Foliar application of γ-aminobutyric acid (GABA) improves vegetative growth, and the physiological and antioxidative potential of *Daucus Carota* L. under water deficit conditions

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Abstract

Scarcity of water is one of the most serious concerns in plant biology with diverse implications at all the levels of molecular, biochemical, and physiological phenomena of plant growth, development, and consequently the productivity. Most of the strategies to induce or enhance drought tolerance in plants are unreasonably expensive and/or time-consuming. Some studies conducted in the recent past have shown that plant growth regulators (PGRs) may induce/improve physiological tolerance in plants to cope with adverse environmental conditions including drought. The present study was aimed at investigating the effects of foliar spray of GABA (0, 1,

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2, and 4 mM) applied 20 days following the germination of seeds, on vegetative growth, morphological characteristics, integrity of cell-membrane, and the levels of photosynthetic pigments and enzymatic antioxidants in carrot cvs. Supertaj and Bharat, grown under 100% and 50% field capacity of soil moisture. The treated and untreated (control) carrot plants were harvested and analyzed 2 weeks following the GABA application. The results revealed that foliar application of GABA improved the vegetative growth and significantly increased the levels of free amino acids, plastid pigments, enzymatic antioxidants, and the relative water content in the root crop grown under 50% field capacity of soil moisture, compared to control. Additionally, the GABA application decreased the electrolyte leakage of ions and melondialdehyde (MDA) content in carrot leaves. The carrots harvested from GABA-treated or untreated (control) plants were not significantly different for their protein contents. In conclusion, the incorporation of GABA in the production management of carrots may help plants to mitigate the adverse effects of water deficit stress.

Keywords: oxidative stress; enzymatic antioxidants; malondialdehyde; membrane permeability; chlorophyll

1. INTRODUCTION

Scarcity of water is one of the major abiotic stresses in agriculture that earnestly limits the growth, development, and productivity of all crops, around the globe [1, 2]. A multitude of physiological and metabolic phenomena in plants is

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affected by the drought stress. Decreases in the photosynthetic activities [3], relative water content and the rate of usual transpiration [4], and over-production of reactive oxygen species (ROS) [5] that causes serious damages to DNA, RNA, protenins and cellular membranes, are some of the deleterious effects caused by drought stress in plants. Thus the oxidative stress is considered as one of the major causes of physiological injuries in plants when grown under water-deficit conditions. To mitigate the damaging effects of ROS, many plant species activate their defense systems [6] by producing enzymatic and non enzymatic antioxidants such as peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), tocopherol, glutathione, ascorbic acid, along with an exhaustive list of similar molecules. Karimi et al. [7] proposed that improving the free radical scavenging activity of plants through the enhamcment of these biological molecules is an effective physiological strategy to cope with drought stress. In addition to other protective measures, the levels and the activities of these antioxidants determine the ability of plants to grow and survive under both biotic and abiotic stresses.

A large volume of studies conducted in the recent past have shown that certain plant growth regulators (PGRs), when applied exogeneously, play their important role in regulating the responses of plants to mitigate abiotic stress signals through various tolerance mechanisms [7]. Among the PGRs already tested on diverse plant species in this context, GABA – a 4-carbon non-protein amino acid, has emerged as a promising multiprotactant with the ability to rapidly induce tolerance in plants against a variety of abiotic stresses such as mechanical injury or stimulation,

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hormonal toxicity, water logging, extreme temperatures, heat shocks, salt and/or drough stresses 0[8-14]. In plants, GABA is involved in a multitude of physio-biochemical functions so as to regulate their growth and tolerance against various abiotic stresses. It seems to be an integral part of sustenance of cytosolic pH, C-N flux for tricarboxylic acid cycle, osmotic pressure, regulation of redox status, and carbon/nitrogen metabolism [10]. It has been shown to alleviate chilling injury in zucchini [15], cucumber [16], peach [17], banana [18], litchi [19] fruits, and tomato seedlings [20]. Vijyakumari and Puthur [21] demonstrated that GABA helps to improve drought tolerance in black pepper (*Piper nigrum* L.) by reducing the rate of lipid peroxidation and inhibiting the photosynthetic and mitochondrial activities during osmotic stress. Similar results were reported by Li et al. [22] in creeping bentgrass (*Agrostis stolonifera* L.) grown under drought conditions. These reports suggest that GABA could be a potential means of enhancing tolerance in plants against various abiotic stresses.

Carrot (*Daucus carota* L.) is an important short-cycle nitrophilous vegetable [23] which is grown, consumed and relished worldwide. Being a rich source of vitamins, minerals, carbohydrates, proteins, fibers, carotenoids, and phenolic pigments, carrot increases resistance in the human body against many infectious and cardiovascular diseases [24] probably by increasing total antioxidant capacity and reducing MDA content in the blood plasma [25]. Due to the functional properties of carrots, their consumption is steadly increasing worldwide and so is its demand in the local and international markets. The optimum growth and

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productivity of the tuberous crop require appropriate scheduling of irrigation including supply of sufficient water, specifically during the sensitive stages of root growth and development. Depending upon the period of crop cycle (usually 100-140 days), the filed production of carrot requires the supply of water in the range of 6000-9000 m^{-3} hac^{-1}, with an average pan evaporation rate of 6-7 mm d^{-1} [26]. Low availability of moisture especially during the sensitive stages of growth adversely affect the biomass and quality of carrots [23].

