic conditions lead to limitation of the ground water causing serious concerns. The present research study was aimed at assessing growth and physio-biochemical responses of barley to calcium chloride ($CaCl_2$) solution (10 mM) applied through roots under induced drought stress for 5, 10, and 15 days. $CaCl_2$, being an enhancer of osmolytes and antioxidant enzymes, counteracts the damaging effects caused by abiotic stresses. A pot experiment was conducted by sowing barley under induced drought stress for 5, 10, and 15 days, respectively. Plants exposed to different levels of the induced drought stress condition were treated with 10 mM of $CaCl_2$ solution via roots during the seedling stage. Results indicated that water-limited conditions negatively affected plant growth parameters including final emergence percentage, final germination percentage, and mean emergence time. Moreover, absolute growth rate, relative growth rate, and net assimilation rate were significantly improved under 5, 10, and 15 days of drought stress supplemented with $CaCl_2$ solution. Under drought conditions, an increase was observed in hydrogen peroxide (H_2O_2), glycine betaine (GB), and proline (PRO) content, and in ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and lipid peroxide (LPO) activities. H_2O_2 and LPO showed a significant decline with $CaCl_2$ application under induced drought stress regimes. On the contrary, GB, PRO, APX, CAT, POD, and SOD contents of root and leaf were significantly improved with $CaCl_2$ application under induced drought stress. In conclusion, $CaCl_2$ solution effectively curbed the damages caused by oxidative stress via accumulating osmolytes and scavenging reactive oxygen species by activating the antioxidant enzymatic defence system of barley.

Key words: centrifugation, climate change, drought, Peshawar, spectrophotometry

HIGHLIGHTS

- The agriculture sector of Pakistan has been negatively affected by changing climatic regimes.
- The groundwater table of Peshawar has been declining 1–3 feet/year for the last two decades.
- $CaCl_2$ application improved AGR, RGR, and NAR under drought stress conditions.
- H_2O_2 and LPO contents were reduced with $CaCl_2$ application.
- Osmolytes content and antioxidant enzymes were improved with $CaCl_2$ application under drought stress.

GRAPHICAL ABSTRACT



ABBREVIATIONS

FEP	final emergence percentage
FGP	final germination percentage
MET	mean emergence time
AGR	absolute growth rate
RGR	relative growth rate
NAR	net assimilation rate
H_2O_2	hydrogen peroxide
GB	glycine betaine
PRO	proline
APX	ascorbate peroxidase
CAT	catalase
POD	peroxidase
SOD	superoxide dismutase
LPO	lipid peroxide
ROS	reactive oxygen species
$CaCl_2$	calcium chloride

1. INTRODUCTION

1.1. Physio-biochemical responses of plants to drought stress

Drought can be defined as 'a prolonged shortage in the water supply, whether ground water, surface water, or atmospheric water'. Limited water availability is becoming a threatening issue all over the globe, and water stress conditions have intense negative effects on agriculture and ecosystems (Shao *et al.* 2008). It is believed that water scarcity is one of the most dangerous environmental stresses which drastically reduces plant growth and production up to 45% throughout the world in all the

cultivated lands (Mirzaee *et al.* 2013). Drought stress affects plant growth and development by declining the rate of photosynthesis. The major factor responsible for slowing the rate of photosynthesis is stomatal closure during abiotic stress regimes which ultimately leads to reduced stomatal CO_2 fixation (Rasouli *et al.* 2021). Plant growth, total biomass, and yield are affected by several environmental biotic factors such as microorganisms, anthropogenic activities, and abiotic factors like salinity, drought, temperature, toxicity of heavy metals, and oxidative stress (Jaleel *et al.* 2007a, 2007b). Plants confront water-deficit conditions mainly due to the following two factors: (a) when the rate of transpiration is very high or (b) it is difficult for the roots to absorb water from the soil. Both these conditions mostly coincide in arid and semiarid climates of the world (Jaleel *et al.* 2007a, 2007b). Turgidity of the cell is maintained by the formation of osmolytes, and osmotic potential being an adaptive mechanism in encountering the loss of turgor pressure under the drought stress is regulated by osmolytes accumulation in the cytoplasm (Manivannan *et al.* 2007).

Production of osmolytes such as protein, glycine betaine (GB), and proline (PRO) helps to combat the negative impacts of oxidative stress by providing suitable environmental conditions compatible with the structure and function of macromolecules (Dawood 2016). Owing to their non-toxicity, osmolytes have no effect on metabolic activities of plants due to their very low molecular weight and high solubility rate, which is why their presence even in a very large amount does not interfere with the normal cellular physio-biochemical processes (Slama *et al.* 2015). Previous studies showed that calcium is involved in the regulatory mechanisms, defence, and signalling that tend to adjust plants to droughts, cold, salt, and heavy metal stress (Xu *et al.* 2013). Plants facing abiotic stresses activate strong antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APX), peroxidase (POD), and non-enzymatically through vitamins and phenolics compounds (Al-Hassan *et al.* 2015).

Calcium (Ca^{++}), being a macro-nutrient, plays a key part in plant growth regulation. It has a crucial role in controlling the structure and function of cell membranes. Calcium (Ca^{++}) by binding to phospholipids upholds the structure of lipid bilayers thus providing structural integrity to plasma membranes. Besides, Ca^{++} has been found to hamper the adverse impacts of oxidative stress via regulating plant water relations and antioxidant metabolism (Ahmad *et al.* 2015a, 2015b). Ca^{++} is involved in regulating various cellular functions as a secondary messenger (Cousson 2009). As a secondary messenger, it helps in modulating important physiological functions such as nutrient uptake, and changes in cell status to assist the plant in counteracting the negative impacts of oxidative stress (Colorado *et al.* 1994). Adding Ca^{++} supplements to irrigated water alleviates the adverse effects of water deficiency and decreases growth inhibition in plants. They also allow potassium ion transport and K^+/Na^+ selectivity in Na^+-stressed plants. Interaction of calcium and sodium ions on plant growth and ion relation is well recognized. Furthermore, it is well known that calcium ions increase the levels of osmolytes such as GB and PRO in abiotic stress conditions (Gobinathan *et al.* 2011).

Barley is an important cereal crop and is used as feed for animals in Pakistan. It is cultivated in temperate regions throughout the world at high altitudes as well as in plain areas. It belongs to the family Gramineae and is considered an important edible crop among communities of marginal areas, the highlands of central Asia, Horn of Africa, Andean countries, and the Baltic States. Barley is cultivated in environments ranging from the deserts of Middle East to high elevation of Himalayas (Hayat *et al.* 2012). It is the most important alternative food source in dry areas of the world (Barriga & Anj 2010; Khan & Bajwa 2010).

1.2. Future prospects of climate change in Peshawar (Pakistan)

Changing environmental conditions are becoming a hindrance to meeting the increasing demand for food and sustainable agriculture. Changes in climatic conditions lead to fluctuations in temperature, droughts, floods, earthquakes, and other environmental calamities, ultimately leading to shrinking crop productivity (Shah *et al.* 2021). In Pakistan, the duration of summer is longer than winter, and the temperature ranges between 30 and 45 °C in Peshawar as well as in other cities of Khyber Pakhtunkhwa. The temperature in Pakistan is expected to raise approximately 3 °C by 2040 and 5–6 °C by the end of this century (IUCN 2009). Pakistan has been impacted by the effects of environmental changes to a great extent and this enhanced susceptibility of the country to the danger of changing environment has broadly been recognized (Chaudhary 2017; Ghulam *et al.* 2017; Saeed & Athar 2018; Ali *et al.* 2020). Pakistan has witnessed 0.5 and 0.8 °C expansion in normal mean and extreme temperature, over the period 1961–2010 (Ali *et al.* 2020). While the future expansion in temperature is projected to be higher than the worldwide normal, the northern parts situated at a higher altitude are probably going to encounter significantly raised surface air temperature (5.8 °C) (Ali *et al.* 2015). Pakistan's population is quickly expanding with a growth rate of 2.1%, which is higher than the global growth rate of 1.1%. Considering the current situation of

environmental fluctuations, it is anticipated that 2.1 million hectares of arable land of Pakistan will be impacted by drought by 2025 (World Bank 2018). In general, during the summer and spring seasons Peshawar experiences drought periods due to a higher rate of transpiration and an increase in temperature (Farooq *et al.* 2009).

1.3. Impact of climate change on agriculture and hydrology

The majority of the South Asian countries including Pakistan have been recognized as drought susceptible zones exposed to the adverse consequences of climate change (Biemans *et al.* 2013). Exponential population growth rate, lack of water accessibility, soil degradation, and urbanization alongside environmental changes are the global challenges posing a threat to food security (Lal 2013). For the last decades, the agriculture sector of Pakistan has been adversely affected by changing climatic conditions, which might lead to food insecurity by 2030, bringing about a spike in food prices (Bandara & Cai 2014). Drought periods, floods, extreme temperatures and changes in precipitation patterns have a direct adverse impact on crop production. A research study carried out on wheat production of Swat, Charsadda, and Peshawar districts in Khyber Pakhtunkhwa, Pakistan, suggested that short-lived and high yielding varieties should be introduced in mountainous regions due to the prevalent condition of global warming in these areas (Hussain & Mudasser 2007). Moreover, due to climatic fluctuations a pronounced decline has been forecasted in the production of rice and wheat, which are the staple foods of Pakistan, mainly grown in the following districts: Peshawar, Charsadda, Mardan, and Swabi within Khyber Pakhtunkhwa (Ahmad *et al.* 2015a, 2015b; Shakoor *et al.* 2015).

