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PHENOLIC COMPOUND VANILLIC ACID MODULATES GROWTH AND DEFENSE SYSTEM OF CABBAGE (*BRASSICA OLERACEA* VAR. *CAPITATA* L.) UNDER DROUGHT STRESS

ABDUL RAHMAN, NUDRAT AISHA AKRAM* AND MUHAMMAD IQBAL

Department of Botany, Government College University, Faisalabad, 38000, Pakistan
**Corresponding author's email: nudrataauaf@yahoo.com*

Abstract

The primary purpose of this investigation was to evaluate the influence of externally applied vanillic acid (VA) on growth, osmoprotectants accumulation, and the oxidative defense system of two cabbage cultivars (Cbs-174F1 and Hcb-1040-B) under water stress. The imposition of scarcity of water at the rate of 60% field capacity led to a notable decrease in cabbage plant biomass along with their lengths. Furthermore, a remarkable reduction was observed in total chlorophyll and chlorophyll a and b pigments in both leaves and heads of the cabbage under water scarcity. Concurrently, there was an increase in leaf free proline, total phenolics, total soluble proteins, hydrogen peroxide (H_2O_2) and the activities of peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD) under drought stress. Foliar-applied vanillic acid (2.0 and 4.0 mM) resulted in significant enhancement in all the observed growth attributes. Moreover, there was an improvement in chlorophyll and ascorbic acid contents, along with the activities of POD, CAT and SOD enzymes in both leaves and heads of cabbage, particularly in limited supply of water. Foliar spray of VA also led to an increased accumulation of proline, total phenolics, and total soluble proteins in both leaves and heads of both cabbage cultivars. VA application also resulted in reducing the accumulation of hydrogen peroxide and relative membrane permeability. In a comprehensive view, the positive influence of externally applied VA on cabbage plant growth had been due to enhanced osmoprotectants accumulation and antioxidant defense system especially by lowering H_2O_2 and MDA contents under water deficit conditions.

Key words: Water stress, *Brassica oleracea* L., Vanillic acid, Antioxidants, Osmoprotectants.

Introduction

Drought refers to the extended absence of irrigation or rainfall, lasting long enough to significantly reduce soil moisture levels which adversely affect plant growth. Drought stress, on the other hand, arises when the plant loses more water from its surface than its roots can uptake, leading to a decrease in the water content within the plant's tissues, thereby disrupting its normal physiological functions (Pierce *et al*., 2005; Zafar *et al*., 2023). Water deficiency stress presents a worldwide challenge and poses a risk to the growth metrics of cultivable field crops (Jaleel *et al*., 2009; Naz *et al*., 2023). The availability of water stands as a determinant of the success or failure of any crop. Shortage of water and some other environmental stresses are responsible for some adaptation by plants for their survival. These include augmenting root biomass, diminishing shoot growth, and implementing alterations in leaf location, a reduction in leaf size, and falling of leaves from plants (Lukovic *et al*., 2009; Oliveira *et al*., 2018). These plants show reduction in cell size, enlarged vascular tissues, alterations in the xylem or phloem ratio, and decline in both xylem and phloem vessel dimensions in their anatomy (Makbul *et al*., 2011; Boughalleb *et al*., 2014). Moreover, in response to drought or salinity stress, plants notably decrease the diameter of xylem vessels and enhance the epidermal thickening, photosynthetic, and phloem tissues in the above-ground portions (El-Afry, 2012; Iqbal *et al*., 2023). Attributes such as stem length, total leaf area, fruit count per plant, chlorophyll *a* and *b* contents, fruit weight, water use efficiency, and overall crop yield undergo considerable reductions (Mani, 2014).

The destruction of photosystems takes place, resulting in reduced photosynthetic activity in plants due to inadequate water supply (Anjum *et al*., 2011). Drought conditions also induce oxidative damage to carotenes and other key biomolecules (Farooq & Bano, 2006). The production of reactive oxygen species (ROS) and its neutralization difference happened due to shortage of water (Smirnoff, 1998). This phenomenon leads to diverse physiological and biochemical changes that in turn, generate ROS. Drought stress induces leaf deterioration, the closure of stomata, and the breakdown of leaf chlorophyll. These consequences diminish the plant's ability to capture light, and the disturbance in electron movement, hampers photosynthesis, hindering the production and accumulation of energy sources (Wu *et al*., 2023). Additionally, under stress conditions, the photosynthetic system may suffer extensive damage attributed due to generate ROS (Askari & Ehsanzadeh, 2015). Hence, plants can develop specific mechanisms, for example, the activation of antioxidant enzymes like peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), to mitigate the impact of ROS. Alternatively, they may resort to an increase in proline, osmolytes and beta-carotene (Sarker & Oba, 2020a), carotenoids (Askari & Ehsanzadeh, 2015; Sarker & Oba, 2021), phenolics, ascorbic acid, betacyanin, betaxanthin, and flavonoids (Sarker & Oba, 2020b; Sarker and Oba, 2021) as antioxidant metabolites. Several investigations have documented, impact of water scarcity on CAT, peroxidase, and ascorbate peroxidase in various plants used as medicines (Gharibi *et al*., 2019).

Vanillic acid (VA), an oxidized form of vanillin, is a derivative of benzoic acid utilized as a flavoring agent (Kim *et al*., 2010). This compound serves as a natural phenolic acid, representing the conjugated form of vanillate. It presents as a solid with a melting point of 211.5°C and solubility of 1.5 mg/ml at 14°C (Imming *et al*., 2006). The molecular properties of VA,

characterized by its high hydroxylation, equip it to scavenge one or two potent oxidant molecules (Sroka & Cisowski, 2003). In various applications, such as food industry, cosmetics, and pharmaceuticals, vanillin is extensively employed as a flavor enhancer (Imming *et al*., 2006). Furthermore, VA exhibits a range of pharmacological attributes, encompassing effects, protection of cardiac, liver, neurons, inflammation, and antioxidants (Sharma *et al*., 2020). This phenolic compound was initially identified in plants like *Melilotus messanensis* (Macias *et al*., 1997), *Chenopodium murale* (Batish *et al*., 2007), and *Dactylis glomerata* (Parveen *et al*., 2011). As a phenolic compound, VA contributes to allelopathic effects, resulting in reduced root system activity in plants (Chen *et al*., 2011). Diverse sources of VA include a variety of vegetables (broccoli, drumstick, pumpkin, curry, and nettle), fruits (pomegranate and grapes), and herbs and spices (tea, rosemary, cinnamon, thyme, sage, oregano, mint, and ginger) (Shah *et al*., 2014).

Vanillic acid exerts an influence on the growth of diverse plant species by disrupting various physiological processes, encompassing transpiration, water usage, shoot and root development, hindrance of nutrient absorption by roots, diminished photosynthesis, and restricted leaf expansion. Separate treatments involving VA prompted an augmentation in both soluble and cell wall-bound peroxidase enzyme, showing a notable association with a marked reduction in root length (Devi & Prasad, 1996). For example, in sweet potato, exogenous application of VA led to an elevation in phenylalanine ammonia lyase activity (Sato *et al*., 1996). A range of allelochemicals, including VA are believed to contribute to the depolarization of root cell membranes by exhibiting impact on nutrient uptake and plant growth (Baziramakenga *et al*., 1997). The introduction of VA via application leads to an upsurge in the synthesis of other phenolic compounds through the phenylpropanoid pathway (Strack, 1997). Incorporation of VA into lignin due to its use leads in lessening growth (Tan *et al*., 1997). The rigidity of the cell wall is enhanced through the application of vanillic acid, ultimately resulting in growth reduction in plants (Sánchez-Moreno *et al*., 1999).

Cabbage (*Brassica oleracea* var. *capitata* L.) is commonly consumed either in its raw state or prepared through various methods such as boiling, fermentation, or incorporation into salad. With its noted antioxidant, anti-inflammatory, and antibacterial attributes, cabbage has earned a significant place in traditional medicine (Šamec *et al*., 2017). Cabbage boasts substantial quantities of fiber, vitamins, minerals, and compounds that promote health (Nawaz *et al*., 2018). The hypothesis of this study was that if exogenous application of VA can effectively enhance cabbage's ability to withstand drought. Thus, the study's primary aim was to assess how drought stress affected the growth and production of cabbage plants and how externally applied vanillic acid (via foliar application) could regulate growth and key physiological processes in cabbage exposed to drought stress.