As previously documented, GABA may help improving the production and quality of carrots when grown under water-deficit conditions through its role as a bioregulator of various physio-biochemical phenomena [27]. The present study was, therefore, aimed at investigating the effects of exogenous application of GABA on the pattern of root growth, and the levels of photosynthetic pigments and dietary antioxidants.

2. MATERIALS AND METHOD

2.1 Experiment setup, treatments, and sampling

The pot experiment was carried out in the botanical garden of Government College University, Faisalabad, Pakistan. Seeds of two carrot varieties (Supertaj and Bharat) were obtained from Ayub Agriculture Research Institute (AARI) Faisalabad. Before sowing, seeds were surface sterilized with ethanol (70%) and washed with distilled water. Eight (8) seeds were sown in each pot of size 55 cm high and 45 cm in diameter in October, 2017. The pots were employed in completely

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randomized design (CRD). 10 days after germination, the plants were thinned to four per replicate. Twenty (20) days after seed germination, two levels of water [i.e. 100% field capacity (control), and 50% field capacity] were organized to apply to the pots. After the water deficit treatments have properly been established, the aquous solution of GABA (0, 1, 2, or 4 mM) was applied to the carrot foliage till runoff. Two (2) carrot plants from each replication were harvested when the upper visible part of their roots was around 0.75"-1.00" in circumference. The plants were carefully washed, first with tap water and then with distilled water, in the laboratory. Data on various morphological, physiological, and biochemical parameters were recorded as detailed here under:

2.2 Analysis of carrot plants

2.2.1 Physical characteristics

The fresh weight of the root and shoot of carrots in each replication was separately measured immediately after harvest. The length of carrot root and shoot was measured with the help of a meter rod. To record the data for dry weight, both organs were separately ovendried at 60 °C until the stable mass was achieved.

2.2.2 Assessment of physiological components

2.2.2.1 Plastid pigments

The levels of plastid pigments [chlorophyll a (Chl *a*), chlorophyll b (Chl *b*), and total chlorophyll content (TCC)] in the fresh leaves of treated and untreated (control) carrots were analysed by the Arnon's method [28]. Briefly, the fresh leaf sample (0.5 g) of carrot (n = 3) was extracted with 10 mL of acetone (80%) under dim

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light conditions. The optical density (OD) of the supernatant was recorded at 480, 663, and 645 nm.

2.2.2.2 Relative water content

The relative water content (RWC) of carrot leaves in each replication was measured by the method described by Jones and Turner [29] with modifications. Briefly, ten (10) round discs (1 cm diameter), were excised from the young leaves with full expansion. After weighing, the leaf-discs were floated in distilled water at 25 ± 1 °C for 2 h. Then the turgid weight of the discs was recorded after blotted drying. The discs were ovendried at 65 °C until a stable weight was achieved. The RWC of the carrot leaf sample was calculated by the following equation:

$$\text{RWC (\%)} = [(\text{fresh weight} - \text{dry weight}) \div (\text{turgid weight} - \text{dry weight})] \times 100$$

2.2.2.3 Relative electrolyte leakage (REL)

Following the modified method of Guo et al. [30], the permeability of cell-membrane of carrot leaves was estimated as the function of electrolyte leakage (EL), in the present study. In brief, fifteen (15) fully expanded leaves (8-mm diameter) of carrot were cut into small pieces and submerged in distilled water (10 mL) for 10 min. The leaf sample was then shooked on the electrical shaker at 80 rpm for 12 h. The initial electric conductance (EC_0) of the sample was noted. The final electric conductance (EC_f) was recorded after digesting the sample at 100 °C for 25 min. The percent REL was calculated by the following expression:

$$\text{REL (\%)} = (EC_0 / EC_f) \times 100$$

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2.2.3 Chemical analysis

2.2.3.1 Protein content

The concentration of protein in the leaf extract was determined by the Bradford method [31] as described by He [32] in detail. First of all, five (5) standard solutions of Bovine Serum Abumin (BSA) were prepared with known concentrations of protein (i.e. 0, 20, 40, 60, 80, and 100 µg mL^{-1}). In the labelled test tubes, 30 µL of each of the standard solution or protein samples of unknown concentration were added. To plot the standard curve, 30 µL of deionized water was added to a blank test tube. For unknown protein samples, 30 µL of protein preparation buffer was added instead. Then 1.5 mL of Bradford reagent was added to each of the test tubes. Following incubation for 10 min at 25 ±1 °C, the OD of the content was recorded at 595 nm.

2.2.3.2 Total free amino acids content

The level of total free amino acids (TFAA) in carrot leaf sample was estimated by the method described by Hamilton and Van Slyke [33]. The fresh carrot leaves (0.5 g) were chopped and extracted with 0.2 M phosophate buffer (pH 7.0). To the test tube, containing leaf extract (1 mL), 1 mL of pyridine (10%) and 1 mL of ninhydrine (2%) were added. Then the test tubes were placed on water bath for about 30 min. Final volume was made up to 50 ml with dH_2O. The OD was recorded at 570 nm. Total amino acid content expressed as microgram per gram of fresh weight (µg g^{-1} fw) was determined by the following equation:

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TFAA = $(OD_{570} \times \text{Volume of sample} \times \text{dilution factor}) \div (\text{weight of fresh leaves} \times 1000)$

Where TFAA = total free amino acids, and OD_{570} = optical density at 570 nm

2.2.3.3 Malondialdehyde (MDA) content

MDA content was determined by following the method of Camak and Horst (1991) with slight modifications. The fresh leaf sample (1 g) was homogenised in 20 mL of TCA (0.1%), centrifuged at 12000 g for 10 min and the supernatant (1 mL) was dispensed in the test tube containing 4 mL of 20% TCA with 0.5% TBA. The mixture was then heated at 95 °C for 30 min in the water bath. After cooling down on the ice bath, the mixture was centrifuged at 12000 g for 10 min. Then ODs of the supernatant were recorded at 532, 600, and 450 nm. The MDA content was calculated by the following equation [34]:

$$\text{MDA content } (\mu\text{mol } L^{-1}) = 6.45\ (OD_{532} - OD_{600}) - 0.56 OD_{450}$$

The final results were expressed as nanomole malondialdehyde per gram of fresh weight (nmol MDA g^{-1} fw).