The Peshawar Valley is a significant topographical zone in the upper Indus basin in Pakistan. The Kabul River gathers water from the streams and canals of the Peshawar Valley and drains it into the River Indus (Khuram *et al.* 2021). The decrease in water table ranged from 1 to 3 feet each year in the area of study, precipitation has diminished and stream water discharge showed huge disparities in river water volume over the last two decades. Research findings showed three fundamental indicators for drought, including irregular patterns of precipitation, reduced water table, and low surface water accessibility in the area of study (Idrees *et al.* 2022). Groundwater is the exclusive source of water supply in Peshawar with 1,400 public tube wells having an overall release of 8 million gallons/h. Other than these, hand pumps and dug wells are additionally providing fresh water to meet the water needs of the inhabitants. During 1981–2017, the population growth increased the demand for fresh water. In 2014, the extraction of water from ground water source was 105 mm/year, showing high extraction and low restoration of groundwater from precipitation, leading to shrinking groundwater sources, and ultimately low water table (Khan & Ali 2019). In order to fulfil the food requirements of the steadily expanding population, sewage water is being utilized for irrigation practices due to the scarcity of irrigation water in Peshawar, which might cause soil and crops contamination (Perveen *et al.* 2012).

The present research work was aimed at assessing the physio-biochemical and growth responses of barley *Hordeum vulgare* L. to calcium chloride ($CaCl_2$) solution, its efficacy in attenuating the negative impacts of drought-induced stress, and to unveil the degree of effectiveness of $CaCl_2$ solution in regulating key metabolic activities by enhancing the drought tolerance capacity of barley cultivar exposed to varying levels of drought-induced stress conditions.

2. MATERIALS AND METHODS

2.1. Physiography and meteorology of the area of study

A pot experiment was conducted at the Department of Botany, University of Peshawar, Pakistan (34 °1′ 33.3012″N and 71 °33′ 36.4860″ E) during the spring season 2020. Peshawar is located in the Iranian plateau and has a tropical climatic condition. It is the biggest and capital city of the Khyber Pakhtunkhwa province. To the west, The Federally Administered Tribal Areas (FATA) is located and at its North Mohmand Agency shares the boundary. Likewise, Kohat district is located at its southern side. Nowshera and Charsadda districts are connected towards its north-east and north side. Peshawar is spatially extended out to 1,257 km^2 with an elevation of 340 m/1,115.49 feet (Figure 1). The maximum average temperature in summer goes above 40 °C and the minimum average temperature reaches 25 °C. The wind speed is around 5 knots in December to 24 knots in mid-June. In same manner, the relative humidity varies from 45% in June to 75% in August (Mehmood *et al.* 2016). Peshawar gets precipitation both in summer and winter. Relatively, average winter precipitation is on the higher side compared to summer precipitation, the annual mean monthly temperature ranges from 10.2 to 31.3 °C and mean annual rainfall is 384 mm (Nicol *et al.* 1999; Salma *et al.* 2012). Figure 2 depicts land cover of the area of study generated from Landsat data for the period 1990, 2000, 2010, and 2019, respectively.

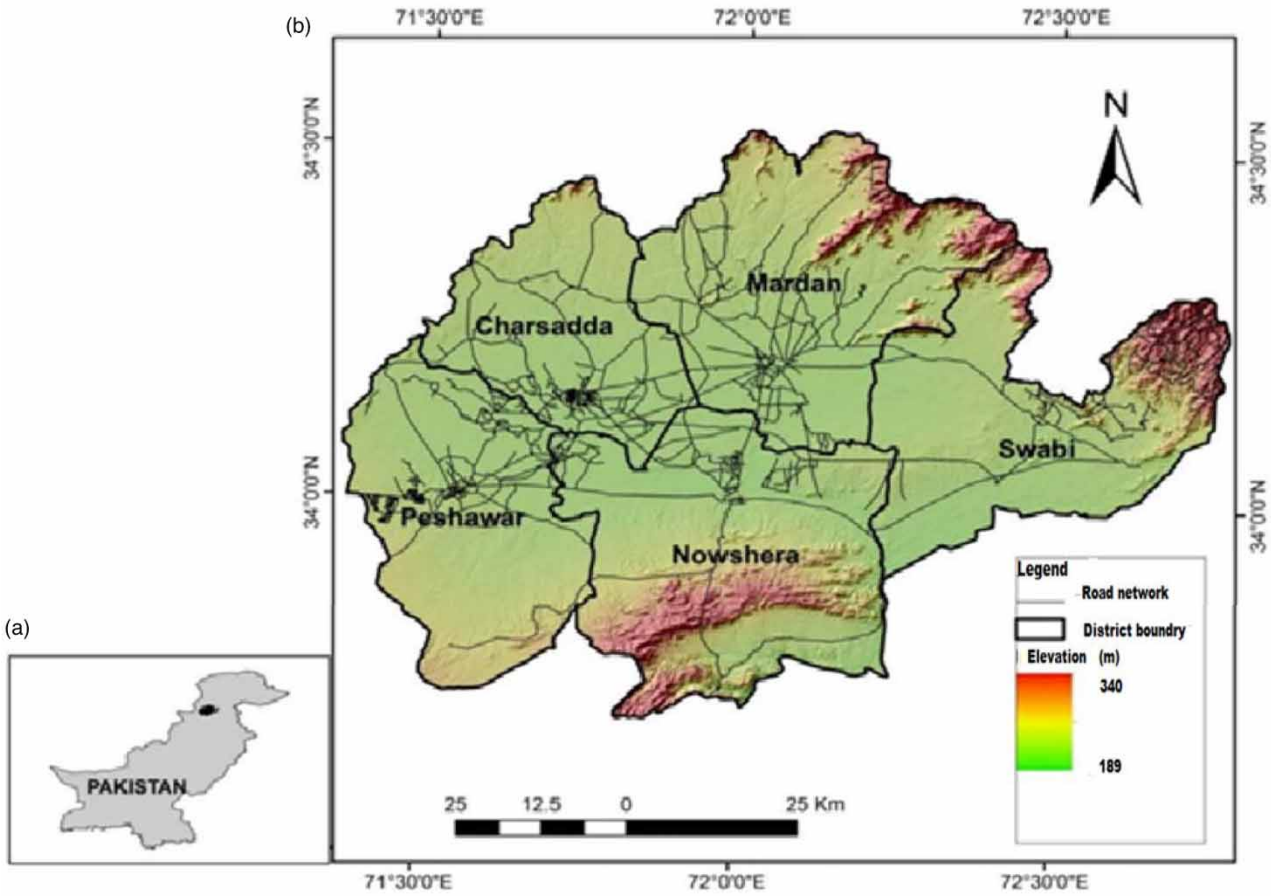


Figure 1 | Spatial map of the study area: (a) map of Pakistan and (b) map of Peshawar. *Source*: Shafique *et al.* (2014).

2.2. Experimental design

Seeds of barley were collected from National institute of Agriculture and Research Centre (NARC), Islamabad. Surface-sterilized seeds were sown in pots of 20 cm height, 18 cm upper and lower diameter and 2 cm thickness containing clay soil, pure sand, and farmyard manure at 1:1:1. Pots were placed 5 cm apart from each other and arranged in a randomized complete block design (RCBD). Twenty seeds were sown per pot and watered normally until complete germination. Germination indices were observed meticulously and noted on a regular basis up to their vegetative maturity. Unwanted weeds were removed periodically and thinning of seedlings was maintained throughout the growing period. $CaCl_2$ solution (10 mM) was prepared by dissolving 1.5 g of solid $CaCl_2$ in 1 l of distilled water. The trial comprised a total of 12 sets and each set had three replicates. After 30 days of germination, the drought period was induced by exposing three sets of trial to 5, 10, and 15 days, respectively; $CaCl_2$ solution (10 mM) was applied through roots to all the three sets exposed to varying levels of drought-induced stress conditions. Three sets of trials were taken as the control group while three sets were kept as control + drought of 5, 10, and 15 days, respectively. The remaining three sets were applied with $CaCl_2$ solution (10 mM) without exposing to drought conditions. At the end of the drought stress periods, four plants were uprooted from each replicate, washed, and dried for the purpose of determination of growth and physio-biochemical attributes (Figure 3).

