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The modified qualities of basil plants by selenium and/or ascorbic acid

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Abstract: The current research aimed to evaluate the possible impacts of foliar supplementations of selenium (Se) and/or ascorbic acid (AsA) on basil. Seedlings were foliarly treated with 4 concentrations of Se (0, 30, 60, and 120 mg L⁻¹) and/or 2 levels of AsA (0 and 200 mg L⁻¹), 2 times with 2-week intervals. In contrast to Se₃₀, chl_a contents were significantly decreased by Se₁₂₀ compared with the control. Se applications had a reducing effect on chl_b contents. Se and/or AsA treated plants exhibited higher contents of carotenoid. The antioxidative system was affected by the applied treatments as it was indicated by the induced peroxidase activities, higher ascorbate and glutathione contents, and improved antioxidant activities in Se and/or AsA supplemented samples, among which the highest amounts were found in Se₃₀-AsA and Se₆₀-AsA groups. Se and/or AsA utilizations resulted in stimulations in activities of phenylalanine ammonia lyase as well as increases in soluble phenol content and proline concentrations. The supplementations of AsA and/or Se led to increases in proline contents. According to these findings, the foliar supplementations of Se and AsA at appropriate concentrations trigger desirable effects on plant metabolism, thereby improving nutritional value and resistance to stress conditions.

Key words: Antioxidant, ascorbate, biofortification, heavy metal, *Ocimum basilicum*, selenium

1. Introduction

Ascorbic acid (AsA) is one of the most critical low molecular weight antioxidants and has control over crucial processes, including the cell cycle and cell wall expansion (Noctor and Foyer, 1998). Ascorbic acid, a water soluble compound, is known as a major redox buffer, one of the most abundant antioxidants, and a required cofactor for several enzymes. Its multiple implications in various critical aspects of key physiological processes like cell division, growth, signal transduction, photosynthesis, and hormone biosynthesis are well documented (Gallie, 2013). Ascorbate is regarded as the first line of defense against detrimental effects of active oxygen species (AOS), and protects plant cells from various abiotic and biotic oxidative stressors (Suza et al., 2010). It has been stated that foliarly supplemented ascorbate may be an effective treatment for improving tolerance in stress exposed plants in arid and semi-arid regions (Dolatabadian et al., 2009). In addition, it has been proposed that various crucial compounds, including phenolics, carotenoids, tocopherols, chlorophyll, and ascorbic acid all possess a key role in the prevention of different diseases related to oxidative stress such as cardiovascular diseases, cancer, atherosclerosis, inflammation, neurodegenerative diseases, diabetes, and other chronic diseases (Šircelj et al., 2010).

Selenium (Se), a natural element in soil, has not yet been proven as an essential nutrient for plants, in contrast with other kinds of organisms (Lindblom et al., 2013), and further studies are needed to clarify the effects of selenium on different aspects of plant metabolism and life cycle. Both selenium deficiency and toxicity are proposed as vital issues worldwide (Terry et al., 2000), mainly because of a narrow boundary between its requirement and toxicity levels (Germ et al., 2007; Pilon-Smits and Quinn, 2010). There is a positive correlation between the likelihood of some important issues such as cancers and selenium deficiency (Diwadkar-Navsariwala et al., 2006; White and Broadley, 2009). The production of crops fortified with Se, especially foliar supplementation, has been introduced as an alternative way of increasing the intake of Se in the human diet (Kápolna et al., 2012). Currently there is growing interest in Se and its effects on plant metabolism, biofortification, phytoremediation, and plant tolerance to stress conditions. The underlying physiological and molecular mechanisms responsible for the recorded positive responses in exogenously Se-treated plants have not been completely understood yet (Habibi, 2013).

Plants are exposed to various severe physicochemical forms of stress that adversely influence growth, metabolism, and yield (Bai et al., 2013). It is obvious that

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salinity or high EC conditions, which adversely affect plant growth and productivity, cause ion toxicity, osmotic stress, nutrient deficiency, oxidative stress, and changes in metabolism (Nazar et al., 2011). There are many efforts to achieve suitable exogenous treatments to minimize the detrimental effects of stress on crop production.