2.2.4 Biochemical analysis

2.2.4.1 Superoxide dismutase (SOD)

The activity of superoxide dismutase (SOD, EC 1.15.1.1) was measured in terms of the inhibition rate of nitroblue tetrazolium (NBT), using xanthine oxidase as a source of hydrogen peroxide. The method described by Czégény et al. [35] was followed while the optical density (OD) was recorded at 560 nm. One Unit SOD activity depicted enzyme quantity that caused 50% photochemical inhibition of NBT.

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2.2.4.2 Guaiacol peroxidase (POD)

The activity of guaiacol peroxidase (POD, EC 1.11.1.7) was measured by the method described by Hameed and Sheikh [36] with modifications. The assay solution (3 mL) contained 50 mM of phosphate buffer (pH 5.0), 20 mM of guaiacol, 40 mM of H_2O_2, and 0.1 mL of enzyme extract. The increase in OD due to the production of tetra-guaiacol was monitored at 470 nm every 20 sec for 1 min and the difference of 0.01 depicted the units of POD activity per min.

2.2.4.3 Catalase (CAT)

Based on the conversion of hydrogen peroxide (H_2O_2) into water and oxygen, catalase (CAT, EC 1.11.1.6) activity was measured by the method described by Aebi [37]. Briefly, 3 mL of the reaction mixture contained 50 mM phosophate buffer (pH 7.0), 5.9 mM H_2O_2 and 0.1 mL of the enzyme extract. The CAT activity was assayed at 240 nm against the consumption of H_2O_2 every 20 sec. The change in absorbance of 0.01units min^{-1} was taken as one unit of the CAT activity.

2.3 Analysis of data

The data acquired during the course of present investigation was statistically analysed using 3-way ANOVA by SAS 9.2 software [38] and the means were compared by the Tukey test at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

Plants invoke a variety of complex mechansims to deal with biotic and abiotic stress cues/signals by themselves. When exposed to stress, a series of chemical,

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biochemical and/or physiological mechanisms are elicited. Synthesis of cyoprotectant molecules, increase in tissue-specific scavenging capacity against excessive ROSs, decrease in malondialdehyde content, and production of nitrogenous compounds with low molecular weight are some conspicuous examples of protective/defensive mechanisms involved in minimizing the deleterious effects of stressing agents in plants. In response to environmental anxiety, plants start accumulating GABA in their affected tissues quickly which help them adapting stress conditions to some extent [14]. Intensive studies in the recent past have demonstrated that endogenous levels of GABA can remarkeably be increased by exogenous application of GABA [39, 40].

With an exhaustive list of physiological functions, the role and importance of GABA in animal physiology is well-documented [15]. Comparatively, the role of GABA in plants has not so been scrutinized. It is, however, known that various abiotic stress stimuli such as environmental extremes, drought, salinity, mechanical damages, hypoxia, UV-irradiation, heavy metals, etc., increase the level of GABA in plants [15] probably by increasing the activities of tissue-specific GABA transporters [41].

It has been shown that exogenous application of GABA helps coping a variety of plant species with adverse effects of these stress cues [42] either by increasing the antioxidant metabolism [16], the osmotic adjustment [22], maintaining or improving the integrity of cell membrane [43], or by inhibiting the formation of malondialdehyde (MDA) during lipid peroxidation [44]. In carrots, most of the

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previous studies were focused on managing the damaging effects of abiotic stresses by increasing the level of their tolerance through conventional breeding [45], activation of the molecular network including signal transduction [46], production of stress-specific metabolites [47], expression of genes related to unambiguous stresses [48], development of functional and/or regulatory genes [49, 50], and chemical priming [51, 52]. Most of these management strategies are either practically complex or beyond the financial affordability of the growers thereby warranting to explore simple and cheaper alternatives with commercial implications. The present study was undertaken to investigate the efficacy of exogenous application of GABA in curtailing the damaging effects of drought in carrots. The results have been presented and discussed here under:

3.1 Vegetative growth and other morphological characteristics

Though, the scarcity of water (i.e. 50% FC in the present investigation) significantly ($P \leq 0.001$) decreased the fresh and dry weights of roots and shoots in both cultiavrs of carrot at harvest, it had more detrimental effects on the lengths of shoots compared to the roots (Table 1). The foliar application of GABA improved ($P \leq 0.001$) the vegetative growth of carrot plants grown under the water-defdicit conditions, irrespective of the level of GABA applied, in the present investigation. However, the foliar application of 2 mM GABA surpassed other treatments in improving the lengths and fresh and dry weights of roots and shoots of carrot plants grown under 50% FC condition, regardless of the cultivars. Remarkably, Supertaj

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showed better response to GABA spray applications, in terms of root and shoot growth, compared to Bharat cv. grown either 50% FC or 100% FC conditions.

