JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2024, VOL. 7(3), 263-276

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Influence of an exogenous application of glycine betaine and methionine on biochemical and morphological traits of basils (*Ocimum basilicum* L)

Mohammad Hossein Aminifard[1,*], Abbas Jorkesh[2], Hamide Fatemi[3] and Samane Mohammadi[3]

[1]*Department of Horticultural Science, Faculty of Agriculture, University of Birjand, Birjand, Iran*
[2]*Department of Horticultural Science, Faculty of Agriculture, University of Guilan, Rasht, Iran*
[3]*Department of Horticultural Science, College of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran*

ARTICLE INFO

Original Article

Article history:
Received 19 January 2024
Revised 26 March 2024
Accepted 3 April 2024

Keywords:
Chlorophyll
Total antioxidants
Total phenol
Weight

DOI: 10.22077/jhpr.2024.5779.1293
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**

Department of Horticultural Science, Faculty of Agriculture, University of Birjand, Birjand, Iran.

Email: mh.aminifard@birjand.ac.ir

ABSTRACT

Purpose: This experiment was carried out to examine the impacts of glycine betaine (GB) and methionine (Met) on basil plants' biochemical and morphological traits in two experiments under greenhouse conditions at Guilan University, Iran. **Research method:** Two completely randomized plans were used for the experiment, each involving three replications. The experiment factors during the first experiment were various amounts of GB (0, 50, 100, and 150 mg. L^{-1}), and in the second experiment, we utilized four Met quantities (0, 50, 100, and 150 mg L^{-1}). **Findings:** The results showed that GB utilized at 150 mg L^{-1} led to the maximum leaf fresh and dry weight, stem dry weight, chlorophyll a, chlorophyll b, total antioxidants, and leaf calcium and nitrogen content. The treatments with GB had a 1000 seed weight higher than the control. According to the results, leaf fresh and dry weight, root dry weight, and chlorophyll a and b in control were significantly higher than other Met treatments. Root fresh weight and the florets number per plant in control and 50 mg L^{-1} Met were significantly higher than in other treatments. Besides, the 50 mg L^{-1} Met treatments resulted in higher total phenol, antioxidants, and leaf phosphorus content than the control. **Research limitations:** No limitations were found. **Originality/Value:** The findings of this experiment demonstrate that the use of Met in greenhouse conditions does not have significant effects on basil plants, but GB has significant effects.

INTRODUCTION

Basil (*Ocimum basilicum*) belongs to the botanical family Lamiaceae, as noted by Zangeneh et al. (2019), encompassing a total of 250 genera and an astonishing 7000 species (Pandey et al., 2014). Basil, also known as the genus *Ocimum*, garners significant attention due to its extensive diversity. Approximately 30 species make up its entirety, with a variety that includes herbs and bushes. The distribution of this species spans tropical and sub-tropical regions in Africa, Central Asia, and South America (Singh et al., 2018; Hamoody et al., 2020).

Glycine betaine, otherwise referred to as N, N, N -trimethylglycine, is a preferred compatible solute for many prokaryotes and perhaps the most widely utilized osmolyte in the plant and animal kingdoms (Arafa et al., 2007). As well, GB, as one of the compatible solutes, contributes to salinity by the osmotic adjustment in plants (Ashraf & Harris, 2004), safeguarding the proteins (through sustaining the composition of enzymes such as Rubisco) (Bohnert & Jensen, 1996), protecting the membrane structure (Crowe et al., 1992), Safeguarding cytoplasm and chloroplasts against the detrimental effects of Na^+ (Rahman et al., 2002), Safeguarding the photosynthetic process (Sakamoto & Murata, 2002), and through its role as a sweeper of oxygen radicals (Smirnoff & Cumbes, 1989). The content of GB differs significantly among plant species due to its non-toxic, environmentally safe, and water-soluble nature (Makela et al., 1998). Not all plants can accumulate GB naturally. Although some transgenic plants capable of accumulating GB have been successfully constructed (Sakamoto & Murata, 2002; Sakamoto & Murata, 2000). The association of GB in the regulation of the activities of antioxidant enzymes has been described (Wang et al., 2010). The effectiveness of an exogenous application of GB depends on the type of species, the developmental stage of the plant, the application level, the number of applications, etc. (Ashraf & Foolad, 2007).