Basil is known worldwide as an important medicinal crop. In regard to the above highlighted significance of Se and AsA, the present research was conducted to evaluate the possible impacts of foliar supplementations of these compounds on the growth, physiology, and nutritional value in basil grown under high EC conditions.

2. Materials and methods

2.1. Preparation, experimental design, and treatments

The pot experiment was carried out in a complete randomized design with eight treatment groups and three replications. The sweet basil seeds (*Ocimum basilicum*) were surface sterilized in a NaOCl solution for 10 min and washed thoroughly with distilled water four times. The electrical conductivity (EC) and pH of soil were examined using a pH meter and an EC meter; a pH of 7.68 and an EC of 4.1 dS m⁻¹ were recorded. The seedlings of 4 leaves grown under natural conditions were treated with four concentrations of Se (0, 30, 60, and 120 mg L⁻¹) and/or two levels of AsA (0 and 200 mg L⁻¹). The foliar applications of Se and/or AsA were done two times with 2-week intervals. Plants were grouped in eight treatment groups and named as follows:

C: control, AsA: ascorbic acid of 200 mg L⁻¹, Se₃₀: selenium of 30 mg L⁻¹, Se₃₀-AsA: the mixed treatments of selenium (30 mg L⁻¹) and ascorbic acid (200 mg L⁻¹), Se₆₀: selenium of 60 mg L⁻¹, Se₆₀-AsA: the combined treatments of selenium (60 mg L⁻¹) and ascorbic acid (200 mg L⁻¹), Se₁₂₀: selenium of 120 mg L⁻¹ and Se₁₂₀-AsA: the simultaneous application of selenium (120 mg L⁻¹) and ascorbic acid (200 mg L⁻¹).

Plants were harvested 2 weeks after the last treatments for the biochemical analysis.

2.2. Measurements of photosynthetic pigments and leaf characteristics

Photosynthetic pigments, extracted by 80% (v/v) acetone, were measured by a spectrophotometer (GENESYS-10UV) and their concentrations were calculated according to the equations of Lichtenthaler and Wellburn (1983) as follows:

$$\text{Chla} = 12.21 (A_{663}) - 2.81 (A_{646})$$

$$\text{Chlb} = 20.13 (A_{646}) - 5.03 (A_{663})$$

$$\text{Carotenoids} = (1000 A_{470} - 3.27 [\text{Chla}] - 104 [\text{Chlb}]) / 227$$

The various leaf characteristics, including leaf areas, and fresh and dry mass were determined.

2.3. The quantification of proline contents

Proline content was quantified according to the method previously described by Bates et al. (1973). Briefly, proline extraction was done using sulfa salicylic acid (3% (w/v)). The mixture containing the tissue extract, glacial acetic acid, and ninhydrin reagent was incubated for 1 h at 100 °C. After immediate cooling on ice, the mixture was mixed with toluene and vortexed. The absorption of the toluene phase was measured at 520 nm. Finally, proline contents were calculated based on the proline standard curve and expressed in µg g⁻¹ fw.

2.4. Enzyme extraction

Enzymes were extracted at 4 °C in a mortar and pestle using 0.1 M phosphate buffer at pH of 7.5, containing 0.5 mM Na₂-EDTA and 0.5 mM ascorbic acid as an extraction buffer. The homogenates were centrifuged for 15 min at 4 °C and the supernatants were applied as enzyme extracts.

2.5. Determination of peroxidase activities

Peroxidase activity was assayed as previously described by Abeles and Biles (1991). The reaction mixture consisted of acetate buffer (0.2 M, pH 4.8), 3% H₂O₂, and 0.04 M benzidine in 50% (v/v) methanol. The reaction was started by adding the enzyme extract. The peroxidase activity was expressed as ΔA min⁻¹ g⁻¹ fw.

2.6. Measurement of ascorbate and reduced glutathione (GSH) contents

Spectrophotometric quantification of ascorbate and reduced glutathione (GSH) was performed according to the method previously described by Prieto et al. (1999) based on the reduction of Mo(VI) to Mo(V) by a sample analysis and the subsequent formation of a green phosphate-Mo(V) at an acidic pH. An aliquot of 100 µL of water extract was mixed in a microtube with 1 mL of reagent solution containing 0.6 M H₂SO₄, 28 mM NaH₂PO₄, and 4 mM (NH₄)₆Mo₇O₂₄·4H₂O. The samples were incubated at 95 °C for 90 min, and following cooling to room temperature the absorbance of the aqueous phase was measured at 695 nm. Finally, ascorbate and GSH contents were calculated using molar absorptions.