Table 1. Effects of foliar application of γ-aminobutyric acid (GABA) on morphological characteristics of carrot grown under water-deficit conditions.

Cultivars	Treatments		Shoot			Root		
	Drought condition Field capacity (%)	GABA (mM)	Length (cm)	Fresh weight (g)	Dry weight (g)	Length (cm)	Fresh weight (g)	Dry weight (g)
Supertaj	100 (control)	0	20.01^{cd}	10.71^{cd}	0.71^{b}	10.50^{c}	6.57^{bc}	0.90^{bc}
		1	21.50^{bc}	14.11^{b}	0.80^{b}	13.12^{b}	8.01^{b}	1.11^{b}
		2	27.12^{a}	18.01^{a}	1.02^{a}	16.01^{a}	10.12^{a}	1.35^{a}
		4	23.50^{b}	12.11^{bc}	0.70^{b}	12.51^{b}	7.01^{bc}	0.86^{cd}
	50 (water-deficit)	0	13.50^{f}	6.17^{e}	0.35^{c}	7.12^{e}	2.50^{f}	0.60^{e}
		1	17.50^{de}	8.01^{de}	0.45^{c}	8.01^{d}	4.51^{de}	0.65^{de}
		2	19.01^{cd}	10.12^{cd}	0.65^{b}	9.51^{c}	5.50^{cd}	0.85^{cd}
		4	16.12^{ef}	7.03^{e}	0.35^{c}	7.50^{de}	3.15^{ef}	0.55^{e}
Bharat	100 (control)	0	17.50^{ab}	6.41^{bc}	0.60^{ab}	8.11^{c}	4.12^{cd}	0.60^{abc}
		1	20.88^{a}	8.17^{b}	0.45^{bc}	10.50^{b}	7.11^{b}	0.85^{ab}
		2	22.22^{a}	12.11^{a}	0.75^{a}	12.02^{a}	9.01^{a}	0.92^{a}
		4	15.51^{c}	7.01^{bc}	0.50^{c}	11.01^{ab}	5.03^{c}	0.56^{bc}
	50 (water-deficit)	0	11.52^{d}	2.90^{e}	0.25^{c}	5.2^{e}	2.11^{e}	0.30^{c}
		1	14.50^{cd}	5.51^{cd}	0.35^{bc}	6.5^{d}	3.12^{de}	0.45^{c}
		2	16.11^{bc}	6.02^{cd}	0.55^{c}	7.5^{cd}	3.51^{cde}	0.51^{bc}
		4	15.01^{c}	4.05^{de}	0.28^{c}	6.5^{d}	2.01^{e}	0.35^{c}
Sources of variance			Significance					
Cultivars			31.51***	121.85***	31.54***	98.13***	29.43***	41.64***
Drought			106.24***	152.05***	49.05***	407.70***	199.0***	62.90***
GABA			16.35***	31.7***	9.20***	46.02***	6.77***	11.56***
Cultiuvars × Drought			1.47^{ns}	6.81*	3.73^{ns}	4.20*	0.43^{ns}	0.53^{ns}
Cultivars × GABA			2.18^{ns}	0.50^{ns}	1.35^{ns}	2.66^{ns}	0.09^{ns}	0.57^{ns}
Drought × GABA			1.64^{ns}	3.31*	2.26^{ns}	4.44**	2.64^{ns}	1.24^{ns}
Cultivars × Drought × GABA			3.28*	0.32^{ns}	1.91^{ns}	0.64^{ns}	1.67^{ns}	0.05^{ns}

The values followed by different letters within the same column are significantly different at $P \leq 0.05$. *, **, and *** in ANOVA table based on error mean square values indicate the differences at $P \leq 0.05$, 0.01, and 0.001, respectively.

As shown in Tables 1, growing of carrots under water-deficit conditions (50% FC in the present investigation) exhibited detrimental effects on their fresh and dry biomass while the roots suffer the most, regardless of the cultivars. The

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foliar application of GABA substantially negated the hostile effects of drought on the vegetative growth of carrots from both cultivars studied, irrespective of the doses of the non-protein amino acid used. These results were in agreement with those recently reported in muskmelon [8] and white clover [53] grown under saline soil, brown mustard [54] under heavy metals, and rice [55] grown under ammonium toxicity. The foliar application of GABA might have increased the fresh biomass of carrots by inciting the cell division and/or expansion probably through maintaining the metabolic balance at the tissue level. Increased level of fresh biomass, in turn, might have increased the dry biomass of roots and shoots taken from Supertaj and Bharat cultivars.

3.2 Permeability of cell membrane and relative water content

The leaf-discs excised from carrot plants, grown under 50% FC (deficit water condition), had significantly higher levels of REL (45-55%) and lower RWC (28-37%), compared to those taken from the plants grown under 100% FC (control) condition which exhibited around 29-34% and 45-55% of REL and RWC, respectively, regardless of the cultivars, investigated (Table 2). The leaf discs from Supertaj cultivar exhibited lower REL and higher RWC, compared to those excised from Bharat cv. regardless of the growth conditions for the availability (FC) of water. Foliar application of GABA did not affect the REL of leaf-discs of carrot but significantly increased their RWC, under water deficit (50% FC) condition, irrespective of the cultivars studied and the levels of GABA applied. However, the foliar application of 2

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mM GABA was found more effective to negate the deleterious effects of drought on REL and RWC in carrot grown under water-deficit conditions. Additionally, Supertaj cv. of carrot was found more responsive to foliar spray application of GABA, compared to Bharat within the same context.