Methionine (Met), an essential amino acid, is the primary limiting sulfur amino acid because it can be converted by animals to cysteine, and thus, it meets the requirements for these two amino acids (WHO, 2007). Met is a 4-carbon amino acid synthesized from independently derived components (Kim & Leustek, 2000). Moreover, the sulfur-containing amino acid methionine is a primary metabolite in plant cells. It is a protein constituent and the precursor of S-adenosyl-L-methionine (SAM), the most biological methyl-group donor (Kim & Leustek, 2000). Met serves as a precursor of ethylene in model systems, as well as in fruits and other plant tissues (Lieberman, 1979). In apple tissue, the conversion of methionine to ethylene represents the significant metabolism of the Met (Burg & Clagett, 1967). Met is a precursor of ethylene in several higher, including climacteric tissues of apples, avocados, bananas, and tomatoes (Burg & Clagett, 1967; Lieberman et al., 1966; Mapson et al., 1970). Nitrogen is of vital importance for plant growth due to being a part of amino acid, protein and chlorophyll molecule (Zeraatgar et al., 2019; Roy et al., 2022). In this experiment, we studied the effects of an exogenous GB and Met on the biochemical and morphological traits of basils (*Ocimum basilicum* L.) plants under greenhouse conditions.

MATERIALS AND METHODS

Two completely randomized designs were utilized for conducting the experiments in three replications (with five pots). The experiment factors in the first research were GB (0, 50, 100, and 150 mg L^{-1}), however different amounts of Met (0, 50, 100, and 150 mg L^{-1}) were used in the second one.

Foliar spraying of experimental solutions was done once in the morning every two weeks, starting from the four-leaf stage until the beginning of May. For this purpose, glycine betaine and methionine were thoroughly mixed with water and sprayed entirely on the plant with a manual sprayer so that the surface of the shoot, stem, and leaf was covered entirely. In each pot, 20-25 mL of the respective solution was employed during every instance of foliar application. On the 6th of January, Isfahan PakanBazr Seed Company cultivated basil seeds that were ready for growth. For the planting process, pots measuring 22 cm in height and 33 cm in diameter were employed.

The seedbed was a combination of cow manure, soil, and sand with a ratio of 2:1:1. Soil organic matter was measured by the hot oxidation method. To determine the number of mineral elements, the soil samples were transported to the laboratory and after drying and grinding the samples, nitrogen was prepared by the more digestible method, and other elements were prepared by the dry digestion method (Hosseini et al., 2021). The amount of absorbed nitrogen was determined by the Kjeldahl method using a micro Kjeldahl device, phosphorus was determined by the Elsen method using a spectrophotometric device at a wavelength of 660 nm, and potassium was determined by the photometric film method (FP7 model). Soil acidity was determined by preparing saturated mud and using a pH meter. The significant properties of the soil utilized within the pot are recorded in Table 1. Fertilizers are not used in the plant growth cycle except for cow manure. Irrigation of the plants was done every three days. After 15 days of greening, the plant thinning process took place to ensure only six plants remained in each pot. The greenhouse temperature during the day and at night was 25°C and 15°C, respectively, while the plant was growing. In addition, the CO_2 concentration measured 350 ppm, the relative humidity stood at 40%, and the photoperiod consisted of 16 hours of light followed by 8 hours of darkness.

On May 4, uprooting four plants from the soil in each pot was conducted to identify several surface and underground vegetative indicators of basil. The estimated indicators included leaf, root, and stem weight (using a scale with an accuracy of one thousandth gram). To determine the dry weight of the root, stem, and leaf the plant samples were placed in an oven at a temperature of 70°C for 48 hours, weighed (Azarmi-Atajan et al., 2023). The height of the plant from the soil surface to the top plant was measured with a ruler (Fani, 2023). Using a ruler with an accuracy of one millimeter, measure the diameter of its main stem. Using the two remaining plants, various reproductive traits were evaluated involving the number of flowers per plant. Pigment content evaluation encompassed chlorophyll a, chlorophyll b, and carotenoid according to the technique detailed in the findings of Minguez-Mosquera and Prez-Galvez (1998). To specify the chlorophyll content, 100 mg of leaf tissue was poured into a microtube, and 1,200 µL of 80% acetone was combined and shaken well. 300 µL of the upper phase of the solution was removed, and 2700 µL of 80% acetone was combined with it, and the absorbance was read with a spectrophotometer at wavelengths of 470, 663.2, and 646.8. The amount of total chlorophyll was calculated using the following equation (1).

$$\text{Chl a} = 12.25\,A_{663.2} - 2.79A_{646.8} \qquad \text{Chl b} = 21.5A_{646.6} - 5.1A_{663.2}$$

$$\text{T Chl} = \text{Chl a} + \text{Chl b} \qquad (1)$$

Table 1. Various soil chemical indicators are utilized for basil cultivation in pots.