Material and Methods

To evaluate how different levels of vanillic acid concentrations improved cabbage's ability to withstand scarcity of water, a pot experiment was conducted. The trial was arranged during October to February, 2022 in a completely randomized design with three factor factorials [drought (2 levels) x cultivars (2) x levels of VA (3)] with four replications of each treatment. There were a total of 48 (2 x 2 x 3 x 4 = 48) experimental units. The pots were placed in the experimental site of Government College University, Faisalabad, Pakistan. The seedlings of cabbage variety, Hcb-1040-B and Cbs-174 F1, were acquired from the Vegetables Section of Ayub Agricultural Research Institute, Faisalabad, Pakistan. The soil was added to 48 plastic pots, each having 6.0 kg of loam-sandy soil. The physiochemical properties of the soil were as follows: pH 8.7, EC 2.53 dS m^{-1}, phosphorus 6500 mg/l, 34% moisture and 0.83% humus. During the experimental period, the average (day + night) temperature was 18°C in the winter season, the overall raining percentage was 11.8 mm and 78.34% moistness. Thirty-five day-old seedlings of cabbage were transplanted into plastic pots. After thinning, each pot contained three young plants of the same size. After 20 days of transplanting, drought stress treatment, including water scarcity with 60% field capacity (F.C.) and control (100% F.C.) was initiated and maintained at required levels on the basis of soil saturation percentage. These levels took 20 days to attained the desired field capacities. At the same time, three levels of vanillic acid (0, 2, and 4 mM along with Tween-20 (0.1%) were applied to plants through their leaves as spray. After 15 days of spray, two plants were carefully harvested and stored at -20°C for storage purpose. The remaining plant was allowed to grow until head formation. However, data for the following growth and physio-biochemical attributes were determined.

Morphological attributes: The uprooted two plants from each replicate (pot) were put on blotting paper after being cleaned with distilled water to remove extra water contents. The roots and shoots were separated and the lengths of roots and shoots measured. Subsequently, their fresh weights were recorded. Then, the shoot and root samples were subjected to air-drying prior to being placed at 70°C in an oven till their constant dry weight, masses of completely dehydrated plants were recorded.

Relative water contents (RWC): Fresh leaves subsequently submerged in a water container once their fresh weights had been determined. Following a three-hour immersion in water, the turgid weights of these samples were recorded. Subsequently, all samples underwent a 72 h oven drying process, and their dry weights were recorded. The calculation of relative water contents was performed in accordance with the methodology outlined by Jones & Turner (1978).

Relative membrane permeability (RMP): A newly plucked leaf containing 500 mg was cut into pieces and placed in 10 ml of deionized water. Two hours later, EC was measured. The samples were left undisturbed overnight, and their EC_1 values were recorded. Subsequently, the samples were autoclaved for 30 minutes,

let it to room temperature, and their EC_2 was determined. The calculation of RMP was performed using the formula as delineated by Yang *et al*., (1996).

Chlorophyll (*a* and *b*) contents: Adhering to the methodology established by Arnon (1949), a freshly plucked leaf weighing 0.5 g was thoroughly blended in a pre-chilled pestle and mortar with 80% volume-by-volume acetone. The samples that had been equipped were then kept at 4°C for a whole day. Then recorded the absorbance at 645 nm and 663 nm with a UV-Visible spectrophotometer.

Proline: To assess proline content according to the methodology outlined by Bates *et al*., (1973), the extract was obtained from blended recently harvested foliage with 10 ml of 3% sulphosalicylic acid, and subsequently material which obtained was filtered properly. An aliquot, 2 ml from extract was reacted with 2 ml acidic ninhydrin and 2 ml of glacial acetic acid. This fusion was placed in an ice bath after it had been brought to boil using water bath. The absorbance of the top layer was subsequently measured at 520 nm after 4 ml of toluene had been given to each sample.

Ascorbic acid: A new leaf (500 mg) was blended using 10 ml trichloro-acetic acid (6%). Subsequent steps were conducted in accordance with the method introduced by Mukherjee & Choudhuri (1983). To 2 ml each leaf extract, 2 ml of dinitrophenyl hydrazine (2%; v/v) were added. To this mixture, 1.0 ml of 10% thiourea (weight/volume) was poured in it. Once the samples had been brought to a boil in a water bath for a period of fifteen minutes, they were let cool down at room temperature. Then, 5 ml of H_2SO_4 (80%; volume/volume) were put in to the treated sample, and the absorbance measured at 530 nm.

Total phenolics: A newly harvested leaf (250 mg) was extracted in 5 ml of 80% acetone according to the method developed by Julkenen-Titto (1985). Following the centrifugation 2 ml of dH_2O were used to homogenize one milliliter of the sample. These samples were treated with a 20% sodium carbonate solution (5 ml) and 1 ml of Folin-Ciocalteu's phenol reagent was added to the specimen extract. Subsequently, spectrophotometry was carried-out at 750 nm to determine total phenolic contents.

Total soluble proteins: A method for determining the total soluble proteins as proposed by Bradford (1976) was employed. In a cuvette, 0.1 ml of enzyme extract and 5 ml (Bradford reagent) were combined, and the absorbance at 595 nm was measured using spectrophotometry.

Hydrogen peroxide (H_2O_2): The procedure defined by Velikova *et al*., (2000) followed for the determination of H_2O_2. A 500 mg leaf sample were mixed using five milliliters of 0.1% TCA (trichloroacetic acid) in a cooled pestle and mortar. Subsequent filtering, 5 ml of a phosphate buffer and 1.0 ml of 1 M potassium iodide were added to 1.0 ml of the resultant supernatant. A UV-Vis spectrophotometer was used to measure the mixtures absorbance at 390 nm after they had been vigorously stirred.

Activities of enzymatic antioxidants: A newly harvested leaf was stored for seven-day period in an extremely low refrigerator. A leaf sample weighing 0.5 g was subjected to extraction using 10 ml K buffer with 50 mM and 7.8 pH. Following centrifugation, the resulting fusion was stored in sanitized Eppendorf tubes for evaluating the catalase, peroxidase, and superoxide dismutase enzymes' activity. The SOD activity was assessed following (Giannopolitis & Ries, 1977), whereas the working of POD and CAT enzymes were observed based on the methodology outlined by Chance & Maehly (1955).

Statistical analysis

The data of different above-mentioned attributes were examined through Co-Stat v. 306, and obtained analyses of variance (ANOVA). The DMR test was used for assessing the mean values of each treatment, and the least significant difference was used to compare the mean values at the 5% probability level.

Results

Leaf characteristics: In this study, we observed that shoot and root fresh and dry weights and the lengths of both cultivars of cabbage (*B. oleracea*), Hcb-1040-B and Cbs-174F1 were suppressed significantly ($p \leq 0.001$) under water stress (60% field capacity). However, no major change was experiential on root length in scarcity of water. Foliar applied vanillic acid (VA; 2.0 mM and 4.0 mM) improved the underground and aerial parts, fresh and dry weights as well as shoot and root sizes of both cabbage cultivars under water deficit and control regimes (Table 1; Fig. 1). Exogenously applied 4.0 mM level of VA was more effective in improving the biomass of both cabbage cultivars at 100% and 60% field capacities. Regarding shoot and root lengths under water-deficit stress, as well as root fresh and dry weights, cv. Cbs-174F1 performed moderately better than the other variety of cabbage. In terms of shoot dry and fresh weights, both cabbage cultivars responded similarly to exogenous vanillic acid treatment under drought conditions.

Water deficiency had a substantial ($p \leq 0.001$) adverse effects on chlorophyll *a, b* & total chlorophyll concentrations in cabbage varieties. Foliar administration of vanillic acid worked well for improving these photosynthetic pigments of both cabbage cultivars at 100% field capacity and drought stress. Of both VA levels, 4.0 mM level was most effective in raising the concentrations of chlorophyll pigments of both cabbage cultivars (Table 1; Fig. 2). On the other hand, the chlorophyll a/b ratio of two cabbage cultivars did not show any noticeable variation. Both cabbage cultivars responded similarly to externally administered VA under drought stress (Table 1; Fig. 2).