2.3. Soil texture, pH, electrical conductivity, and elemental analysis

Analysis of the soil used for plant growth showed sandy loam texture, which was assessed with the help of the hydrometer method (Gee & Bauder 1979). Electrical conductivity (EC) of the soil recorded was 2.57 ds/m, pH 6.5

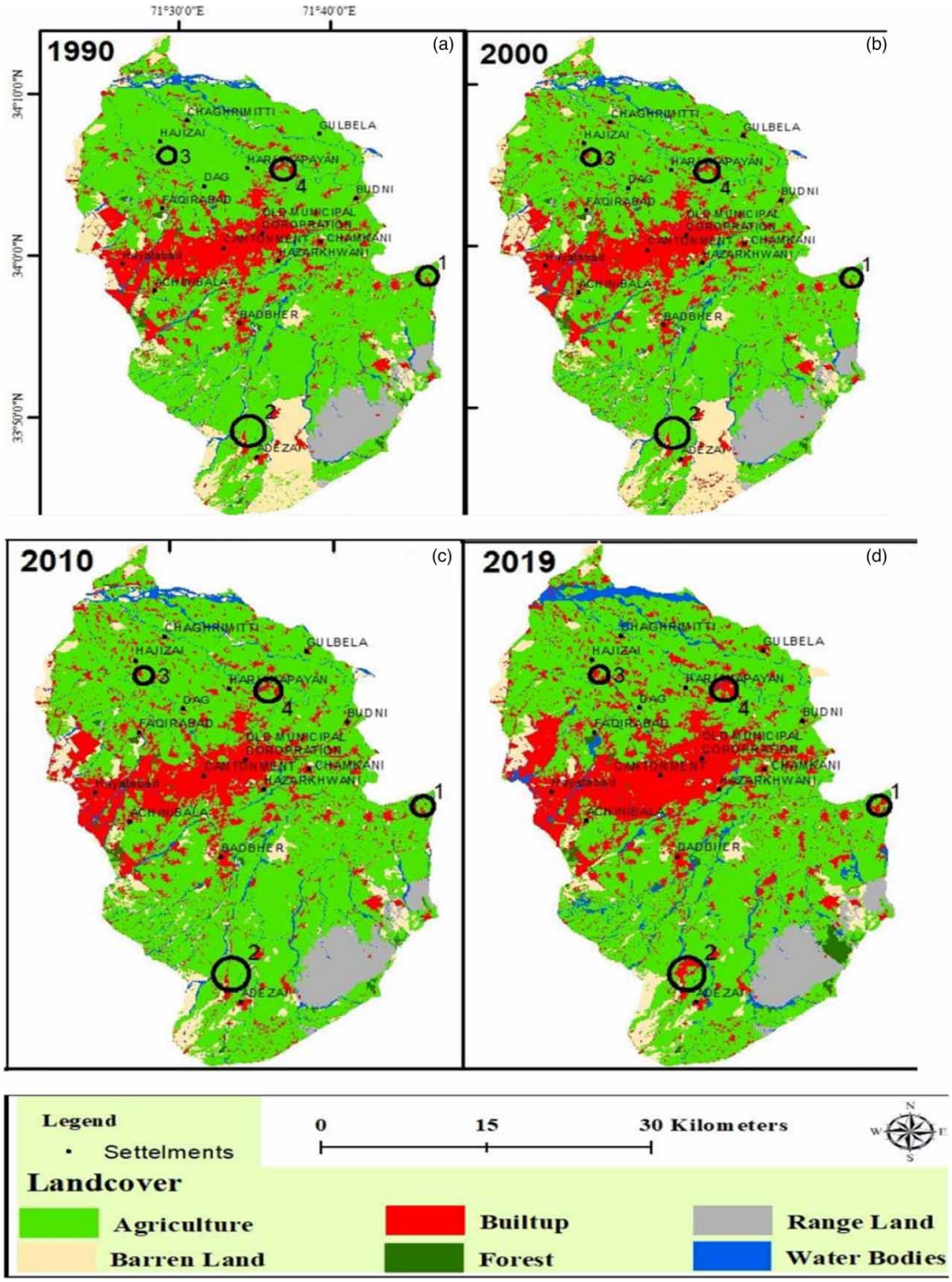


Figure 2 | Depicts land cover generated from Landsat data for the following period: (a) 1990, (b) 2000, (c) 2010, and (d) 2019. *Source*: Ahmad *et al.* (2022).

(McLean 1982). Elemental analysis of the soil showed nitrogen (N) 5.22 g/kg (Keeney & Nelson 1982), organic carbon (C) 26.3 g/kg (Nelson & Sommers 1982), potassium (K) 88.4 mg/kg (Hanway & Heidel 1952), and phosphorus (P) 7.7 mg/kg (Jackson *et al.* 1973).

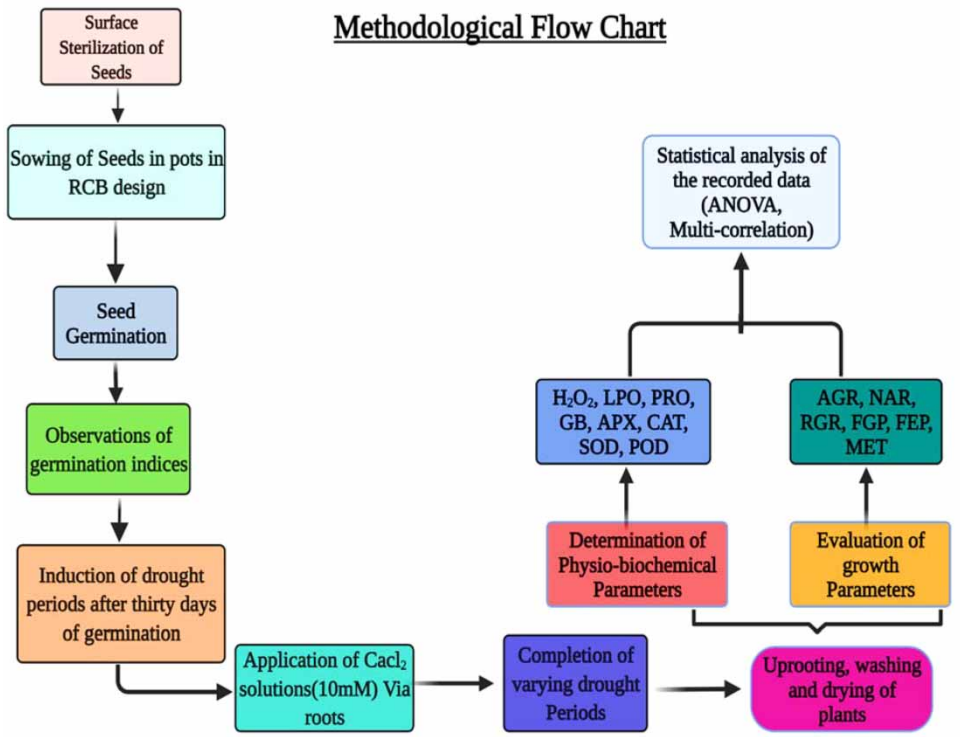


Figure 3 | Methodological flow chart of the designed experiment.

2.4. Measurement of growth attributes

Growth attributes, namely absolute growth rate (*AGR*), net assimilation rate (*NAR*), and relative growth rate (*RGR*) were calculated by following the formulas suggested by Ghule *et al.* (2013).

$$AGR = \frac{H_2 - H_1}{t_2 - t_1} \tag{1}$$

H_1 and H_2 denoted seedling height (cm) at time t_1 and t_2.

NAR is the amount of increase in plant dry weight per unit of assimilatory surface per unit time (g/cm /day).

$$NAR = \frac{W_2 - W_1 \times (\log_e A_2 - \log_e A_1)\ (\text{g/cm /day})}{t_2 - t_1\ A_2 - A_1} \tag{2}$$

A_1 and A_2 denoted leaf surface area in cm^2 and W_1 and W_2 represented dry plant matter, estimated in grams at time t_1 and t_2.

$$RGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1} \tag{3}$$

W_1 and W_2 denoted plant dry weight (g) at time t_1 and t_2, $\log_e$ is natural logarithm.

FGP was estimated by the suggested formula of Al-Ansai & Ksiksi (2018).

$$FGP = \frac{\text{The total seeds germinated at end of trial}}{\text{Number of initial seeds sown}} \times 100 \tag{4}$$

Final emergence percentage (*FEP*) and mean emergence time (*MET*) were calculated by following the formula of Kader (2005).

$$FEP = \frac{\text{Final no. of seedlings emerged}}{\text{Total no. of seeds sown}} \times 100 \quad (5)$$

$$MET = \frac{\Sigma Dn}{\sum n} \quad (6)$$

where '*D*' is the number of days counted from the beginning of emergence and '*n*' is the number of seeds that had emerged on the day '*D*'.

3. ASSESSMENT OF PHYSIOLOGICAL PARAMETERS

3.1. Estimation of hydrogen peroxide content

Hydrogen peroxide (H_2O_2) content was analysed by the methodology of Velikova *et al.* (2000). Fresh leaf and root material (0.5 g) were taken and grounded in 5.0 ml (0.1%) of trichloro acetic acid (TCA). The mixture was centrifuged for 10 min and 0.5 ml of supernatant was collected, 0.5 ml of phosphate buffer and 2.0 ml of potassium iodide were added to supernatant. Optical density (OD) readings were recorded at 390 nm.

3.2. Estimation of lipid peroxidation

3.2.1. Procedure

Lipid peroxidation (LPO) levels were measured by following the methodology of Yang & Miao (2010). Foliar and root material (0.5 g) were homogenized in 10 ml of trichloroacetic acid (TCA) and the samples were centrifuged for 10 min. After centrifugation, 1.0 ml of supernatant was collected and 4.0 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA was mixed with it. The mixture was heated at 90 °C for 1 h. OD was noted at 532 nm.