2.7. Antioxidant activity (free radical scavenging capacity)

Free radical scavenging capacity was estimated on the basis of the scavenging activity of DPPH by measuring the decrease in absorbance at 517 nm. The antioxidant activity of the leaf methanolic extract was evaluated by the DPPH free radical scavenging assay as previously described by Oraghi Ardebili et al. (2012). The inhibition percentage of the DPPH free radical was calculated by the following formula:

2.8. Determining the phenylalanine ammonia lyase (PAL) activity

The reaction mixture for PAL activity consisted of 6 µM

phenylalanine, Tris-HCl buffer (0.5 M, pH 8), and 200 μ L of enzyme extract. After 60 min at 37 °C, the reaction was ended by adding 50 μ L of 5 N HCl. PAL activities were analyzed (the rate of conversion of L-phenylalanine to trans-cinnamic acid) at 290 nm. PAL activity was determined by measuring the amount of cinnamic acid produced and expressed in μ gCin min⁻¹ μ g⁻¹ fw, according to the method described by Beaudoin-Eagan and Thrope (1985).

$$\text{Inhibition percentage} = \left(\frac{\text{Ablank} - \text{Asample}}{\text{Ablank}} \right) \times 100$$

2.9. Determining the total soluble phenols

Total soluble phenolics in the leaf ethanolic extracts were assessed using the Folin–Ciocalteu reagent procedure. Tannic acid was applied as a standard compound.

2.10. Statistical analysis

All data were subjected to analysis of variance (ANOVA) and differences between groups were assessed using Duncan's multiple-range test with SPSS software.

3. Results and discussion

3.1. Leaf characteristics

Significant increases in the number of leaves were recorded in Se and/or AsA sprayed plants, especially the former ones, among which the highest amount was in Se₃₀-AsA (Table 1). The results presented in Table 1 indicate that in contrast to Se of 30, two other used levels of Se adversely affected the leaf area and fresh and dry mass as compared with the controls. Se₁₂₀ caused necrotic lesions on the leaves and limited growth, which were signs of Se toxicity at this level. In nonaccumulator plants, stunting, necrotic lesions on the leaves, and decreased root growth have been mentioned as symptoms of Se toxicity (Matich et al., 2009). Plant growth was influenced by the applied

concentrations of AsA and/or Se, especially the latter one, as it was indicated by the altered leaf characteristics. The alterations in cell division, the cellular differentiation process, metabolism, and/or hormonal balance may be responsible for the recorded changes in various leaf characteristics. It has been documented that Se has a modifying effect on some phytohormones (especially salicylic acid, jasmonic acid, and ethylene) (Tamaoki et al., 2008) and a regulating effect on plant nutrition (Feng et al., 2013). The foliarly simultaneous applications of Se₃₀ and AsA was the most effective treatment to improve growth and development of basil plants, while two other used levels of Se adversely affected some parameters related to growth. The growth-promoting effects of Se have been recorded for various plants, including lettuce (Xue et al., 2001), green tea leaves (Hu et al., 2003), potatoes (Turakainen et al., 2004), soybeans (Djanaguiraman et al., 2005), and barley (Habibi, 2013). On the other hand, inhibitory effects of Se at inappropriate concentrations on growth and yield have also been reported for various plants, such as mustard (Fargašová, 2003), lettuce (Xue et al., 2001; Ramos et al., 2010), and tartary buckwheat (Breznik et al., 2004). The mitigating effects of exogenous ascorbic acid have been recorded in various plants exposed to environmental stressors, including wheat (Athar et al., 2008), common bean (Saeidi-Sar et al., 2013), mung bean (Kumar et al., 2011), and apple (Bai et al., 2013).