Analysis of variance of data revealed that water deficit regime (60% F.C.) significantly ($p \leq 0.001$) increased the relative membrane permeability (RMP) of both cabbage cultivars (Table 1; Fig. 3). However, both levels of VA were effective significantly ($p \leq 0.001$) in decreasing the RMP of the cabbage plants. No significant difference was experiential between both cabbage cultivars in relation to RMP under different treatments.

Table 1. Mean square values obtained from analysis of variance of data for different morpho-physiobiochemical characteristics of leaf of drought stressed cabbage (*Brassica oleracea* var. *capitata* L.) plants treated with foliar-applied vanillic acid.

Source of variations	df	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight
Cultivars (Cvs)	1	0.927ns	2.412***	0.344ns	0.120**
Drought (D)	1	586.9***	2.726***	16.65***	0.214***
Vanillic acid (VA)	2	175.2***	1.889***	2.898***	0.081**
Cvs x D	1	1.238ns	0.052 ns	0.077ns	0.021ns
Cvs x VA	2	8.399ns	0.037 ns	0.025ns	0.004ns
D x VA	2	5.060ns	0.029ns	0.444ns	0.001ns
Cvs x D x VA	2	10.47ns	0.058ns	0.006ns	0.002ns
Error	36	15.61	0.117	0.204	0.011
		Shoot length	**Root length**	**Chlorophyll *a***	**Chlorophyll *b***
Cultivars (Cvs)	1	3.758*	207.8***	1.564***	0.979***
Drought (D)	1	32.25***	3.542ns	1.939***	0.337***
Vanillic acid (VA)	2	5.616**	29.66*	0.234*	0.038*
Cvs x D	1	1.639ns	21.84ns	0.106ns	0.002ns
Cvs x VA	2	0.503ns	0.775ns	0.008ns	0.002ns
D x VA	2	0.041ns	92.51***	0.018ns	0.003ns
Cvs x D x VA	2	0.025ns	13.42ns	0.001ns	0.002ns
Error	36	0.807	6.525	0.044	0.010
		Total chlorophyll	**Chl. *a/b* ratio**	**Relative membrane permeability**	**Proline**
Cultivars (Cvs)	1	5.018***	2.137**	0.149ns	2.050***
Drought (D)	1	3.896***	0.016ns	1.724***	17.26***
Vanillic acid (VA)	2	0.462***	0.013ns	1.013***	4.017***
Cvs x D	1	0.075ns	0.827ns	0.304*	8.391***
Cvs x VA	2	0.006ns	0.074ns	0.005ns	0.146ns
D x VA	2	0.024ns	0.147ns	0.062ns	0.158ns
Cvs x D x VA	2	0.006ns	0.006ns	0.022ns	0.215ns
Error	36	0.037	0.259	0.073	0.084
		Total phenolics	**H_2O_2**	**Ascorbic acid**	**Total soluble proteins**
Cultivars (Cvs)	1	5.250*	132.7***	0.131ns	0.273ns
Drought (D)	1	77.83***	10.47**	3.720***	410.7***
Vanillic acid (VA)	2	20.50***	9.684***	1.409***	101.9**
Cvs x D	1	1.659ns	0.292ns	0.145ns	0.229ns
Cvs x VA	2	2.923ns	0.136ns	0.245ns	8.778ns
D x VA	2	1.351ns	0.072ns	0.023ns	1.660ns
Cvs x D x VA	2	0.083ns	0.041ns	0.087ns	2.033ns
Error	36	0.908	0.989	0.078	14.24
		Catalase	**Peroxidase**	**Superoxide dismutase**	
Cultivars (Cvs)	1	0.411ns	84.21ns	3.196***	
Drought (D)	1	6.168***	775.2***	4.083***	
Vanillic acid (VA)	2	3.128***	748.7***	0.924**	
Cvs x D	1	0.039ns	511.5***	0.046ns	
Cvs x VA	2	0.394ns	14.37ns	0.010ns	
D x VA	2	0.005ns	3.109ns	0.002ns	
Cvs x D x VA	2	0.033ns	5.443ns	0.079ns	
Error	36	0.315	27.63	0.111	

ns = No-significant; *, ** and *** = Significant at 0.05, 0.01 and 0.001 levels, respectively

A noticeable ($p \leq 0.001$) rise in the accumulation of proline & total phenolics was noted in both cabbage cultivars in response to drought stress (Fig. 3). Foliage application of both levels of vanillic acid was effective in enhancing ($p \leq 0.001$) proline as well as total phenolics in the cabbage plants under water deficit stress. Of both VA levels, 4.0 mM VA was most effective in promoting the accumulation of these metabolites (Table 1). Of both cabbage cultivars, cv. Hcb-1040-B was inferior to cv. Cbs-174F1 in accumulating proline under water shortage. While total phenolics were higher in cv. Hcb-1040-B particularly under water-deficit stress (Fig. 3; Table 1).

Under water stress, there was a notable ($p \leq 0.001$) increase in the accumulation of hydrogen peroxide (H_2O_2), ascorbic acid (AsA), and total soluble proteins. Nevertheless, exogenously used 4 mM VA was more effective that the other VA level in reducing the H_2O_2 level and increasing AsA and total soluble proteins in cabbage under water-deficit conditions (Table 1; Fig. 3). The cv. Cbs-174F1 cabbage outperformed the other cultivar in accumulating H_2O_2 contents, while AsA and total soluble proteins remained unaffected under both water regimes.

Under water limited conditions, cabbage plants exhibited a substantial ($p \leq 0.001$) rise in the activities of SOD and POD enzymes (Table 1). The administration of VA significantly enhanced the SOD and POD activities in cabbage experiencing water deficit stress (Table 1; Fig. 4). Data indicated that treating plants with 4.0 mM of VA increased POD activity in water stressed plants.