3.3. Quantification of PRO content

3.3.1. Procedure

PRO content was evaluated by using the method of Tian *et al.* (2019). Fresh leaf and root content (0.5 g) were chopped in 5.0 ml of 3% sulfosalicylic acid, the mixture was filtered, and 2.0 ml of filtrate was taken. 4.0 ml of glacial acetic acid and 4.0 ml of acid ninhydrin were added to the filtrate. The mixture was heated at 100 °C for 1 h, and after heating 4 ml of toluene was added. OD readings were noted at 520 nm.

3.4. Quantification of GB content

3.4.1. Procedure

The amount of GB was determined using the methodology of Di-Martino *et al.* (2003). Plants foliar and root material (0.5 g) were chopped in 5 ml of distilled water. The mixture was passed through Watt man filter paper by diluting the filtrate with H_2SO_4 solution. Samples were centrifuged and cold KI–I_2 was added to each sample. 1.0 ml of supernatant was collected and OD was measured at 365 nm.

Activities of antioxidant enzymes

3.5. Determination of APX activity

3.5.1. Procedure

APX activity was determined by following Salimi *et al.* (2016). Fresh leaf and root material (0.5 g) were chopped in 5.0 ml of phosphate buffer and the samples were spun in a centrifuge machine for 10 min. After centrifugation, 0.1 mM H_2O_2, 0.1 mM EDTA, 0.6 mM ascorbic acid, and 0.1 ml of enzyme extract were added to each sample. Finally, OD reading was taken at 290 nm.

3.6. Determination of CAT activity

3.6.1. Procedure

CAT approximation was determined by pursuing the method of Sewelam *et al.* (2017). Root and fresh foliar material (0.4 g) were grounded in 5 ml of phosphate buffer and the samples were kept in a centrifuge machine for 10 min at 1,000 rpm. After centrifugation, 0.1 ml of supernatant was taken and 2.0 ml of H_2O_2 and 0.1 ml of sodium phosphate were added. OD was measured at 240 nm.

3.7. Estimation of SOD activity

3.7.1. Procedure

SOD activity was determined by Bhardwaj *et al.* (2018). Fresh leaf and root samples (0.5 g) were grounded in 5.0 ml of phosphate buffer and the samples were spun in a centrifuge machine for 10 min. 0.1 ml of supernatant was taken from each sample and 0.72 ml of nitro blue tetrazolium (NBT), 0.72 ml of ethylenediamine tetraacetic acid (EDTA), 0.72 ml of ethanol, and 0.72 ml of riboflavin were added. OD was recorded at 560 nm.

3.8. Quantification of POD activity

3.8.1. Procedure

POD activity was determined using the methodology of Latef (2011). Root and leaf material (0.2 g) were chopped in 5.0 ml of phosphate buffer and centrifugation was done for 10 min. Moreover, 20 mM of guaiacol and 10 mM of H_2O_2 were added to 0.1 ml of supernatant of each sample. At the end, OD was noted at 470 nm.

3.9. Statistical analysis

The experiment comprised two factors including varying levels of induced drought stress (5, 10, and 15 days) and $CaCl_2$ solution (10 mM). The experimental design followed was the randomized complete blocked design (RCBD). Statistical analyses, including analysis of variance (ANOVA) and multi-correlation were performed through statistical software programmes: Statistix 10 and SPSS Statistics 24. Standard errors and mean values were determined and the least significance difference (LSD) test was performed and indicated by letters (a–i).

4. RESULTS

4.1. Effect of $CaCl_2$ on agronomic attributes of barley under water-deficit stress

Inferences from statistical analysis (Tables 1 and 2) revealed significant ($P \leq 0.05$) improvement in growth parameters including AGR, RGR, and NAR, under 5, 10, and 15 days of exposure to induced drought stress supplemented with 10 mM of $CaCl_2$

Table 1 | Effect of calcium chloride solution on absolute growth rate, relative growth rate, and final emergence percentage under varying levels of induced drought stress

	Days	Treatments	AGR	RGR	FEP
T1		Control	1.546 ± 0.121^{def}	7.875 ± 2.005^{cde}	96.666 ± 38.944^{ab}
T2	5 days	Drought	1.006 ± 0.087^{efg}	4.812 ± 0.114^{ef}	61.666 ± 4.714^{b}
T3	5 days	Drought + calcium chloride	0.813 ± 0.073^{a}	5.695 ± 0.658^{ab}	73.333 ± 4.721^{b}
T4		Calcium chloride	1.441 ± 0.101^{a}	8.719 ± 1.082^{ab}	70.2 ± 4.082^{bc}
T5		Control	0.061 ± 1.058^{ghi}	1.612 ± 0.493^{fg}	65.1 ± 29.533^{bc}
T6	10 days	Drought	0.541 ± 0.114^{hi}	0.629 ± 0.193^{g}	65.11 ± 8.164^{bc}
T7	10 days	Drought + calcium chloride	0.413 ± 0.275^{ab}	0.721 ± 0.302^{a}	71.666 ± 36.591^{bc}
T8		Calcium chloride	0.506 ± 0.245^{ab}	2.272 ± 0.601^{ab}	68.333 ± 6.236^{bc}
T9		Control	3.666 ± 1.546^{b}	17.039 ± 4.341^{b}	65.112 ± 31.885^{bc}
T10	15 days	Drought	2.346 ± 0.073^{cd}	9.149 ± 0.597^{c}	60.12 ± 4.082^{c}
T11	15 days	Drought + calcium chloride	1.693 ± 0.378^{bc}	10.945 ± 0.978^{b}	60.11 ± 30.912^{c}
T12		Calcium chloride	3.373 ± 0.405^{a}	12.072 ± 2.765^{a}	65.111 ± 4.082^{bc}

AGR, absolute growth rate; RGR, relative growth rate; FEP, final emergence percentage.
(Mean ± standard error) letters (a–i) indicating least significance difference among the mean values at $p \leq 0.05$.

Table 2 | Effect of calcium chloride solution on final germination percentage, net assimilation rate, and mean emergence time under varying levels of induced drought stress

	Days	Treatments	FGP	NAR	MET
T1		Control	76.666 ± 8.498^{b}	1.774 ± 0.233^{cd}	1.133 ± 1.331^{ab}
T2	5 days	Drought	91.666 ± 4.714^{cd}	1.034 ± 0.095^{ef}	3.144 ± 3.349^{cd}
T3	5 days	Drought + calcium chloride	73.333 ± 6.236^{abcd}	1.291 ± 0.135^{a}	0.288 ± 0.317^{cd}
T4		Calcium chloride	70 ± 4.082^{bc}	2.154 ± 0.073^{a}	1.777 ± 1.938^{bcd}
T5		Control	65 ± 4.082^{bc}	0.512 ± 0.451^{fg}	0.611 ± 0.735^{d}
T6	10 days	Drought	$65 + 8.164^{bcd}$	0.151 ± 0.287^{h}	2.21 ± 2.336^{def}
T7	10 days	Drought + calcium chloride	71.666 ± 8.498^{cd}	0.142 ± 0.218^{ab}	1.655 ± 1.963^{abc}
T8		Calcium chloride	68.333 ± 6.236^{bc}	0.087 ± 0.238^{a}	1.388 ± 1.963^{ab}
T9		Control	65 ± 4.082^{bc}	4.301 ± 0.614^{b}	1.377 ± 1.541^{abcd}
T10	15 days	Drought	60 ± 4.082^{c}	1.728 ± 0.195^{cd}	0.6 ± 0.734^{bcd}
T11	15 days	Drought + calcium chloride	60 ± 8.164^{c}	2.104 ± 0.135^{ab}	2.833 ± 3.252^{abc}
T12		Calcium chloride	65 ± 4.082^{bc}	5.754 ± 0.555^{a}	2.166 ± 2.301^{abc}

FGP, final germination percentage; NAR, net assimilation rate; MET, mean emergence time.

solution in comparison with the control and the rest of the groups. On the contrary, these parameters were noted to be negatively affected in the group which was exposed to varying levels of induced drought stress with no $CaCl_2$ application. Moreover, growth parameters including FEP, final germination percentage (FGP), and MET exposed to 5, 10, and 15 days of the induced drought stress condition responded negatively. Moreover, application of 10 mM $CaCl_2$ solution did not cause any significant improvement in these parameters under varying levels of induced drought stress as compared to the control group.

4.2. Effect of $CaCl_2$ on physiological attributes of barley under water-deficit stress

4.2.1. Effect on H_2O_2 concentration

Varying levels (5, 10, and 15 days) of induced water stressed condition significantly ($P \leq 0.05$) boosted the concentration of H_2O_2 content in both leaf and root. Comparing with control group and rest of the groups, $CaCl_2$ (10 mM) applied under all the drought levels lowered down the quantity of H_2O_2 content in leaf and root (Figure 4; Table 3).