Organic carbon (%)	Nitrogen (%)	Calcium (%)	Phosphorous (%)	Potassium (%)	pH	Texture
1.2	1	0.48	0.19	0.55	7.1	Loam Sandy

The method of Jones (2001) was used to calculate the nitrogen, phosphorus, and calcium percentages in leaves. The Bakhshi and Arakava (2006) method was also utilized to define the antioxidant capacity and phenolic content during leaf extract extraction. This way, 2 gr of leaves were chopped separately by a sharp knife. Afterwards, an addition of 4 mL of extraction solvent containing 85% HPLC methanol and 15% acetic acid took place. Following that, the samples were stored at a temperature of 4°C for one whole day and night. In the next step, the samples were poured into a tube and centrifuged at 10,000 rpm for 10 minutes. About 200 µL of the floating phase of each sample was filtered using a 0.45 µm disposable syringe head filter. Phenols were then evaluated using the Folin-Ciocalteau method described by Tavarini et al. (2008). 0.5 mL of plant sample was transferred to the test tube, and after 5 minutes, 0.5 mL of Folin Cicalto was added to it, and then 2 mL of sodium bicarbonate (255 g/L) was added to it and shaken. The solution was kept at room temperature for 10 minutes, and it received an addition of 15 mL of deionized water. Then, it was centrifuged for 40 minutes in a bain-marie at a temperature of 40 degrees Celsius. To achieve this goal, a spectrophotometer with Specifications T80+PG Instrument UV/Vis Spectrometer was used to calculate the absorbance at 760 nm, and the quantity was identified as mg Gallic acid per 100 g of fresh weight (Dorostkar et al., 2022). Sanchez-Moreno et al. (1999) have provided a detailed explanation of the measurement of antioxidant capacity performed using the DPPH free radical scavenging method. For this purpose, 50 µL of the diluted extracts of the samples were poured into tiny test tubes, and 950 µL of standard DPPH 0.1 solution was added to them. The resulting solution was vortexed, and subsequently, it was placed in a dark room at room temperature for half an hour. The control sample included one milliliter of 0.1 standard DPPH solutions. Then, the absorbance of the control and the sample was determined using a spectrophotometer at a wavelength of 517 nm. To end, the use of SAS 9.2 software was employed to perform data analysis, at a 5% probability level, Tukeys HSD test was utilized to determine significant differences among the means.

RESULTS AND DISCUSSION

Vegetative and reproductive growth

Data in Table 2 shows that most morphological and physiological values were significantly increased by an exogenous GB application. Data inTable 3 showed that GB amino acids at 150 mg L^{-1} resulted in an effect with increment in leaf fresh and dry weight, however, the lowest amount was recorded in the control group. Moreover, the maximum and minimum root fresh weights were obtained by 150 and 50 mg L^{-1} GB usage, respectively. Also, the highest stem dry weight was obtained in plants undergoing treatment with 150 mg L^{-1} GB; comparatively, the 0 and 50 mg L^{-1} GB exhibited the lowest levels. In our results, applying external 100 mg L^{-1} of GB produced positive results and enhanced the main stem diameter and the lowest value recorded in the control (Table 3). Data presented in Table 3 shows that GB applied at 50, 100, and 150 mg L^{-1} resulted in the maximum 1000-seed weight, although the minimum amount was reported in the control. In addition, the treatment with 50 mg L^{-1} of GB presented more florets than the control. In general, glycine betaine in plants increases the osmotic potential, and as a result, the cell mass increases with the absorption of water by the plant. The rate at which plants grow and develop is directly influenced by the speed of production and enlargement of new cells and plants can divide cells only in the state of edema, by creating a state of edema by GB cell division increases and plant growth in the state of foliar spraying has caused the matter. The effect of amino acids of GB on the plant height, stem fresh weight, root dry weight, and the number of lateral branches per plant was insignificant (Table 2).

Table 2 demonstrates Met's impact on both vegetative and reproductive growth. The highest values of leaf fresh and dry weight (14.67 and 2.41 g.plant^{-1}) respectively and root dry weight (1.66 g.plant^{-1}) were found in control treatment. Meanwhile, the least values were obtained in 150 mg L^{-1} Met applications. Also, the minimum root fresh weight and the florets number per plant were recorded in 150 mg L^{-1} Met, and the maximum was observed in treatment control and 50 mg L^{-1} Met foliar application (Table 3). Plant height, stem fresh and dry weight, the number of lateral branches per plant, and 1000-seed weight unaffected by Met foliar spray (Table 2). It is likely that the increase in ethylene production is the reason for the decrease in plant growth due to the use of methionine. Morphological characteristics at the reproductive stage, including the number of flowers, significantly increased by an exogenous application of GB on tomatoes (Rezaei et al., 2012). Makela et al. (1998) observed that applying exogenous GB resulted in a substantial enhancement of growth and yield in both greenhouse and field-cultivated tomatoes. Increasing the concentrations of GB by maintaining photosynthetic capacity and the membrane structure improved plant performance (Cha-um et al., 2019). The obtained results were consistent with Seifolahzadeh et al. (2013) findings, who showed that in basil plants, the use of Met resulted in a decrease in plant growth, leading to a reduction in the leaves number, fresh weight, leaf dry weight, and plant height compared to the control. However, our results disagree with Khattab et al. (2016), who compared to the control treatment, the usage of Met in a gladiolus plant showed a significant increment in the number of florets per spike. Correspondingly, Met raised the shoot dry weight in corn (Chen et al., 2005). Likewise, Shekari and Javanmardi (2017) study indicated that Met at 200 mg L^{-1} improved the root dry weight by 177% in Broccoli. Furthermore, the plant growth measurements of squash as expressed by length, fresh and dry weight of the whole plant, and its leaves and shoots are influenced by amino acid treatments (Abd El-Aal et al., 2010). In a recent study, the foliar application of 20 mg/L L-methionine increased the shoot fresh weight, shoot dry weight, shoot length, and root length of Bitter gourd, also known as *Momordica charantia* L, which is a vegetable with a bitter taste (Akram et al., 2020).