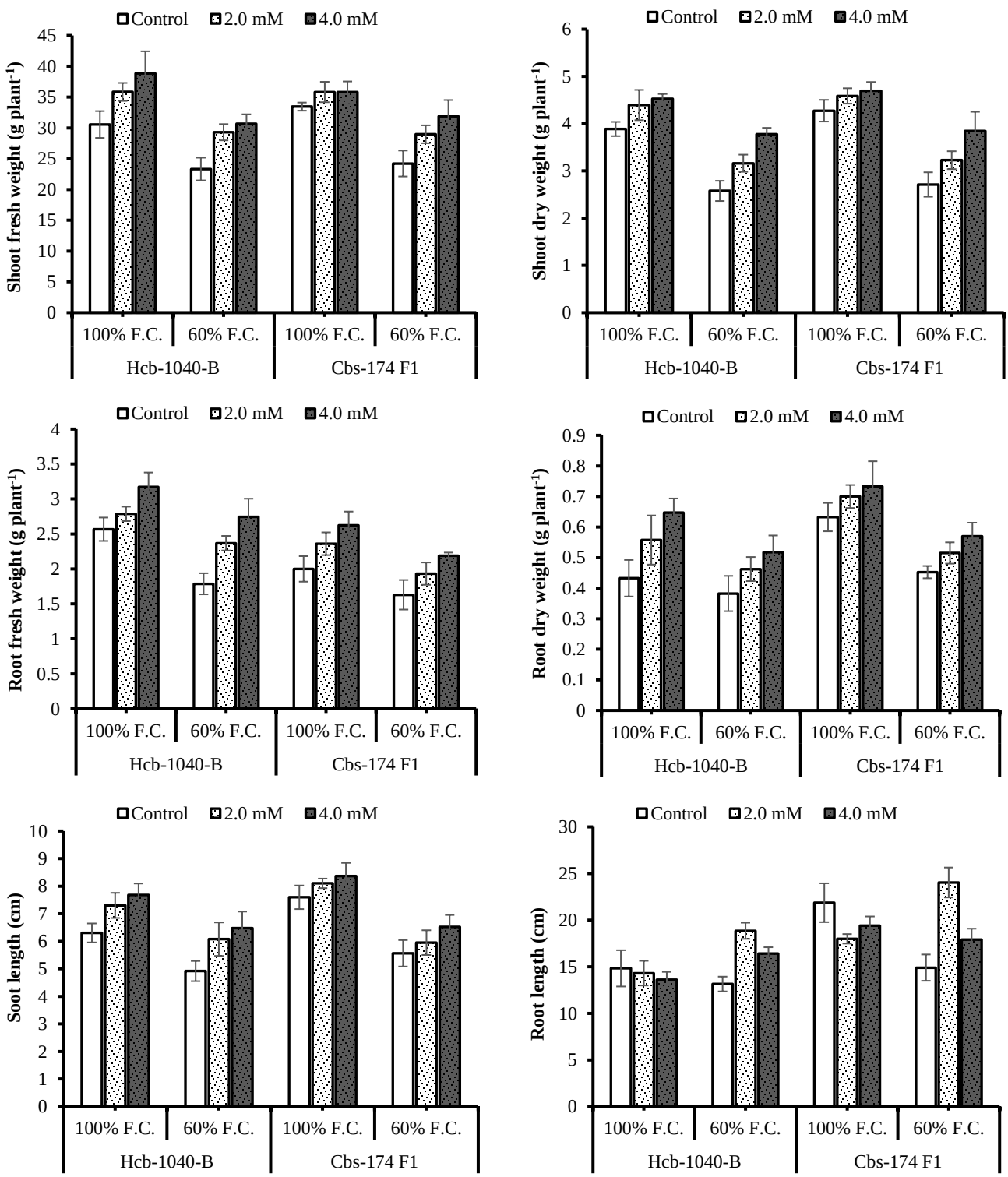

Fig. 1. Shoot and root fresh and dry weights, shoot and root lengths of drought-stressed and non-stressed plants of two cabbage (*Brassica oleracea* var. *capitata* L.) cultivars subjected to folar-applied vanillic acid (0, 2.0 and 4.0 mM) (Means ± S.E.).

The activities of peroxidase and catalase enzymes were significantly elevated during drought stress ($p \leq 0.001$), while a decrease in SOD activity in the leaves of cabbage plants (Table 1). The exogenous administration of VA, especially 4.0 mM tended to upregulate ($p \leq 0.001$) the activities of all observed enzymes, particularly under scarcity of water (Table 1; Fig. 4). Under both water regimes, notably all antioxidant enzyme functions were increased by 4.0 mM VA. Although these cabbage cultivars responded similarly to several exogenous treatments, cv. Cbs-174F1 showed superior SOD enzyme activity.

Head characteristics: A substantial ($p \leq 0.001$) decrease in the levels of a, b, and total chlorophyll was noted under drought stress in the heads of both cabbage cultivars (Table 2; Fig. 5). Moreover, under water-limited circumstances, exogenously applied VA as a foliar spray markedly ($p \leq 0.001$) increased the synthesis of

chlorophyll *a, b* and total in two cabbage cultivars. It was observed that VA as 4.0 mM upregulated the photosynthetic pigment levels more effectively than the other VA level. However, chlorophyll *a/b* ratio of two cabbage verities increased under drought conditions, while it remained unaffected considerably due to exogenous supplementation of VA in both cabbage cultivars (Table 2; Fig. 5). Of both cabbage cultivars, cv. Hvb-1040-B had superior performance in terms of all above-mentioned pigments under water limited regimes.

Under water stress regime, the head proline and H_2O_2 concentrations increased ($p \leq 0.001$) significantly in both cultivars of cabbage. Exogenously used VA was considerably efficient in increasing the proline ($p \leq 0.001$) concentration, while reducing the H_2O_2 contents in the heads of both cabbage cultivars under scarcity of water (Table 2; Fig. 6). Of both VA levels, 4.0 mM VA responsible for significantly enhancing proline and decreasing H_2O_2 accumulation in heads of cabbage.

Ascorbic acid and total phenolics in the heads of both cabbage cultivars increased ($p \leq 0.001$) significantly under limited availability of water (60% field capacity). Exogenously applied VA also considerably ($p \leq 0.001$) boosted the ascorbic acid and total phenolics, particularly at 4.0 mM VA under scarcity of water (Table 2; Fig. 6). The accumulation of AsA was similar in both cabbage cultivars, while, total phenolics were higher in cabbage cultivar Hcb-1040-B than that the other cultivar under drought stress conditions.

Total soluble proteins significantly ($p \leq 0.001$) decreased in the heads of cabbage plants under scarcity of water. A substantial improvement in soluble proteins was observed as a result of exogenous administration of vanillic acid at both VA concentrations tested here. The 4.0 mM was the most beneficial particularly for cv. Hcb-1040-B at 60% field capacity (Table 2; Fig. 6).

Drought stress remarkably enhanced ($p \leq 0.01$) the activities of SOD, POD and CAT enzymes in the heads of both cabbage cultivars (Fig. 7; Table 2). Applying VA foliarly was also successful in enhancing the activities of all the earlier-mentioned enzymes in the heads of both cabbage cultivars under both water regimes. Both varieties of cabbage responded similarly in terms of SOD activity, while cv. Hcb-1040-B was better than the other cabbage cultivar in enzymatic activities of catalase and peroxidase in foliarly administrated VA and scarcity of water.

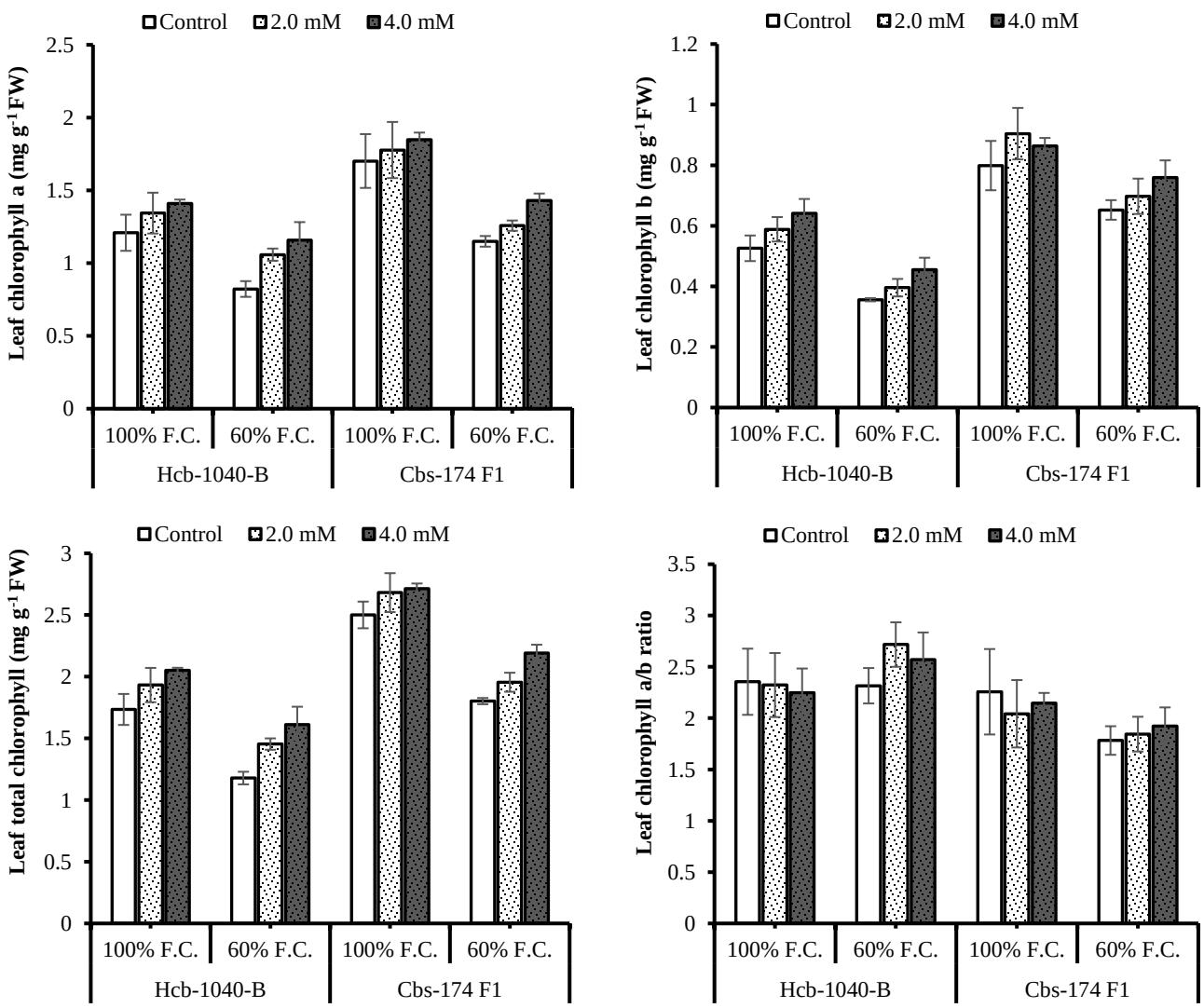

Fig. 2. Leaf chlorophyll *a*, *b*, total chlorophyll and chlorophyll *a/b* ratio of drought-stressed and non-stressed plants of two cabbage (*Brassica oleracea* var. *capitata* L.) cultivars subjected to folar-applied vanillic acid (0, 2.0 and 4.0 mM) (Means ± S.E.).