Table 2. Effects of foliar application of γ-aminobutyric acid (GABA) on electrolyte leakage and relative water content in the leaves of carrot grown under water-deficit conditions.

	Treatments		Electrolyte leakage	Relative water content
Cultivars	Drought	GABA		
	(% field capacity)	(mM)	(%)	(%)
Supartaj	100 (control)	0	30.01^{b}	50.72^{a}
		1	31.12^{b}	52.13^{a}
		2	30.50^{b}	55.03^{a}
		4	29.05^{b}	51.32^{a}
	50 (deficit)	0	50.01^{a}	30.26^{c}
		1	48.05^{a}	34.13^{bc}
		2	45.42^{a}	37.24^{b}
		4	49.35^{a}	36.14^{b}
Bharat	100 (control)	0	32.07^{b}	45.09^{c}
		1	34.23^{b}	47.03^{ab}
		2	32.63^{b}	48.13^{a}
		4	30.50^{b}	46.17^{bc}
	50 (deficit)	0	55.01^{a}	28.05^{g}
		1	52.41^{a}	30.24^{f}
		2	53.40^{a}	32.08^{e}
		4	51.85^{a}	34.18^{d}
Sources of variance			Significance	
Cultivars			17.37***	33.33***
Drought			526.69***	454.11***
GABA			1.13^{ns}	6.41***
Cultiuvars × Drought			2.77^{ns}	1.84^{ns}

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Cultivars × GABA	0.56^{ns}	0.52^{ns}
Drought × GABA	1.53^{ns}	1.92^{ns}
Cultivars × Drought × GABA	0.56^{ns}	0.13^{ns}

The values followed by different letters within the same column are significantly different at $P \leq 0.05$. *, **, and *** in ANOVA table based on error mean square values indicate the differences at $P \leq 0.05$, 0.01, and 0.001, respectively.

Under drought stress, the integrity/stability of cell membrane plays a vital role in improving stress tolerance in plants. In addition to other factors, ROSs stimulate lipid peroxidation that ultimately results in physiolocal damages to the cell membrane. REL is considered as a valid indicator of assessing the extent of damages to the cell membrane. In the present study, drought (50% FC) induced electrolyte leakage whereas the foliar spray of GABA effectively improved the stability (decrease in REL) of cell membrane of carrot leaves (Table 2). The affirmative role of exogenous application of GABA in alleviating the danmaging effects of drought stress by improving the integrity of cell-membrane has also been demonstrated in muskmelon [8], white clover [56], creeping bentgrass [57], and perennial ryegrass [58]. These results suggest a possible role of GABA in protection of the cell membrane of carrot leaves from the damaging effects of drought stress probably by the antioxidative mechanism that resulted in the reduced levels of REL. A further study may help to confirm the protecting role of GABA against drought stress at cellular level.

RWC is an appropriate measure to indirectly evaluating the physiological consequences of drought in plants at cellular level. The higher levels of RWC of leaf discs from carrot plants grown under water-deficit (50% FC) conditions, in response to GABA foliar spray, compared to control (Table 2), were in line with

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those previously reported by Jungklang et al. [59] in *Curcuma alismatifolia* Gagnep., Zhou et al. [60] in hybrid bermudagrass [Cynodon dactylon (L.)], and Krishnan et al. [58] in perennial ryegrass (*Lolium perenne* L.). The role of GABA as a promising osmolyte under drought conditions is well-documented [22, 61] while the osmolytes are known to maintain the turgor pressure of cells and to protect their membranes from dehydration [58].

3.3 Plastid pigments

As shown in Fig 1, the fresh leaves taken from carrot plants grown under 50% FC (i.e. water-deficit condition) had significantly ($P \leq 0.001$) lower levels of chl *a*, chl *b*, and TCC at harvest, compared to those taken from the plants grown under 100% FC. The foliar application of GABA significantly ($P \leq 0.001$) increased the levels of Chl *a* and Chl *b* (Fig 1a, and 1b) in fresh leaves of carrot plants grown under water-deficit (50% FC) conditions, at harvest, irrespective of the levels of GABA applied or the cultivars studied in the present investigation. The level of TCC in fresh leaves of carrots were, however, not significantly affected by GABA foliar spray (Fig 1c). The foliar application of GABA @ 2mM was found more effective in increasing the levels of Chl *a* and Chl *b* in fresh leaves of carrot grown under 50% FC condition, compared to other two levels (1 mM, and 4 mM) while Supertaj cultivar was found more responsive to GABA application, compared to Bharat cv, in the present investigation. These results are coherent to the findings of previous studies carried out in various field and horticultural crops [57, 62-65].