3.2. Photosynthetic pigments

In contrast to Se₃₀, and especially its combined treatments with ascorbate, Chla contents were significantly decreased by the highest applied concentration of Se (Se₁₂₀) as compared with the controls (Table 2). Chla contents were slightly affected by the foliar application of ascorbate (Table 2). All the applied levels of Se had significant reducing effects on Chlb contents (Table 2). In comparison

Table 1. The effects of different concentrations of selenium (Se) and/or ascorbic acid (AsA) on various leaf characteristics.

	The numbers of leaves	Leaf area (cm ²)	Leaf fresh mass (g)	Leaf dry mass (g)
C	12.00 ^d	4.05 ^b	0.19 ^b	0.013 ^c
AsA	14.00 ^c	4.02 ^b	0.19 ^b	0.014 ^{bc}
Se ₃₀	14.66 ^c	3.91 ^{bc}	0.18 ^b	0.015 ^b
Se ₃₀ -AsA	18.00 ^a	4.35 ^a	0.23 ^a	0.019 ^a
Se ₆₀	16.66 ^b	3.63 ^d	0.15 ^d	0.013 ^d
Se ₆₀ -AsA	16.00 ^b	3.75 ^{cd}	0.17 ^c	0.014 ^{bc}
Se ₁₂₀	14.66 ^c	2.65 ^f	0.11 ^e	0.008 ^e
Se ₁₂₀ -AsA	14.00 ^c	2.83 ^e	0.12 ^e	0.009 ^e

*Mean values followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 2. The effects of different concentrations of selenium (Se) and/or ascorbic acid (AsA) on photosynthetic pigments.

	Chla ($\mu\text{g g}^{-1}\text{fw}$)	Chlb ($\mu\text{g g}^{-1}\text{fw}$)	Carotenoid ($\mu\text{g g}^{-1}\text{fw}$)
C	318.42 ^c	154.99 ^{ab}	50.76 ^c
AsA	330.26 ^{bc}	160.86 ^a	61.18 ^b
Se ₃₀	335.57 ^b	132.43 ^{bc}	64.59 ^b
Se ₃₀ -AsA	370.23 ^a	127.32 ^c	69.52 ^{ab}
Se ₆₀	299.23 ^d	122.11 ^c	65.95 ^b
Se ₆₀ -AsA	333.58 ^{bc}	124.71 ^c	68.38 ^{ab}
Se ₁₂₀	260.55 ^f	117.48 ^c	66.94 ^b
Se ₁₂₀ -AsA	282.59 ^e	109.35 ^c	75.19 ^a

*Mean values followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

with control samples, Se and/or AsA foliarly treated plants, especially the mixed ones, exhibited higher contents of carotenoid (Table 2). In contrast to Se of 120, two other used levels of Se had improving (Se₃₀) or slightly reducing (Se₆₀) effects on Chla contents. The modified photosynthetic pigments found in Se and/or AsA-treated plants could be caused by changes in nutrition, redistribution of minerals, and/or the antioxidant system. It is obvious that many plants, especially crops like basil, are sensitive to high EC conditions and stress induced photoinhibition may lead to damage in the photosynthetic apparatus. Thus, the Se and/or AsA-improved carotenoids may act as a critical mechanism to mitigate signs of stress and photoinhibition on the photosynthetic apparatus mainly via the xanthophyll cycle. The involvement of the xanthophyll cycle in the protection of the photosynthetic structure by dissipating excess energy is well understood (Ramachandra Reddy et al., 2004; Li et al., 2010). Se-caused alterations in various photosynthesis related parameters have been recorded in different plant species like lettuce (Xue et al., 2001), soybean (Djanaguiraman et al., 2005), and potatoes (Germ et al., 2007). Selenium toxicity is attributed to its similarity to sulfur (S). Nonfunctional proteins and enzymes resulted from the nonspecific incorporation of sulfur by Se into proteins (Pilon-Smits and Quinn, 2010; Lindblom et al., 2013). It has been suggested that there is a close relationship between photosynthesis and the ascorbate pool size (Ramachandra Reddy et al., 2004). Ascorbate is involved in the xanthophyll cycle (cofactor for the violaxanthin deepoxidase) and the regeneration of α -tocopherol (vitamin E) (Gallie, 2013). Therefore, its pool size may serve as an underlying mechanism in plant protection against photoinhibition, especially under stress

conditions. It has been reported that the exogenously supplemented ascorbate in plants exposed to abiotic stressors had a vital impact on the prevention of lipid peroxidation and the restoration of the plasma membrane (Bai et al., 2013; Saeidi-Sar et al., 2013).