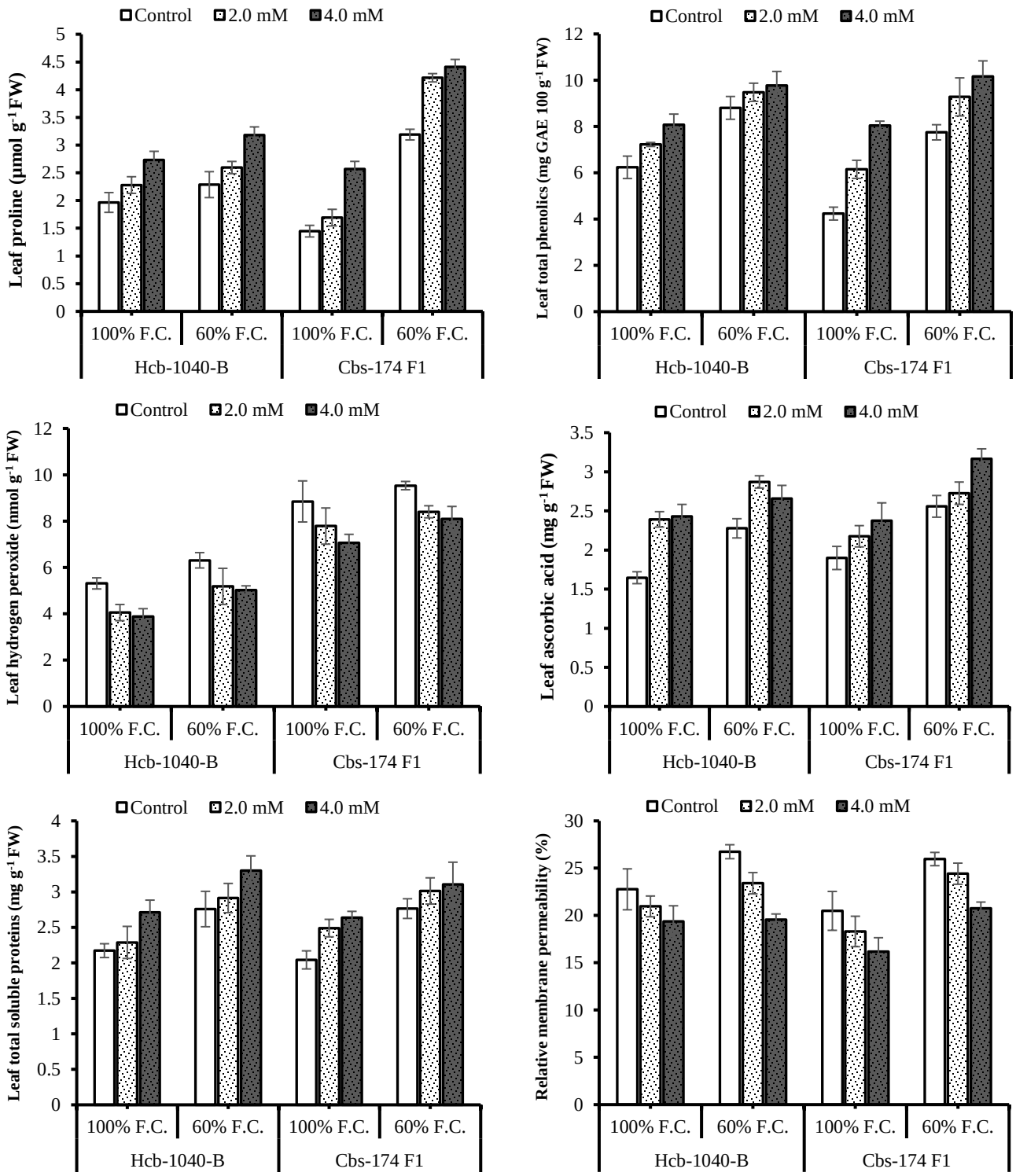

Fig. 3. Leaf relative membrane permeability, proline, total phenolics, hydrogen peroxide, ascorbic acid and total soluble proteins of drought-stressed and non-stressed plants of two cabbage (*Brassica oleracea* var. capitata L.) cultivars subjected to folar-applied vanillic acid (0, 2.0 and 4.0 mM) (Means ± S.E.).

Discussion

Plants undergo exposure to a range of environmental stresses during their growth and development, whether in natural ecosystems or agricultural contexts. Notably, drought has emerged as a formidable environmental stressor, exerting a significant impact on plant growth and yield productivity. Drought stress is believed to cause a marked reduction in water balance, cell enlargement, and photosynthesis as well as closure of stomata resulting in disturbance of growth and metabolism (Ahmed *et al*., 2017; Jabeen *et al*., 2021). In various preliminary studies, the application of external chemicals to the foliage has been investigated as a strategy to increase crop yield in unfavorable growth environments. An earlier study revealed that an external application of 2% chlorocholine chloride to buckwheat seeds led to significant elevation in the amounts of different phenolic acids, including trans-

ferulic, ferulic, vanillic, and salicylic acids, as well as total phenols in the leaves and stems (Sytar *et al*., 2014). Moreover, in other studies it has been found that the levels of phenolic compounds, including phenolic acids and flavonoids were affected by shortage of water in leaves of tobacco and wheat (Ma *et al*., 2014).

These phenolic compounds have the ability to scavenge reactive oxygen species (Quan *et al*., 2016) and mitigate DNA damage, protein denaturation, and lipid peroxidation (Król *et al*., 2014). Though, in this investigation, role of exogenously applied vanillic acid (VA; 2.0 and 4.0 mM) in drought stress resilience of cabbage (*Brassica oleracea* L.) was assessed. Phenolic compounds such as vanillic acid represent the most prevalent and significant groups of plant secondary metabolites known for their antioxidant properties (Quan *et al*., 2016). Nevertheless, vanillic acid was applied externally in the current investigation, acting as a growth stimulant, significantly alleviated the detrimental effects of drought on cabbage plants. Vanillic acid's efficacy as a natural antioxidant in plants has been highlighted in diverse studies addressing various stressors, such as stress from salt (Parvin *et al*., 2020) and stress due to cadmium (Bhuyan *et al*., 2020. These findings underscore the versatile potential of vanillic acid in mitigating oxidative stress across different environmental challenges. The findings from Quan & Xuan (2018) research, showcasing enhanced drought tolerance in rice through the foliar administration of VA, show the effectiveness in this compound in drought stress amelioration. It is likely that the foliar application of exogenous vanillic acid acted as a catalyst for the synthesis of osmoprotectants (Quan & Xuan, 2018)). This in turn could have contributed to an augmented drought resistance mechanism, ultimately improving the ability of cabbage plants to survive in soil conditions with limited water availability.

In the present investigation, the impact of water deficit conditions was evident in a discernible decline in plant growth. This was manifested through noticeable reduction between the fresh and dry weights of the cabbage plant's shoot and root. These findings are in accordance with earlier studies that have consistently observed diminished growth in various crops under water stress conditions. Notable examples include mungbean (Sadiq *et al*., 2019), *Brassica oleracea* (Latif *et al*., 2016), *Zea mays* (Shafiq *et al*., 2019), *Helianthus annuus* (Kosar *et al*., 2018), and canola (Shafiq *et al*., 2014). The consistent pattern underscores the widespread impact of water stress on plant growth across different crop species. Across these investigations, a consistent observation has been the connection between water scarcity responsible for decrease in growth of plant and various factors. Oxidative stress, malnutrition, hormonal changes, protein degradation or suppression, enzyme deactivation, and disruptions in secondary metabolism are some of these variables (Chen & Murata, 2008; Rezaei *et al*., 2012). In this context, vanillic acid used externally as a growth regulator is the subject of the present investigation, played a pivotal role in lessening the harmful impacts caused by drought on cabbage plants. This intervention resulted in a notable improvement in growth, evident through increased lengths of the roots and shoots in seedlings affected by dehydration, as well as enhanced fresh and dry weights. Notably, several phenolic molecules have shown the ability to promote development in *Solanum lycopersicum*, *Oryza sativa*, and *Triticum aestivum* in scarcity of water. These include quercetin (Parvin *et al*., 2019), apigenin (Mekawy *et al*., 2018), and coumarin (Saleh & Madany, 2015). Furthermore, it is worth noting that exogenous vanillic acid has been found to enhance rice cultivation in submerged environments (Xuan & Khang, 2018).