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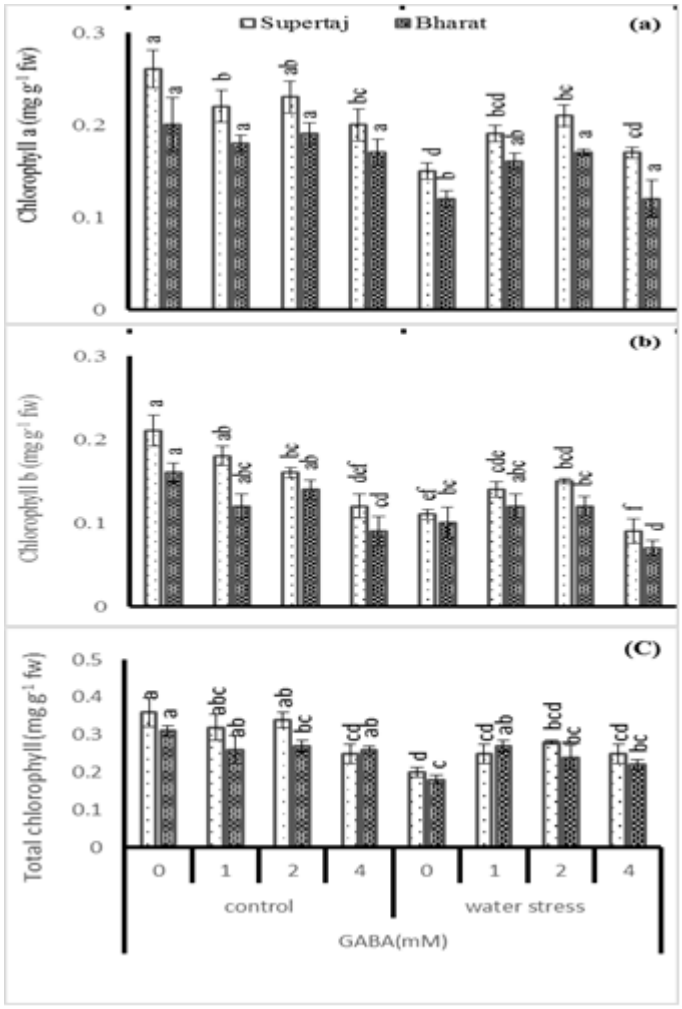

Fig 1. Effects of foliar application of γ-aminobutyric acid (GABA) on the accumulation of chlorophyll a (a), chlorophyll b (b), and total chlorophyll (c) in the leaves of carrot cultivars Supertaj and Bharat, grown under water-deficit conditions. The vertical bars represent standard error (SE) of mean values (n = 3) while those with the same letters do not differ significantly (P ≤ 0.05).

Along with other limiting factors, drought stress inhibits the growth of plants and reduces the rate of their photosynthesis speciously by preventing the enterance of CO_2 gas into the leaves [66]. It has been demonstrated that water stress mainly damages the the organ-specific photosystem II in plants [67] which, under the reduced capacity of CO_2 assimilation, actively regulates the rate of electron transport and the efficiency of photochemical reactions in plants. The lower levels of chlorins (Chl *a* and Chl *b*) in the leaves from carrot plants grown under water-deficit (50% FC)

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conditions, compared to those taken from the plants grown under full water (100% FC) conditions may be ascribed to the damage of their photosystem II.

The results from the present investigation revealed that foliar application of GABA improved the levels of photosynthetic pigments in the leaves of carrots grown under water-deficit conditions (Fig 1). Li et al. [68] demonstrated that exogenous application of GABA promoted net photosynthesis and formation of chlorophyll in maize. They attributed the improvement of photosynthesis and related pigments in response to GABA application to the maintenance of cell turgor, in addition to regulation of various physio-biochemical reactions within the leaf tissues. In our study, the improved levels of chlorins in carrot leaves under water-deficit conditions imparted by GABA application may also be attributed to their higher cell turgor compared to control or to the membrane protecting role of the amino acid it seemingly played in carrot leaves.

3.4 Activities of antioxidant enzymes

The effects of drought/water-deficit condition and foliar application of GABA on the activities of antioxidant enzymes in carrot are shown in Fig 2.

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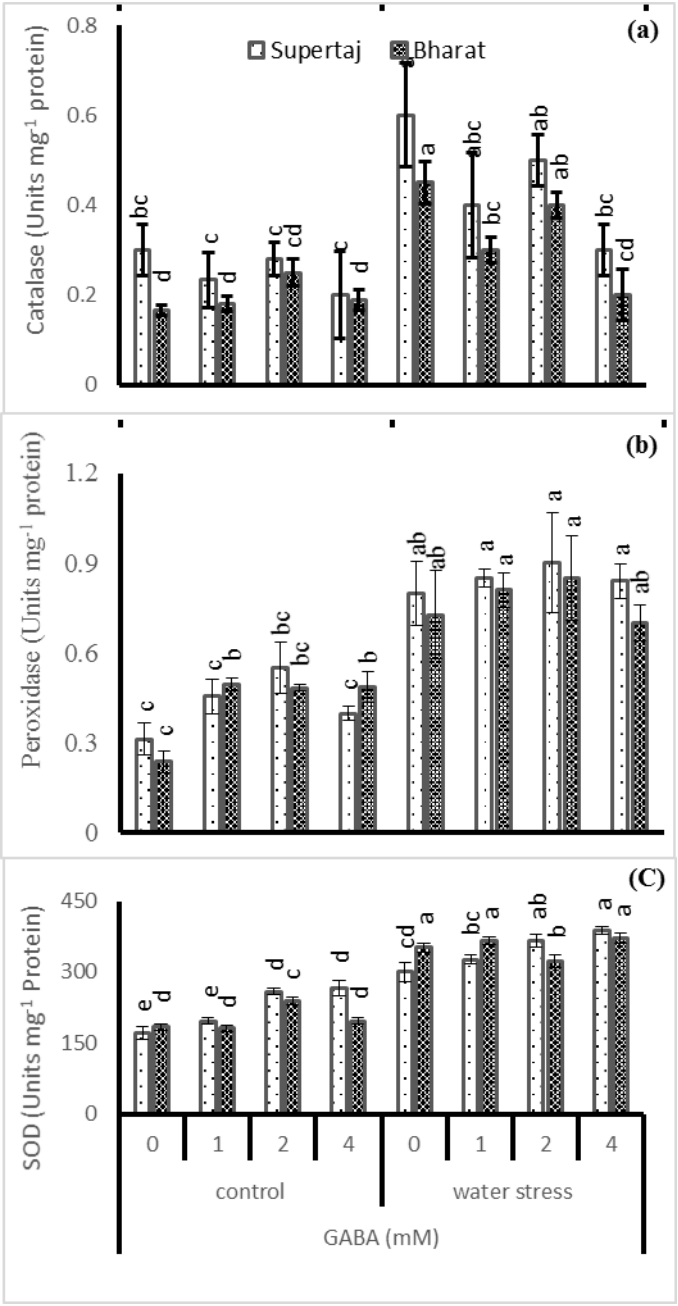