4.2.2. Effect on lipid peroxide content

All the levels of limited water regimes significantly ($P \leq 0.05$) enhanced the concentration of lipid peroxide (LPO) content in foliar material and root. In contrast with control group and all other groups, application of $CaCl_2$ (10 mM) solution under all

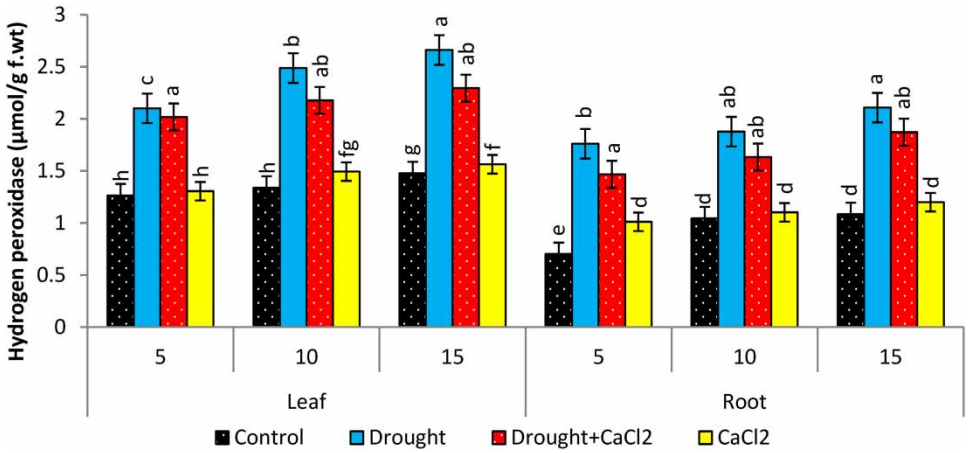


Figure 4 | Effect of $CaCl_2$ on H_2O_2 content in *Hordeum vulgare* L. under varying levels of induced drought stress (mean ± standard error). Letters (a–h) indicate least significance difference among the mean values at $p \leq 0.05$.

Table 3 | Analysis of variance of the measured physio-biochemical parameters with calcium chloride application under varying levels of induced drought stress

Trait	Source of variance	SS	df	MS	*F*	*P*
LPO-L	Drought	0.041	3	0.014	34.622	0.056*
	Treatment	0.049	11	0.004	22.755	0.005
	Drought + Treatment	0.052	12	0.004	46.772	0.008**
	Error	0.002	23	9.180		
LPO-r	Drought	0.051	3	0.017	44.372	0.565*
	Treatment	0.058	11	0.005	21.531	0.089
	Drought + Treatment	0.062	12	0.005	84.310	0.004**
	Error	0.001	23	6.159		
GB-L	Drought	0.086	3	0.029	11.717	0.004**
	Treatment	0.157	11	0.014	45.429	0.367
	Drought + Treatment	0.158	12	0.013	44.224	0.005**
	Error	0.007	23	0.000		
GB-r	Drought	0.097	3	0.032	11.467	0.005**
	Treatment	0.180	11	0.016	49.828	0.007
	Drought + Treatment	0.181	12	0.015	50.476	0.021**
	Error	0.007	23	0.000		
PRO-L	Drought	0.209	3	0.070	14.114	0.008**
	Treatment	0.360	11	0.033	107.824	0.000
	Drought + Treatment	0.362	12	0.030	121.105	0.004**
	Error	0.006	23	0.000		
PRO-r	Drought	0.088	3	0.029	12.977	0.089
	Treatment	0.152	11	0.014	38.543	0.000*
	Drought + Treatment	0.152	12	0.013	36.986	0.002***
	Error	0.008	23	0.000		
H_2O_2-L	Drought	3.478	3	1.159	7.191	0.041*
	Treatment	8.537	11	0.776	187.277	0.059
	Drought + Treatment	8.580	12	0.715	294.871	0.021*
	Error	0.056	23	0.002		
H_2O_2-r	Drought	3.012	3	1.004	8.120	0.000*
	Treatment	6.387	11	0.581	23.928	0.023
	Drought + Treatment	6.410	12	0.534	21.966	0.003**
	Error	0.559	23	0.024		
APX-L	Drought	0.051	3	0.017	28.553	0.521
	Treatment	0.052	11	0.005	6.316	0.065
	Drought + Treatment	0.060	12	0.005	12.929	0.007**
	Error	0.009	23	0.000		
APX-r	Drought	0.003	3	0.001	9.424	0.013*
	Treatment	0.005	11	0.000	5.599	0.005
	Drought + Treatment	0.005	12	0.000	5.220	0.005**
	Error	0.002	23	7.397		
CAT-L	Drought	0.133	3	0.044	47.245	0.032*

(*Continued.*)

Table 3 | Continued

Trait	Source of variance	SS	df	MS	*F*	*P*
	Treatment	0.158[a]	11	0.014	63.158	0.000
	Drought + Treatment	0.162	12	0.013	225.285	0.001***
	Error	0.001	23	5.98		
CAT-r	Drought	0.124[a]	3	0.041	39.375	0.098**
	Treatment	0.154[a]	11	0.014	75.660	0.007
	Drought + Treatment	0.157	12	.013	215.540	0.005**
	Error	0.001	23	6.063		
SOD-L	Drought	0.042[a]	3	0.014	14.300	0.000***
	Treatment	0.071[a]	11	0.006	49.051	0.000
	Drought + Treatment	0.073	12	0.006	141.622	0.000***
	Error	0.001	23	4.290		
SOD-r	Drought	0.028[a]	3	0.009	13.012	0.006**
	Treatment	0.049[a]	11	0.004	38.287	0.313
	Drought + Treatment	0.050	12	0.004	79.946	0.001**
	Error	0.001	23	5.22		
POD-L	Drought	0.053[a]	3	0.018	15.359	0.009**
	Treatment	0.086[a]	11	0.008	45.027	0.067
	Drought + Treatment	0.088	12	0.007	75.055	0.004**
	Error	0.002	23	9.779		
POD-r	Drought	0.055[a]	3	0.018	9.314	0.012**
	Treatment	0.113[a]	11	0.010	52.479	0.008
	Drought + Treatment	0.115	12	0.010	80.805	0.001**
	Error	0.003	23	0.000		

LPO-L, lipid peroxide in leaf; LPO-r lipid peroxide in root; GB-L, glycine betaine in leaf; GB-r, glycine betaine in root; PRO-L, proline in leaf; PRO-r, proline in root; H_2O_2-L, hydrogen peroxide in leaf; H_2O_2-r, hydrogen peroxide in root; APX-L ascorbate peroxidase in leaf; APX-r, ascorbate peroxidase in root; CAT-L catalase in leaf, CAT-r catalase in root; SOD-L, superoxide dismutase in leaf; SOD-r, superoxide dismutase in root; POD-L peroxidase in leaf; POD-r, peroxidase in root; SS, sum of square; Df, degree of freedom; MS, mean square; *F*, variation between sample means; *P*, probability value.
*Correlation is significant at 0.05.
**Correlation is significant at 0.01.

the induced drought stress levels did not show any significant decrease in the levels of LPO content in leaf as well as in root (Figure 5; Table 3).

4.2.3. Changes in total PRO concentration

From statistical analysis it was concluded that water-deficit stress conditions increased total PRO levels. On the contrary, $CaCl_2$ application under varying levels of induced drought stress significantly ($P \leq 0.05$) enhanced total PRO content as compared to control, drought, and rest of the groups (Figure 6; Table 3)

4.2.4. Changes in GB levels

Likewise, total PRO content, increase was noted in the levels of GB content. In comparison with the control group and rest of the groups, application of 10 mM of $CaCl_2$ solution under water-stressed conditions showed significant ($P \leq 0.05$) enhancement in GB content (Figure 7; Table 3).

4.2.5. Effect on APX activity

Varying levels of induced drought stress condition in combination with $CaCl_2$ (10 mM) significantly ($P \leq 0.05$) increased APX activity in both root and leaf. The amount of APX increased with increasing the duration of drought stress level. In

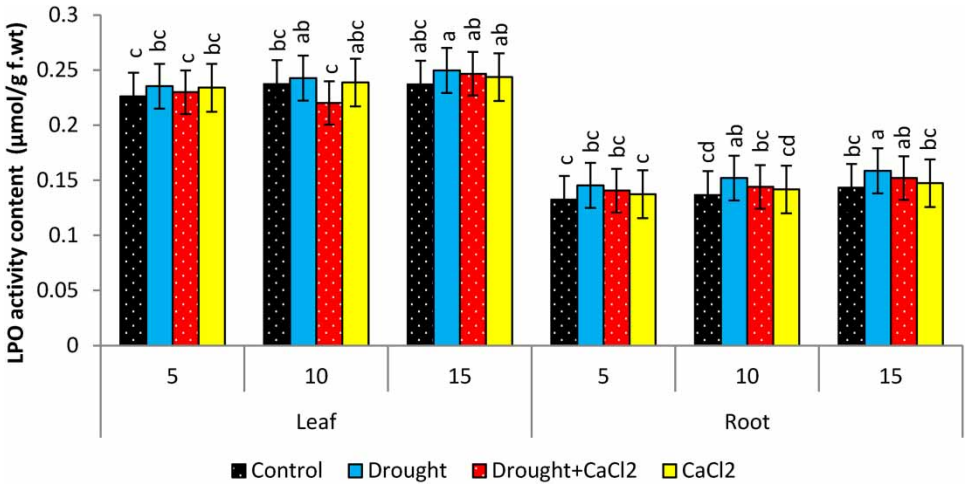


Figure 5 | Effect of $CaCl_2$ on lipid peroxide activity in *Hordeum vulgare* L. under varying levels of induced drought stress (mean ± standard error). Letters (a–d) indicate least significance difference among the mean values at $p \leq 0.05$.