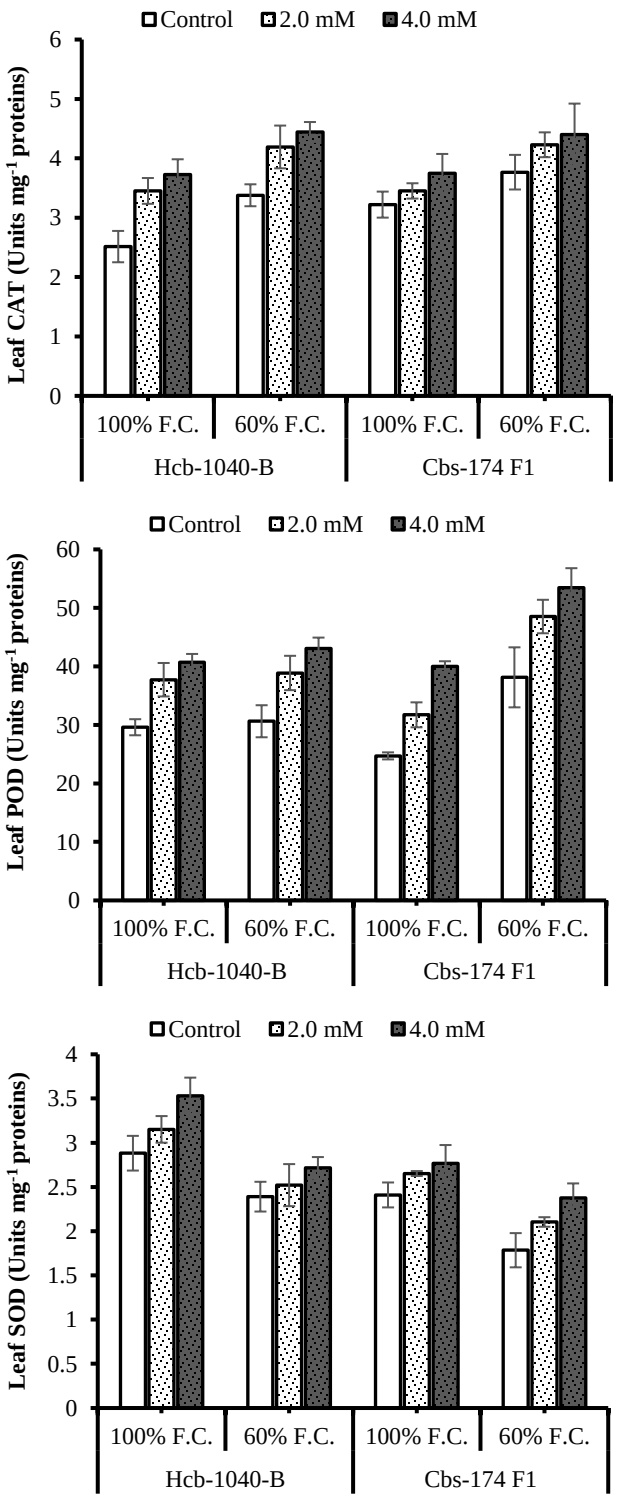

Fig. 4. Activities of leaf catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) enzymes of drought-stressed and non-stressed plants of two cabbage (*Brassica oleracea var. capitata* L.) cultivars subjected to folar-applied vanillic acid (0, 2.0 and 4.0 mM) (Means ± S.E.).

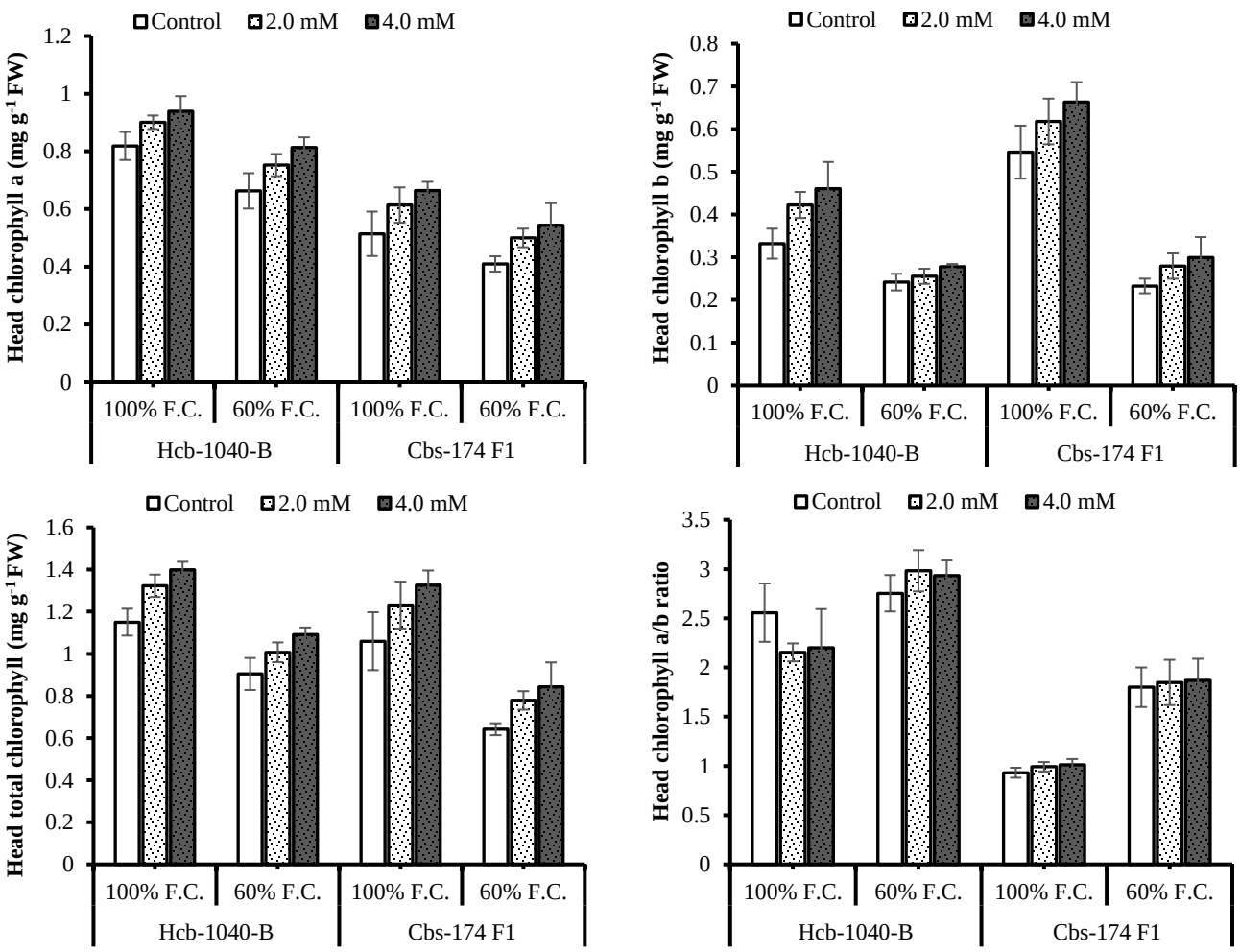

Fig. 5. Head chlorophyll *a*, *b*, total chlorophyll and chlorophyll *a/b* ratio of drought-stressed and non-stressed plants of two cabbage (*Brassica oleracea var. capitata* L.) cultivars subjected to folar-applied vanillic acid (0, 2.0 and 4.0 mM) (Means ± S.E.).