Fig 2. Effects of foliar application of γ-aminobutyric acid (GABA) on the activities of catalase (a), peroxidase (b), and superoxide dismutase (c) in the leaves of carrot cultivars Supertaj and Bharat, grown under water-deficit conditions. The vertical bars represent standard error (SE) of mean values (n = 3) while those with the same letters do not differ significantly ($P \leq 0.05$).

The results revealed significant ($P \leq 0.001$) increases in the activities of all the antioxidant enzymes investigated in carrot grown under 50% FC, irrespective of the cultivars used in the present study. The foliar application of GABA further increased the activities of CAT (Fig 2a) but did not result in any significant changes in

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POD and SOD activities (Fig 2b, and 2c) in carrot grown under water-deficit (50% FC) condition. Compared to other levels, the foliar spray of 2 mM GABA was more effective in increasing the CAT activities in carrot grown under water-deficit conditions, irrespective of the genotypes used. However, Supertaj cultivar was found more responsive to GABA foliar spray, compared to Bharat in the present investigation.

Most of the previous studies on the activities of antioxidant enzymes in various crop plants grown under drought stress presented inconsistent results and thus remained inconclusive [64, 68-70]. In general, the activities of antioxidant enzymes increase in order to minimize the damaging effects of ROSs that are produced excessively under drought stress. In the present investigation, the activities of all the antioxidant enzymes studied, were significantly increased in the leaves of carrot plants grown under water-deficite conditions (Fig. 2). In response to GABA foliar spray to carrots grown under water-deficit conditions, the activities of POD and SOD remained unaffected (Fig 2b, and 2c), however, those of CAT were significantly increased (Fig 2a) at harvest. POD and CAT activities are known to scavenge H_2O_2 whereas SOD is involved in dismutating $O_2^{\cdot-}$ into H_2O_2 and O_2 in plant cells [71]. In carrots, GABA foliar spray may be an effective production strategy to scavenge excessive produced H_2O_2 under water-deficit conditions through increased activities of CAT.

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3.5 Plant metabolites

Growing carrots under water-deficit (50% FC) conditions resulted in higher levels of MDA content in the leaves of both cultivars investigated, compared to control, in the present study (Fig. 3a). However, the leaves taken from Bharat cultivar of carrot had significantly higher levels of MDA content compared to those taken from Supertaj cultivar, irrespective of the growth conditions and the foliar spray applications of GABA. Foliar spray application of 1-2 mM GABA significantly reduced the levels of MDA content in the leaves of Supertaj carrot under both water-deficit (50% FC) and control (100% FC) conditions. Whereas, no significant affect of GABA treatments on MDA content was observed in the leaves from Bharat cultivar of carrot grown under control (100% FC) conditions.

Similar results have previously been reported in a variety of plant species exposed to oxidative stress [8, 44, 72, 73]. The shielding effects of exogenous GABA application on the integrity of cell membrane against against oxidative stress by controlling the rate of lipid peroxidation have also been reported in barley [74] and maize [68] seedlings. In general, exogenous application of GABA on plants grown under water-deficit conditions results in decreased MDA content in their tissues which, in turn, indicates the positive effects of GABA on the structural integrity of cell membrane subjected to oxidative stress.