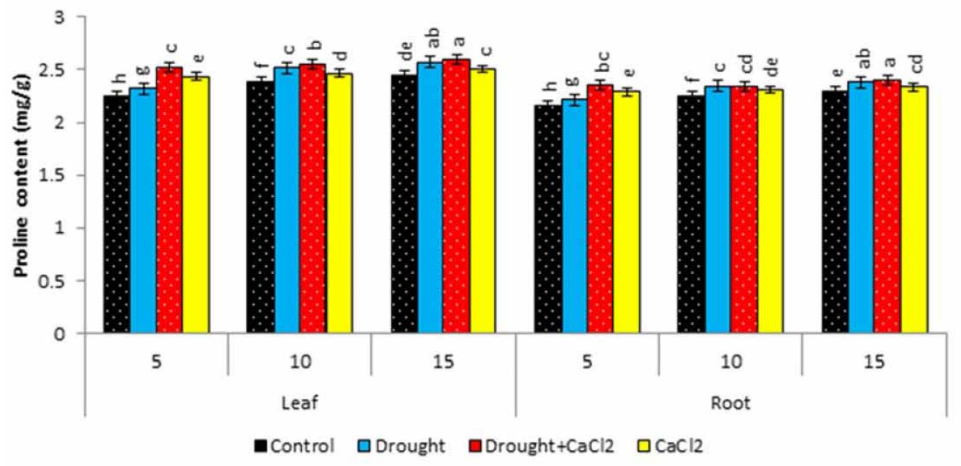


Figure 6 | Effect of $CaCl_2$ on proline accumulation in *Hordeum vulgare* L. under varying levels of induced drought stress (mean ± standard error). Letters (a–h) indicate least significance difference among the mean values at $p \leq 0.05$.

comparison with control group and other groups, higher APX activity was observed in the group which was treated with $CaCl_2$ application under varying levels of induced drought stress condition (Figure 8; Table 3).

4.2.6. Responses of CAT activity

Results from statistical analysis showed that the drought stress regimes adversely affected the plant enzymatic system. Different levels of induced drought stress (5, 10, and 15 days) individually and in combination with $CaCl_2$ (10 mM) application significantly ($P \leq 0.05$) enhanced the activity of CAT in roots and leaves, as compared to the control group and other groups (Figure 9; Table 3).

4.2.7. Responses of SOD activity

Application of 10 mM of $CaCl_2$ via roots had a pronounced effect on the enzymatic system under induced drought stress situations. On exposure to varying drought stress levels and application of $CaCl_2$ (10 mM) solution, a significant ($P \leq 0.05$) increase was noted in SOD activity in both roots and leaves of the studied variety of barley (Figure 10; Table 3).

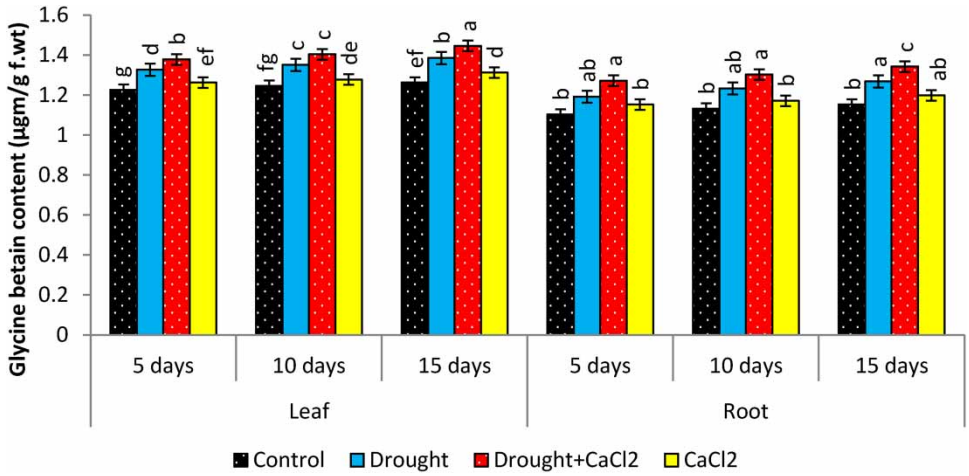


Figure 7 | Effect of $CaCl_2$ on glycine betaine in *Hordeum vulgare* L. under varying levels of induced drought stress (mean ± standard error). Letters (a–g) indicate least significance difference among the mean values at $p \leq 0.05$.

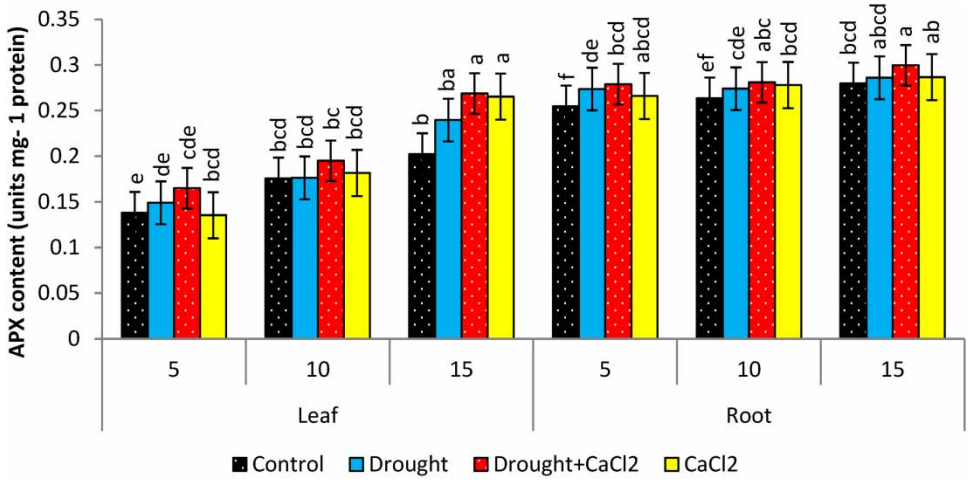


Figure 8 | Effect of $CaCl_2$ on APX activity in *Hordeum vulgare* L. under varying levels of induced drought stress (mean ± standard error). Letters (a–f) indicate least significance difference among the mean values at $p \leq 0.05$.

4.2.8. Effect on POD activity

With increasing levels of induced water-deficit stress condition, POD showed a notable increase. Application of 10 mM of $CaCl_2$ solution under varying drought stress regimes further enhanced POD activity to a significant ($P \leq 0.05$) level both in roots and leaves (Figure 11; Table 3).

4.2.9. Multi-correlation analysis of physiological and biochemical attributes of barley

Multi-correlation analysis among physio-biochemical attributes of barley (Table 4) showed that lipid peroxide in root (LPO-r) significantly ($P = 0.05$) correlated with lipid peroxide in leaf (LPO-L). Likewise, glycine betaine in leaf (GB-L) indicated significant ($P = 0.05$) correlation with LPO-L and LPO-r. Moreover, PRO content in leaf (PRO-L) with GB-L, H_2O_2 in leaf (H_2O_2-L) showed a positive correlation with LPO-L and LPO-r. Similarly, H_2O_2-r showed a positive correlation with LPO-L, LPO-r, and H_2O_2-L. APX-L and APX-r presented a positive and significant ($P = 0.05$) correlation with GB-L, GB-r, PRO-L, and PRO-r. All the enzymes of root and leaves showed a positive and significant correlation with each other and also with osmolyte content of root and leaves which mainly included PRO and GB. From multi-correlation analysis it was suggested that osmoprotectants and antioxidant enzymes work in a synchronized manner and correlated positively during

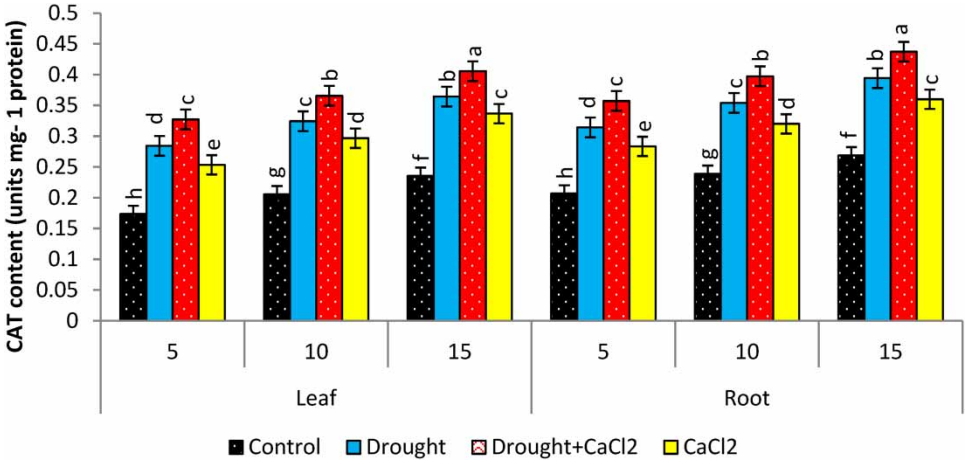


Figure 9 | Effect of $CaCl_2$ on catalase activity in *Hordeum vulgare* L. under varying levels of induced drought stress (mean ± standard error). Letters (a–h) indicate least significance difference among the mean values at $p \leq 0.05$.