3.3. The antioxidative system and antioxidant activities (free radical scavenging capacity)

In comparison with the controls, significant increases in ascorbate and reduced glutathione (GSH) contents were caused by the foliar utilization of Se and/or AsA, especially the combined ones; the highest amounts were found in the Se₃₀-AsA and Se₆₀-As treatment groups (Table 3). The applied treatments of Se and/or AsA had a significant inducing impact on peroxidase activities, with the highest activities recorded in the Se₆₀ group (Table 3). The foliar supplementation of Se and/or AsA, especially the mixed ones, led to significant improvement in antioxidative activities; Se₃₀-AsA and Se₆₀-AsA treatments had the highest results (Table 3). Se and/or AsA induced increases in ascorbate contents paralleled the rises in GSH. These results are in full agreement with Gallie, who reports a coordinate balance between these two antioxidants (Gallie, 2013). It has been hypothesized that alterations in ascorbate levels act as a signal for changes in the GSH pool size (Gallie, 2013). Significantly induced activities of peroxidase, one of the most important antioxidant enzymes, were found in Se and/or AsA treated plants. Moreover, the foliar supplementation of Se and/or AsA, especially the mixed ones, led to significant improvements in antioxidant activities or free radical scavenging capacities, which are of importance for human nutrition and the medicinal industries. The inductions of antioxidant enzyme activities like those of peroxidase, as well as the increase

Table 3. The effects of different concentrations of selenium (Se) and/or ascorbic acid (AsA) on various characteristics related to the antioxidative system in the leaf tissues.

	Inhibition percentage (%)	Peroxidase ($\Delta A \text{ min}^{-1} \text{ g}^{-1} \text{ fw}$)	Ascorbate ($\text{mM g}^{-1} \text{ fw}$)	Reduced glutathione ($\text{mM g}^{-1} \text{ fw}$)
C	17.94 ^d	8.66 ^e	0.28 ^e	0.35 ^e
AsA	31.79 ^{bc}	12.50 ^d	0.42 ^d	0.53 ^d
Se ₃₀	34.51 ^b	11.16 ^d	0.47 ^{cd}	0.60 ^{cd}
Se ₃₀ AsA	40.20 ^a	15.00 ^c	0.56 ^b	0.70 ^b
Se ₆₀	34.56 ^b	19.33 ^a	0.45 ^{cd}	0.57 ^{cd}
Se ₆₀ AsA	43.69 ^a	15.50 ^c	0.62 ^a	0.78 ^a
Se ₁₂₀	27.94 ^c	12.16 ^d	0.42 ^d	0.54 ^d
Se ₁₂₀ AsA	30.25 ^c	17.50 ^b	0.48 ^c	0.61 ^c

*Mean values followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

in ascorbate and GSH concentrations, may relieve stress in plants exposed to unsuitable conditions and promote nutritional values. It has been hypothesized that altering the ascorbate content and/or its redox state may influence other antioxidants (Gallie, 2013). Considering ascorbate involvement in the biosynthesis of certain plant hormones (Prescott and John, 1996), it is possible that enhancing endogenous ascorbate levels may result in modifications in the hormonal balance, signal transduction, and other antioxidant levels. Phytohormones, including salicylic acid, jasmonic acid, and ethylene, are implicated in interactions between selenium and plants by modifying sulfur uptake and metabolism, as well as inducing defense related genes (Tamaoki et al., 2008). The regulating role of Se on the uptake and redistribution of some essential elements like S, Zn, Mn, Cu, and Fe, especially the latter, is introduced as a key mechanism to stimulate the antioxidant system, decrease the levels of reactive oxygen species, and enhance chlorophyll biosynthesis pathways, thereby improving plant resistance to stress (Feng et al., 2013). Considering the pivotal roles of ascorbate and GSH metabolites in antioxidant defenses (Noctor and Foyer, 1998), these compounds play an important role in the protection against oxidative damage under abiotic stress (Bai et al., 2013; Gallie, 2013; Saeidi-Sar et al., 2013).