It is a foundational principle in plant science that chlorophyll molecules play a crucial role in photosynthesis. However, water scarcity can disrupt chlorophyll molecules and impede their synthesis, consequently diminishing the plant photosynthesis rate (Heba & Samia, 2014). This study's findings on scarcity of water led to reduction in chlorophyll content in cabbage plants, particularly in the heads, mirroring observations in cucumber (Naz *et al*., 2016), *Triticum aestivum* (Raza *et al*., 2016), *Raphanus sativus* (Akram *et al*., 2016), and soybean (Heba & Samia, 2014). These studies noted that drought stress resulted in decreased chlorophyll content, subsequently reducing the photosynthetic rate. However, this research contains the exogenous use of vanillic acid pointedly enhanced the chlorophyll content of cabbage due to less availability of water. Same outcomes were reported in rice, where foliar applied low concentration of vanillic acid was found to increase chlorophyll levels (Xuan & Khang, 2018).

The association between an elevation in relative membrane permeability (RMP) and the adaptability of cell membranes to withstand diverse environmental signals, particularly drought stress, is a well-acknowledged phenomenon in both cabbage cultivars. This common occurrence has been previously observed by Elbasyoni *et al*., (2017). However, notably, the application of vanillic acid in our study led to a significant reduction in RMP in cabbage plants under drought conditions.

Compatible solutes or osmoprotectants, such as proline and glycine betaine, are recognized for their role in safeguarding cell components during dehydration and contributing to cell osmotic adjustment (Ashraf & Foolad, 2007). Recent reports indicate that the involvement of proline and GB in scavenging reactive oxygen species (ROS) and regulating water levels becomes significant under stress conditions, as discussed by Asghar *et al*., (2022). Proline, being a main amino acid that is soluble in water, possesses durable hydration capabilities, making it an ideal osmotic medium (Dong *et al*., 2018). The information gleaned from the most recent statement indicated an elevation in proline content in cabbage plants under drought conditions. Similar observations of significantly accumulated proline contents were reported in *Populus euphratica* (Chen *et al*., 2003) and oil palm seedlings (Cao *et al*., 2011) under water deficit conditions. Studies on rice and radish plants (Akram *et al*., 2016; Galahitigama & Wathugala, 2016) demonstrated that enhanced accumulation of proline and GB contents under shortage of water contributed to increased stress tolerance. Moreover, in the present study, when vanillic acid was sprayed on leaves of cabbage plants under both stressed and unstressed conditions, the concentration of proline contents increased substantially.

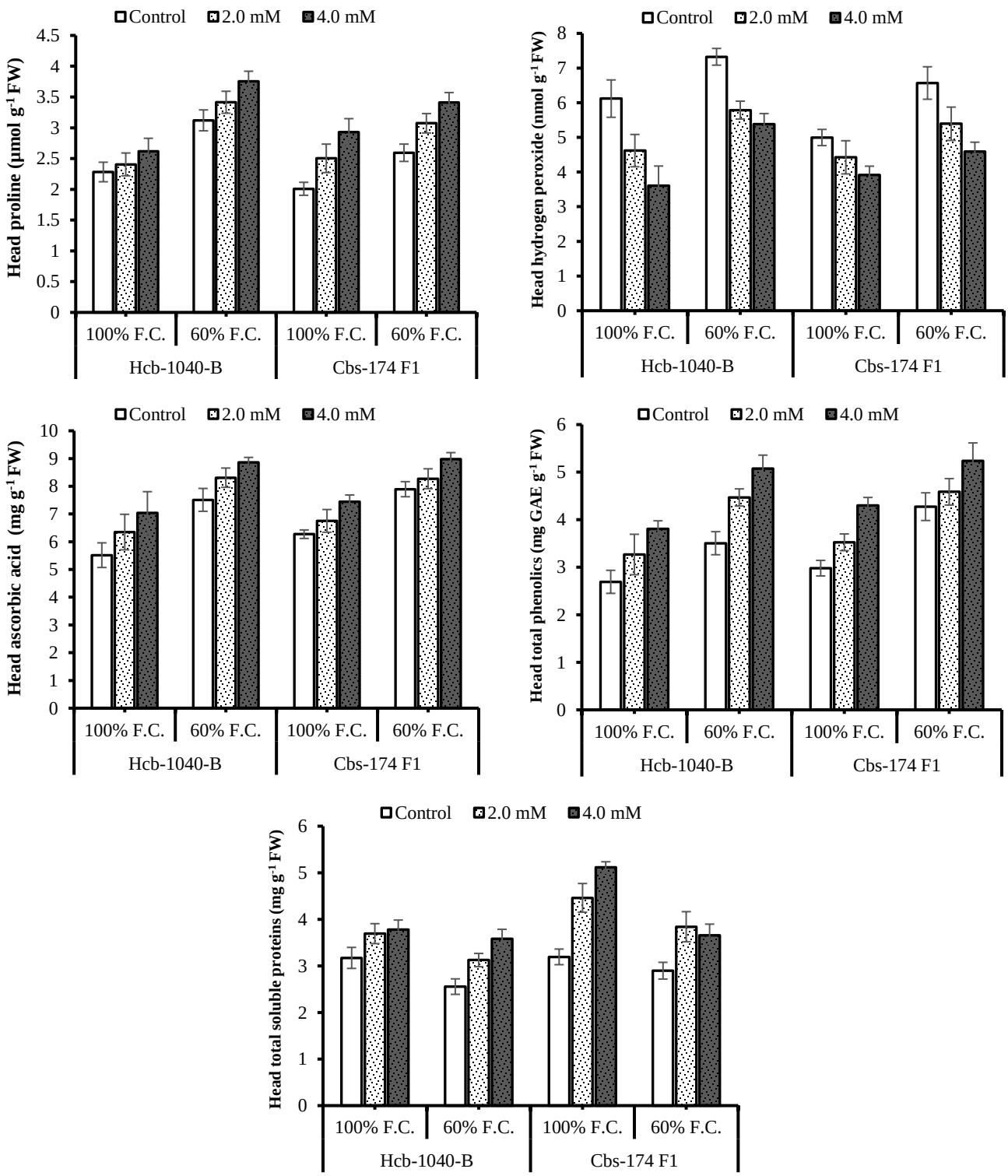

Fig. 6. Head proline, hydrogen peroxide, ascorbic acid, total phenolics and total soluble proteins concentrations of drought-stressed and non-stressed plants of two cabbage (*Brassica oleracea* var. *capitata* L.) cultivars subjected to folar-applied vanillic acid (0, 2.0 and 4.0 mM) (Means ± S.E.).

The plant's defense system against oxidative stress encompasses a diverse range of biomolecules, including both enzymes and non-enzymatic compounds. Ascorbic acid, a prominent non-enzymatic compound, is well-known for its ability for plants to defend from various abiotic stresses through removing oxygen-free radicals from the body efficiently (Shafiq *et al*., 2014). In this study, we observed a similar increase in ascorbic acid content in cabbage plants exposed to drought stress, consistent with earlier findings in maize (Stingu *et al*., 2011). Dolatabadian *et al*., (2010) also reported a significant elevation in ascorbic acid levels in grain corn, particularly under conditions of high drought intensity. Furthermore, we observed that application of VA led to an additional increase in the accumulation of ascorbic acid under drought stress conditions, suggesting a potential role of VA in enhancing plant's antioxidant defense mechanism.