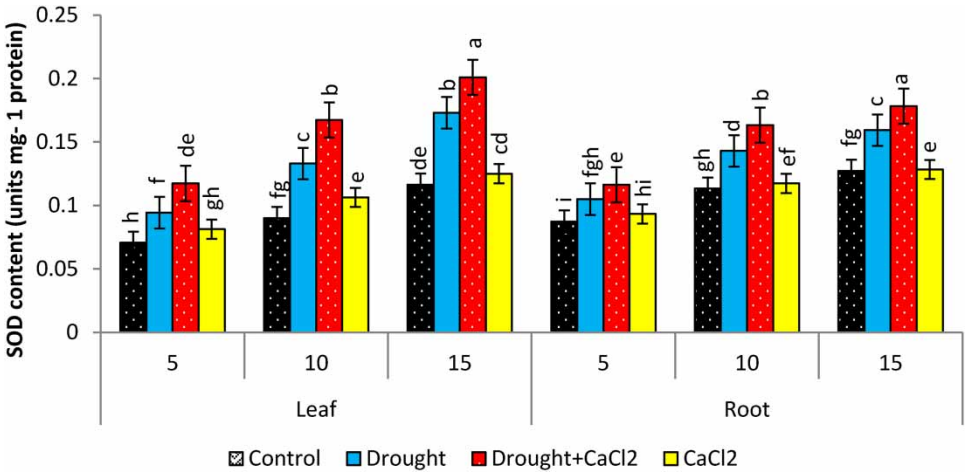


Figure 10 | Effect of $CaCl_2$ on superoxide dismutase activity in *Hordeum vulgare* L. under varying levels of induced drought stress (mean ± standard error). Letters (a–i) indicate least significance difference among the mean values at $p \leq 0.05$.

stress conditions. Beside these results, a negatively significant ($P = 0.05$) correlation was noted between all the antioxidant enzymes with H_2O_2 and LPO content both in root and leaf.

5. DISCUSSION

Continuous changes in environmental conditions have been proving dangerous for biotic components on earth; water-deficit stress is one of the dire consequences of changes in climatic conditions. It is experimentally proven that drought stress impedes plant growth and development. Plants have adopted the internal defence mechanism in the form of antioxidant enzymes and production of osmoprotectants such as PRO and GB, which reduce the damaging effects of oxidative stress by maintaining the salt and water balance (Khan *et al.* 2010a, 2010b, 2010c). Notably, drought-stressed situation results in stomatal closure with reduced carbon dioxide exchange by diminishing the rate of photosynthesis that eventually decreases plant total biomass (Avramova *et al.* 2015).

Interpretation from statistical analysis (Tables 1 and 2) revealed a significant ($P \leq 0.05$) increase in growth parameters including AGR, RGR, and NAR exposed to 5, 10, and 15 days of drought stress supplemented with 10 mM of $CaCl_2$ solution, while these parameters were noted to be affected negatively in the rest of the groups. Moreover, growth parameters including

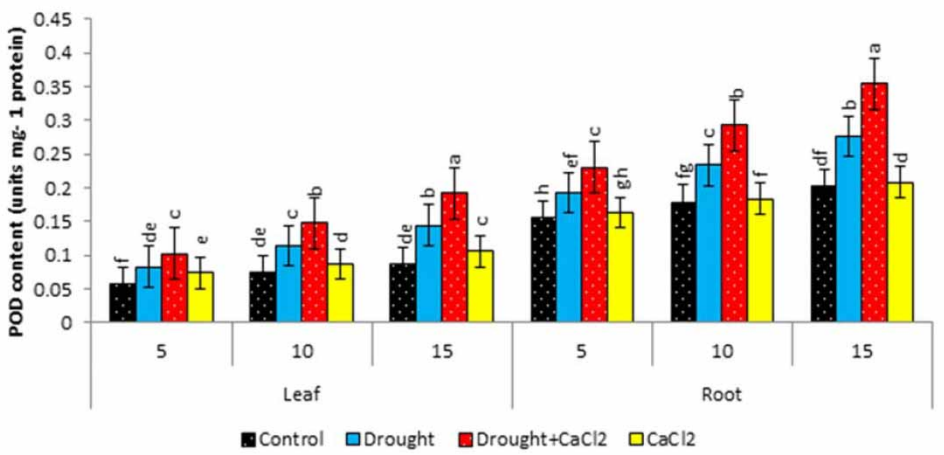


Figure 11 | Effect of $CaCl_2$ on peroxidase activity in *Hordeum vulgare* L. under varying levels of induced drought stress (mean ± standard error). Letters (a–h) indicate least significance difference among the mean values at $p \leq 0.05$.

FEP, FGP, and MET exposed to 5, 10, and 15 days of induced drought stress condition responded negatively. Application of 10 mM $CaCl_2$ solution did not cause any significant improvement in these parameters under varying levels of induced drought stress as compared to the control group. Therefore, it confirmed the results obtained by Abdelly *et al.* (2015) in sunflower; by Ayad *et al.* (2009) in *Geranium* cultivar; by Mekki *et al.* (2015) in cotton plant; and Semida *et al.* (2014) in *Vicia faba* cultivar. Results in Tables 1 and 2 show AGR, RGR, NAR, FEP, FGP and MET, which showed improvement in the control group and affected negatively in the groups which were exposed to varying levels of induced drought stress with no $CaCl_2$ application, thus confirming the same investigation achieved by Sardoei & Mohammadi (2014). Reduction in growth parameters such as AGR, RGR, and NAR are closely related to cytokinesis and cell elongation under drought stress regimes. It is believed that this reduction is due to stomatal closure and diminished photosynthesis, persistent exposure to water limited regimes eventually leading to shrinkage of leaves (Zaheer *et al.* 2019).

Water-deficit condition is characterized by low water content, reduced turgidity, wilting, closure of stomata, and eventually decrease in cell enlargement and growth; plant growth is variously influenced by internal and external factors; despite its genetic makeup, examination of external morphology is an essential tool for the evaluation of crop productivity and total biomass (Sestak *et al.* 1971) Extensive root growth during drought stress regimes is a fundamental adaptation mechanism in drought-tolerant plants; however, in drought susceptible species, the growth of the root system is adversely affected (Passioura 1982). It has been reported that water stress conditions decreased the root length in *Populus* species (Yin *et al.* 2005). Similarly, Stem length decreased in *Albizzia* seedlings under water limited conditions (Sundaravalli & Paliwal 2005). Leaf area and root to shoot ratio are important to maintain control of water use in plants. Leaf area, RGR, and AGR are reported to be significantly reduced under the drought stress (Yadav *et al.* 2005). Reduced leaf area under water-deficient regimes is the main factor behind inefficient photosynthesis and low crop yield (Kramer 1983). Under drought stress conditions reduction in plant height might be linked with poor cell enlargement and growth owing to the low turgor pressure and more leaf senescence (Rane *et al.* 2001). Drought stress suppresses the photochemical proficiency of the photosystem (PS-II) by diminishing electron transport, removal of extrinsic proteins, and release of magnesium and calcium ions from their binding, that ultimately leading to photosynthetic pigments degeneration and low total plant biomass (Wahid *et al.* 2007; Barta *et al.* 2010).

Water-deficient stress condition significantly ($P \leq 0.05$) boosted the concentration of H_2O_2 content in both parts (leaf and root) of barley, whereas comparing with control group and rest of the groups $CaCl_2$ solution (10 mM) under varying levels of induced drought stress condition lowered the quantity of H_2O_2 content in leaf and root (Figure 4; Table 1). Biotic and abiotic stresses induce the overproduction of H_2O_2 in plant cells, H_2O_2 can directly lead to damage cell membranes, proteins, and nucleic acids (Wang *et al.* 2009). Products like MDA, PRO, and H_2O_2 are generally known as stress markers. A pronounced increase has been noted in the levels of MDA, PRO, and H_2O_2 during oxidative stress regimes (Khan *et al.* 2010a, 2010b, 2010c).