3.4. Phenylpropanoid metabolism

The data presented in Table 4 clearly indicate that Se and/or AsA treated samples, especially the mixed ones, had significant stimulating effects on the PAL activities and soluble phenol contents as compared with the controls. Se and/or AsA supplementations, especially the mixed ones, affected phenolic metabolism, as it was shown by exhibiting significantly higher soluble phenol contents and induced

activities of PAL, the key enzyme in phenylpropanoid metabolism. These results might be attributed to the Se-adapted hormonal balance, especially salicylic acid, jasmonic acid, and ethylene, thereby stimulating defense related responses. Inductions of expression of many ethylene and/or jasmonate related genes because of selenate treatment have been documented (Tamaoki et al., 2008). An increase in salicylic acid, a major phenylpropanoid involved in plant acclimatization to various stressors, has been observed in selenium-treated *Arabidopsis* (Tamaoki et al., 2008). Rises in phenylalanine contents have been reported in Se-fertilized plants (Munshi et al., 1990; Ježek et al., 2011). The production of phenylpropanoids, mainly derived from phenylalanine and tyrosine, is triggered as one of the defense mechanisms under a variety of biotic and abiotic stressors (Dixon and Paiva, 1995).

3.5. Proline

In comparison with the controls, the applications of AsA and/or Se led to increased proline contents; Se₃₀-As, Se₁₂₀-As, Se₁₂₀, and Se₆₀-As were the most effective treatments (Table 4). The rise in proline contents, one of the major compatible osmolyte, recorded in the AsA and/or Se supplemented plants, especially the combined ones, may have resulted from modifications in nitrogen metabolism by these compounds. Se-induced modifications in nitrogen assimilation, hormonal status, and/or expressions of proline genes could be responsible for the observed increases in proline contents in the treated plants, thereby easing the possible impacts of the high EC condition on the plants. The multifunctional implications of proline in plant metabolism, such as a protein stabilizer, a hydroxyl radical scavenger, a source of carbon and nitrogen, as well as a cell membrane stabilizer, have been proposed by Çelik

Table 4. The effects of different concentrations of selenium (Se) and/or ascorbic acid (AsA) on proline and soluble phenol contents as well as the activities of phenylalanine ammonia lyase (PAL) in leaf tissues.

	Proline ($\mu\text{g g}^{-1}\text{fw}$)	Phenylalanine ammonia lyase ($\mu\text{gCin min}^{-1}\text{g}^{-1}\text{fw}$)	Soluble phenols ($\text{mg g}^{-1}\text{fw}$)
C	56.31 ^f	3.06 ^d	5.46 ^e
AsA	70.72 ^e	4.84 ^b	6.66 ^d
Se ₃₀	68.51 ^e	3.96 ^c	6.17 ^d
Se ₃₀ AsA	112.51 ^a	5.54 ^a	7.88 ^{bc}
Se ₆₀	73.96 ^e	4.86 ^b	7.41 ^c
Se ₆₀ AsA	81.62 ^d	5.57 ^a	7.39 ^c
Se ₁₂₀	95.51 ^c	3.33 ^d	8.33 ^b
Se ₁₂₀ AsA	103.16 ^b	4.55 ^b	8.98 ^a

*Mean values followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

and Gül Ünsal (2013). The exogenously applied ascorbic acid raised ascorbate, glutathione, and proline contents in mung bean plants exposed to heat stress (Kumar et al., 2011). A Se-altered-nitrogen assimilation process has been reported in barley (Aslam et al., 1990) and wheat (Hasanuzzaman et al., 2010). The increase in nitrate reductase activity in wheat plants has been attributed to the incorporation of selenocysteine to one of the active sites (Hasanuzzaman et al., 2010). Enhancement in amino acid metabolism has been mentioned as an important mechanism by which Se may improve tolerance of plants to stress (Ježek et al., 2011).

In conclusion, these findings indicate that the foliar supplementations of Se and AsA at appropriate

concentrations (depending on the species) trigger desirable effects on plant metabolism such as inducing activities of antioxidant enzymes, increasing nonenzymatic antioxidant compounds like ascorbate and glutathione, expressing defense related genes, stimulating phenylpropanoid metabolism, and enhancing proline contents, thereby improving the plants' nutritional values and their resistance to stress.

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