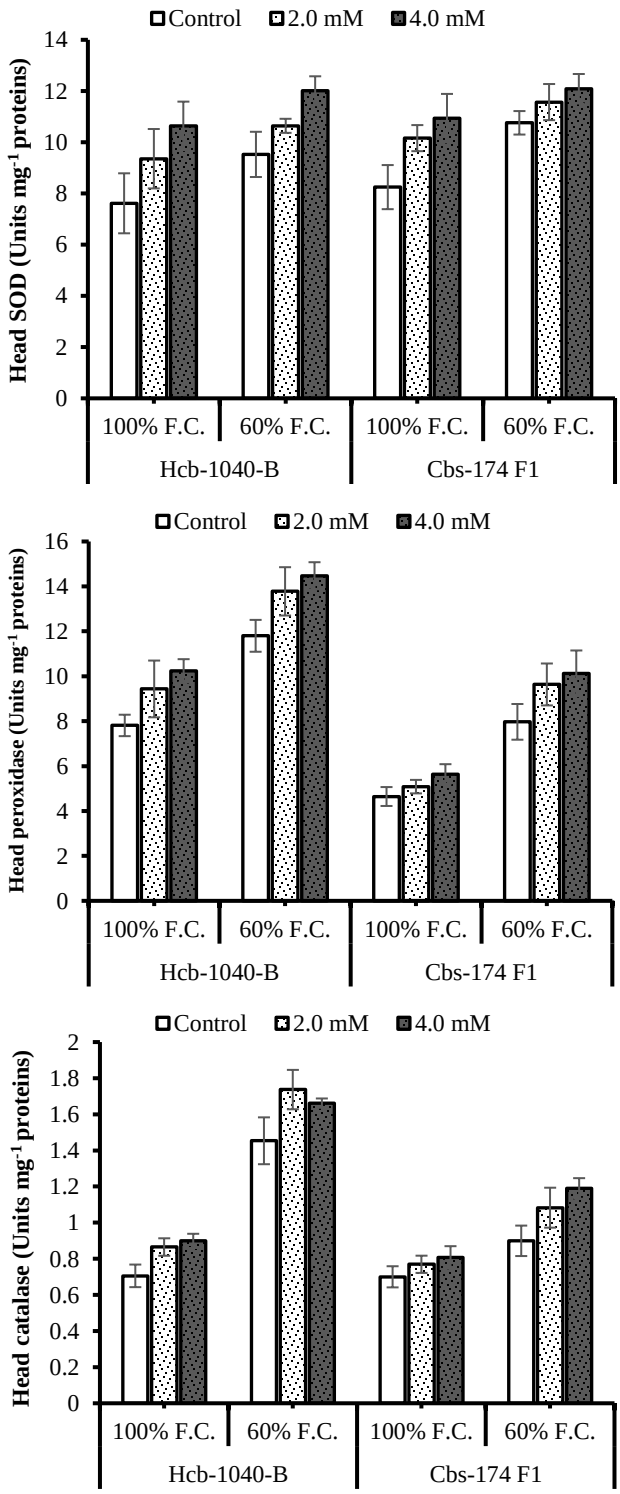

Fig. 7. Activities of head catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) enzymes of drought-stressed and non-stressed plants of two cabbage (*Brassica oleracea* var. *capitata* L.) cultivars subjected to folar-applied vanillic acid (0, 2.0 and 4.0 mM) (Means ± S.E.).

Phenolics, a category of non-enzymatic compounds, play a crucial role in mitigating the harmful effects of ROS generated during stress conditions by donating hydrogen atoms. We observed an increase in the total phenolics of drought-stressed cabbage plants, aligning with previous findings, when sugarcane is stressed by heat (Wahid & Ghazanfar, 2006). Similarly, under drought stress, Gnanasekaran & Kalavathy (2017) found that *Tridax procumbens* vegetative and reproductive parts had higher ranks of total phenolic contents. This suggested a conserved response across different plant species in enhancing phenolic compounds as a protective mechanism during stress. Our observation revealed that exposure to drought stress increased the H_2O_2 contents in both cabbage cultivars, indicating that lipid peroxidation had occurred in response to drought stress. Moreover, the rise in H_2O_2 suggested the possibility of the production of other oxidants, signifying the potential triggering of oxidative stress in cabbage cultivars. Consistent with our findings, previous studies have reported increased accumulation of MDA (malondialdehyde) and H_2O_2 in *Triticum aestivum* (Hamurcu *et al*., 2014), *Cucumis sativus* (Li *et al*., 2011) and *Brassica napus* (Akram *et al*., 2018) plants in shortage of water. It is noteworthy that application of vanillic acid resulted in a reduction of H_2O_2 levels in cabbage crops that are both affected and unaffected from shortage of water. This implied that adding vanillic acid to a plant's diet would help reduce oxidative stress and enhance plant development in both stressful and non-stressful environments. Phenolic compounds, including vanillic acid, play a crucial role in plant antioxidative defense mechanisms, inhibiting lipid peroxidation and contributing to the overall protection against oxidative stress (Xuan & Khang, 2018).

The antioxidative defense mechanisms in plants involves both enzymatic and non-enzymatic components to counter various stresses, including those induced due to scarcity of water (Akram *et al*., 2017). In the present investigation, scarcity of water stress increased the activity of POD, CAT, and SOD enzymes in both cultivars of cabbage when there was insufficient water. This trend is consistent with similar observations in *Zea mays* (Darvishan *et al*., 2013), *Triticum aestivum* (Selote & Khanna-Chopra, 2010), and *Chenopodium quinoa* (Aziz *et al*., 2018), where heightened working of SOD, POD, and CAT were reported under water scarcity. The exogenous application of VA exerted a positive influence in the enzyme production like SOD, CAT, and POD in drought-stressed plants. These align with the findings in *Oryza sativa* under submergence conditions, where it was noted that ROS were suppressed and CAT and SOD enzyme activity were stimulated (Xuan & Khang, 2018). Multiple studies have highlighted the regulatory role of phenolic compounds, including vanillic acid in enhancing the SOD and CAT enzyme activity in plants under stress as part of the ROS detoxification process (Abu El-Soud *et al*., 2013; Li *et al*., 2013; Singh *et al*., 2019; Parvin *et al*., 2019).

Overall, the positive impact of vanillic acid extended beyond reducing the impact of the drought stress in cabbage plants; it also demonstrated improvement in growth and essential physiological and biochemical parameters in stressed and controlled plants. This versatility suggested that the application of vanillic acid can be a valuable strategy for promoting plant growth not only in challenging drought conditions, but also in normal, non-stressed environments. The dual benefits conferred by vanillic acid underscore its potential as a valuable tool for farmers, offering a broad spectrum of advantages to enhance overall plant performance under stress conditions.

Table 2. Mean square values obtained from analysis of variance of data for different physio-biochemical characteristics of head of drought stressed cabbage (*Brassica oleracea* var. *capitata* L.) plants treated with foliar-applied vanillic acid.

Source of variations	df	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Chl. *a/b* ratio
Cultivars (Cvs)	1	0.898***	0.140***	0.328***	16.94***
Drought (D)	1	0.196***	0.705***	1.647***	6.279***
Vanillic acid (VA)	2	0.079**	0.031*	0.209***	0.001ns
Cvs x D	1	0.002ns	0.110***	0.078ns	0.224ns
Cvs x VA	2	9.282ns	9.219ns	3.523ns	0.031ns
D x VA	2	9.305ns	0.005ns	0.004ns	0.111ns
Cvs x D x VA	2	5.549ns	7.517ns	4.667ns	0.121ns
Error	36	0.010	0.006	0.023	0.167
		Proline	**H_2O_2**	**Ascorbic acid**	**Total phenolics**
Cultivars (Cvs)	1	0.386ns	2.906*	1.365ns	1.452*
Drought (D)	1	7.130***	18.08***	36.44***	14.41***
Vanillic acid (VA)	2	1.830***	14.45***	6.557***	6.159***
Cvs x D	1	0.604*	0.291ns	0.403ns	5.208ns
Cvs x VA	2	0.156ns	0.601ns	0.168ns	0.117ns
D x VA	2	0.010ns	0.102ns	0.016ns	0.006ns
Cvs x D x VA	2	0.040ns	0.546ns	0.005ns	0.180ns
Error	36	0.125	0.638	0.676	0.277
		Total soluble proteins	**Superoxide dismutase**	**Peroxidase**	**Catalase**
Cultivars (Cvs)	1	353.1***	5.271ns	199.1***	1.169***
Drought (D)	1	469.4***	30.94**	207.3***	3.580***
Vanillic acid (VA)	2	509.7***	22.91***	17.87**	0.188**
Cvs x D	1	32.88ns	0.083ns	0.013ns	0.737***
Cvs x VA	2	39.15ns	0.674ns	1.046ns	0.011ns
D x VA	2	14.00ns	1.101ns	0.754ns	0.016ns
Cvs x D x VA	2	68.08*	0.171ns	0.269ns	0.009ns
Error	36	18.66	2.577	2.376	0.023ns

ns = No-significant; *, ** and *** = Significant at 0.05, 0.01 and 0.001 levels, respectively

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