Table 4 | Multi-correlation analysis of physiological and biochemical components of *Hordium vulgare* L. with calcium chloride application under varying levels of induced drought stress

Trait	LPO-L	LPO-r	GB-L	GB-r	Pro-L	Pro-r	H_2O_2-L	H_2O_2-r	APX-L	APX-r	CAT-L	CAT-r	SOD-L	SOD-r	POD-L	POD-r
LPO-L	1.0															
LPO-r	0.517*	1.0														
GB-L	0.448**	0.544**	1.0													
GB-r	0.312	0.151	0.085	1.0												
PRO-L	0.551	0.615	0.794**	0.092	1.0											
PRO-r	0.555	0.640	0.755	0.100	0.965	1.0										
H_2O_2-L	0.432**	0.692**	0.779	0.208	0.569	0.547	1.0									
H_2O_2-r	0.515**	0.718**	0.781	0.176	0.635	0.623	0.945**	1.0								
APX-L	0.549	0.472**	0.494**	0.07**	0.664**	0.689**	0.252**	0.349**	1.0							
APX-r	0.461**	0.271**	0.431**	0.019**	0.610**	0.606**	0.209**	0.244**	0.591	1.0						
CAT-L	0.546	0.645**	0.915**	0.051**	0.871**	0.839**	0.750**	0.761**	0.634**	0.646**	1.0					
CAT-r	0.536**	0.647**	0.928**	0.052**	0.871**	0.842**	0.762**	0.775**	0.623**	0.620	0.995**	1.0				
SOD-L	0.524	0.583**	0.880**	0.047**	0.807**	0.773**	0.659**	0.692**	0.700	0.459	0.854	0.867**	1.0			
SOD-r	0.477	0.537**	0.909**	0.082**	0.819	0.761**	0.674**	0.694**	0.613**	0.443**	0.875**	0.886	0.976**	1.0		
POD-L	0.517	0.633**	0.888**	0.011**	0.820**	0.790**	0.700**	0.732**	0.657**	0.445	0.879	0.896	0.971	0.953	1.0	
POD-r	0.482**	0.577**	0.902**	0.005**	0.786**	0.754**	0.704**	0.724**	0.607	0.386*	0.848**	0.872**	0.970**	0.956**	0.983**	1.0

LPO-L, lipid peroxide in leaf; LPO-r lipid peroxide in root; GB-L, glycine betaine in leaf; GB-r, glycine betaine in root; PRO-L, proline in leaf; PRO-r, proline in root; H_2O_2-L, hydrogen peroxide in leaf; H_2O_2-r, hydrogen peroxide in root; APX-L, ascorbate peroxidase in leaf; APX-r, ascorbate peroxidase in root; CAT-L, catalase in leaf; CAT-r, catalase in root; SOD-L, superoxide dismutase in leaf; SOD-r, superoxide dismutase in root; POD-L, peroxidase in leaf; POD-r, peroxidase in root.

*Correlation is significant at 0.05.

**Correlation is significant at 0.01.

Drought being a major abiotic stress causes the over production of H_2O_2 in plants, leading to damage to the plasma membrane and other biomolecules. According to previous findings, H_2O_2 concentration tends to increase with increasing salinity and drought. It is reported that application of $CaCl_2$ solution in combination with NaCl solution slightly reduced H_2O_2 content in various plants exposed to drought stress regimes (Wang *et al.* 2009). Our results were closely parallel with the findings made by Jaleel *et al.* (2007a, 2007b), who studied the effects of $CaCl_2$ solution on *Catharanthus roseus* grown under drought stress and noted a clear reduction in H_2O_2 content.

Varying limited water situation (5, 10, and 15 days) significantly ($P \leq 0.05$) enhanced the concentration of LPO content in foliar material and root of barley. In contrast with the control group and all other groups, treatment with $CaCl_2$ solution (10 mM) in combination with varying levels of induced drought stress decreased the levels of LPO content in leaf and root (Figure 5; Table 3).

LPO is generally quantified in the form of malondialdehyde (MDA) content. Under drought conditions, LPO takes place as a result of oxidative damage to plasma membrane by the production of reactive oxygen species (ROS) and other free radicals (Hernandez & Almansa 2002). $CaCl_2$ is a vital macro-nutrient absorbed by roots and used directly as an osmotic solute for osmotic adjustment. The exogenous application of $CaCl_2$ promoted the defence mechanism in plants under water stress, salinity stress, and heavy metals stress by creating osmolytes and activating antioxidant enzymes, decreasing the LPO of the plasma membrane by connecting different proteins and lipids at the membrane surface (Jiang & Huang 2001). The same results were achieved by Bhardwaj *et al.* (2018); Jaleel *et al.* (2007a, 2007b) in the case of *Triticum aestivum* and *C. roseus*, respectively, applied with 10 mM $CaCl_2$ solution under drought stress conditions.

In the current experimental work, an improvement in PRO content in *H. vulgare* L. seedling under an induced water stress condition in combination with $CaCl_2$ solution was observed. Results showed that water-deficit stress increased PRO content, while $CaCl_2$ (10 mM) solution further raised the levels of PRO content. Interactively, $CaCl_2$ and varying drought stress periods significantly ($P \leq 0.05$) enhanced the concentration of PRO content as compared to control, drought and the rest of the groups (Figure 6; Table 3).

PRO accumulation in the leaves is considered an important adaptation of plants to abiotic stress conditions (Dobra *et al.* 2011). PRO is well known for its osmoprotective property under stress regimes. In plants, an increased level of PRO during drought stress is believed to be an indicator of drought stress tolerance. PRO plays a key role in the mechanism of osmotic adjustment in many crops under severely stressed conditions. The amount of PRO is regulated by two important enzymes: proline oxidase (PROX) and γ-glutamyl kinase (γ-GK) (Ahmad *et al.* (2010)). Parallel to our results, similar findings were noted by Jaleel *et al.* (2007a, 2007b) who carried out studies on *C. roseus* exposed to $CaCl_2$ solution under drought stress. Likewise PRO content, a significant ($P \leq 0.05$) increase was noted in the levels of glycine betaine content under induced water-deficit stress conditions with $CaCl_2$ application (Figure 7; Table 3).

GB is quaternary ammonium compound and acts as an osmotic solute. Glycophytes like oat, tomato, peas, beans, beets, and carrots showed an increase in GB with increasing salinity and drought (Girija *et al.* 2002). Osmotic adjustment is an important physiological event through which plants resist stress conditions. During abiotic stress accumulation of organic solutes such as PRO and GB in the cytoplasm helps in the osmotic adjustment of organic molecules. In resistance to drought, many species of plants accumulated higher content of organic solutes in their tissues (Khan *et al.* 2010a, 2010b, 2010c). The same results were found by Bhardwaj *et al.* (2018); Jaleel *et al.* (2007a, 2007b) in the case of *T. aestivum* and *C. roseus* exposed to 10 mM $CaCl_2$ under drought stress regimes.

Varying levels of induced drought stress conditions in combination with $CaCl_2$ (10 mM) application significantly ($P \leq 0.05$) increased APX, CAT, SOD, and POD activities in *H. vulgare* L. The amount of all the above-mentioned enzymes increased with increasing duration of drought stress. In comparison with the control group the rest of the groups' higher enzymatic activities were observed in 10 and 15-days of drought stress regimes with 10 mM $CaCl_2$ solution (Figures 8–11; Table 3). Under stress conditions, the accumulation of ROS and other free radicals cause corrosive effects on biological membranes and biomolecules. APX, POD, SOD, and other protective enzymes effectively scavenge ROS and other free radicals (Zhang *et al.* 2019). Plant cells have a complex enzymatic as well as a non-enzymatic antioxidant system to prevent cellular damage caused by ROS (Hasanuzzaman & Fujita 2011; Kumar *et al.* 2013), non-enzymatic components like carotenoids, glutathione and tocopherols, coupled with antioxidant enzymes, such as SOD, CAT, and glutathione peroxidase (GPX) scavenge ROS and other free radicals (Ahmad *et al.* 2015a, 2015b). ROS attack proteins, lipids and nucleic acids, and the degree of damage depends on the balance between ROS production and their removal by the antioxidative scavenging systems (Menezes-Benavente *et al.* 2004).

In the drought related studies, during the recovery process $CaCl_2$ showed a significant effect on antioxidant enzymes. The ability of *Camellia sinensis* genotypes to enhance CAT activities during post-stress rehydration and $CaCl_2$ treatment could improve the post-drought recovery process. As *C. sinensis* is a C_3 plant, higher CAT activity could scavenge the H_2O_2 formed in the photorespiratory pathway and thereby reduce photorespiration rate (Jeyaramraya *et al.* 2003). SOD activities were also increased in the tested *C. sinensis* cultivars along with consistently increasing activities of POX and GR (GR). Increased GR activity in stress plants improves stress tolerance and has the ability to alter the redox potential of the important component of the electron transport chain. Glutathione is maintained in a reduced state by GR, higher GR activity induced by $CaCl_2$ in water stressed plants could be an adaptive advantage for resumption of growth after stress conditions (Asada & Takahashi 1987), but an increase in SOD activities after drought during the recovery period could be an adaptation to improve growth after drought stress regimes. Parallel to our findings, Upadhyaya *et al.* (2011) claimed consistent results, suggesting that $CaCl_2$ solution improved the activities of antioxidant enzymes along with growth responses under drought stress conditions.

6. CONCLUSIONS

In conclusion, it has been evident that $CaCl_2$ solution (10 mM) applied via roots effectively curbed the damaging effects of ROS and other free radicals formed during induced drought stress conditions; by regulating key physiological processes such as osmolyte accumulation which mainly include PRO , proteins, GB, and the activation of the antioxidant enzymatic system. In addition, the agriculture sector of Pakistan has been adversely affected by the continuous shifts in climatic conditions that might lead the country towards food insecurity by 2030. Besides, the ground water table of Peshawar has been reducing 1–3 feet annually. The present findings could be helpful for determining the ways in which conditions could be handled to secure the survival and enhance the growth of barley under water-limited conditions. Furthermore, there is still a great need for further research to assess various growth and physio-biochemical responses of barley cultivars to different types of growth regulators under abiotic stress conditions.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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