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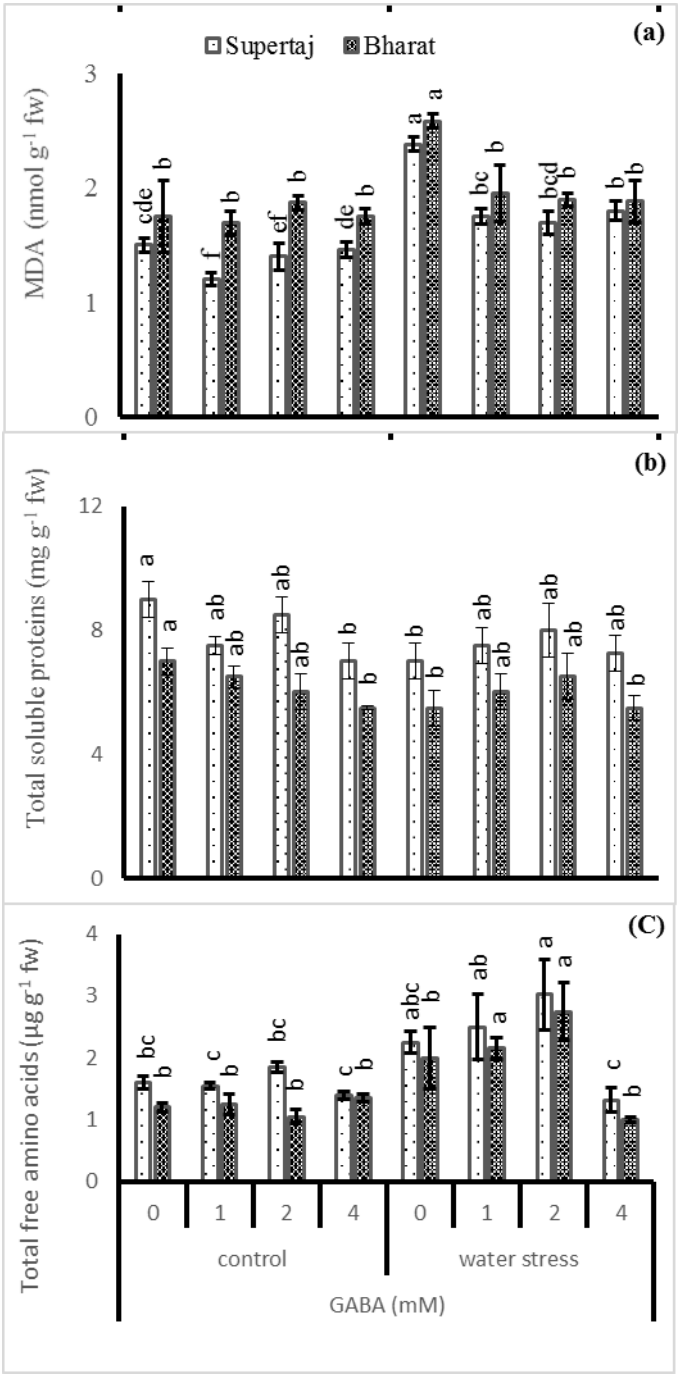

Fig 3. Effects of foliar application of γ-aminobutyric acid (GABA) on the accumulation of malondialdehyde (a), total soluble proteins (b), and total free amino acid content (c) in the leaves of carrot cultivars Supertaj and Bharat, grown under water-deficit conditions. The vertical bars represent standard error (SE) of mean values (n = 3) while those with the same letters do not differ significantly ($P \leq 0.05$).

The fresh leaves from carrot plants grown under 50% FC (water-deficit) condition exhibited significantly lower levels of total soluble proteins (TSP_s), compared to those taken from the plants grown under 100% FC condition, regardless

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of the genotypes used (Fig. 3b). However, Supertaj cultivar of carrot had significantly higher levels of TSP_s in its leaves on fresh weight basis, compared to Bharat cv., both in 50% and 100% FC conditions. The foliar spray of 1-2 mM GABA slightly increased the levels of TSP_s in the leaves of carrot plants grown 50% FC (water-deficit) condition, regardless of the cultivars investigated. Zhen et al. [75] reported that exogenous application of GABA regulated the metabolic pathway of amino acids and promoted protein biosynthesis of proteins in muskmelon grown under chemical [$Ca(NO_3)_2$] stress. Whereas, the levels of soluble proteins were found positely correlated with GABA concentrations in the leaves of mulberry subjected to low temperature storage [76].

In addition to playing an important role in amino acid transport model, various types of proteins help in producing stress response substances and are directly or indirectly involved in the related metabolic pathways. Zhu et al. [14] identified thirty-six (36) proteins involved in amino acid transport system in tea plant (*Camellia sinesis* L.). Any damage to the structure of proteins, either due to stress or other harmful agent, may compromise tissue-specific cellular integrity of plants [77].

Supertaj carrots grown under 50% FC produced higher levels of TFAA content in their leaves, compared to those grown under control (100% FC) conditions (Fig. 3c). Whereas no significant difference in the levels of TFAA content was observed in the leaves of Bharat carrots grown under control (100% FC) or water-deficit (50% FC) conditions in the present investigation. The foliar spray application of 1-2 mM GABA significantly increased the levels of TFAA content in the

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leaves of both carrot cultivars grown under water-deficit (50% FC) conditions.

Accumulation of primary metabolites with low molecular weight such as polysaccharides/sugars, tryptophan, glycolipids, polyols, short peptides, and some amino acids etc, along with their proper balance and intrinsic functions, exhibit their direct role in normal growth, development and reproduction of plants. Those which demonstrate significant accumulation in plant tissues under drought stress are considered as the key metabolites. Ullah et al. [78] identified forty-five (45) significantly active metabolites in seven (7) triticeae species with their possible role of drought tolerance in plants. The present study was, however, limited to estimation of total amino acid content in the leaves of carrot plants grown under water-deficit stress. A detailed metabolite profiling of roots and shoots of carrot under drought stress would help in further understanding the mechanism of drought tolerance in plants.

Conclusions

In summary, the results from the present investigation demonstrated that foliar application of 1, 2, and 4 mM GABA effectively mitigated the physiological and biochemical responses of drought stress in carrot. The positive effects of GABA application in carrot were more pronounced with the application rate of 2 mM than the other doses of GABA applied in the present study. Probing the physiological functions of GABA to regulate tolerance/resitsnace in carrot and other root crops against oxidative stresses, may be an exciting pitch of future research. Further study is also needed to examine the physiological functions of GABA in root crops

including carrot grown under drought conditions and their interaction with productivity and quality of the edible portion at harvest and during cold storage.

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