Table 2. Mean square on the impact of glycine betaine and methionine on both vegetative and reproductive factors in basil.

Treatment	Variation source	df	Plant height	Main stem diameter	Root fresh weight	Root dry weight	Stem fresh weight	Stem dry weight
Glycine betaine	Treatment	3	40.3^{ns}	1.05*	1.4**	0.12^{ns}	1.19^{ns}	0.31*
	Error	8	30.5	0.23	0.18	0.03	0.98	0.04
	CV (%)	11	10.14	13.21	10.15	9.97	11.17	9.08
Methionine	Treatment	3	88.30^{ns}	0.58**	0.48*	0.09*	1.07^{ns}	0.027^{ns}
	Error	8	30.00	0.05	0.07	0.01	0.70	0.021
	CV (%)	11	11.67	9.67	7.54	8.48	11.44	8.96

Table 2. (*Continued*). Mean square on the impact of glycine betaine and methionine on both vegetative and reproductive factors in basil.

Treatment	Variation source	df	Leaf fresh weight	Leaf dry weight	Number of lateral branches per plant	Florets number	1000- seed weight
Glycine betaine	Treatment	3	4.56**	0.5**	0.11^{ns}	7.22*	0.14*
	Error	8	0.52	0.05	0.41	1.5	0.01
	CV (%)	11	5.17	11.55	20.38	12.04	7.98
Methionine	Treatment	3	8.70*	0.14**	0.08^{ns}	7.63**	0.04^{ns}
	Error	8	1.55	0.01	0.33	0.50	0.01
	CV (%)	11	9.58	6.64	23.89	10.74	6.42

ns = non-significant, * and ** represent significance at the 5% and 1% probability levels, respectively.

Table 3. Means comparison of the effect of glycine betaine on several factors for vegetative and reproductive growth of basil.

Treatment	Concentration (mg L^{-1})	Plant height (cm)	Main stem diameter (mm)	Root fresh weight (g.plant^{-1})	Root dry weight (g.plant^{-1})	Stem fresh weight (g.plant^{-1})	Stem dry weight (g.plant^{-1})
Glycine betaine	0	50^{a}	3.02^{b}	3.9^{ab}	1.66^{a}	8.63^{a}	2.02^{b}
	50	53.3^{a}	3.57^{ab}	3.53^{b}	1.87^{a}	8.13^{a}	2.04^{b}
	100	55.6^{a}	4.46^{a}	4.28^{ab}	2.08^{a}	9.36^{a}	2.57^{ab}
	150	58.6^{a}	3.61^{ab}	5.12^{a}	2.08^{a}	9.46^{a}	2.61^{a}
Methionine	0	53.66^{a}	2.96^{a}	4.03^{a}	1.66^{a}	7.96^{a}	1.65^{a}
	50	48^{a}	2.86^{a}	3.95^{a}	1.56^{ab}	7.67^{a}	1.69^{a}
	100	45.33^{a}	2.19^{a}	3.73^{ab}	1.38^{ab}	7.12^{a}	1.72^{a}
	150	40.66^{a}	2.11^{a}	3.14 b	1.26^{b}	6.61^{a}	1.5^{a}

Table 3. (*Continued*). Means comparison of the effect of glycine betaine on several factors for vegetative and reproductive growth of basil.

Treatment	Concentration (mg L^{-1})	Leaf fresh weight (g.plant^{-1})	Leaf dry weight (g.plant^{-1})	Number of lateral branches per plant	Florets number	1000- seed weight (g)
Glycine betaine	0	13^{b}	1.5^{b}	3^{a}	8.33^{b}	1.42^{b}
	50	13.5^{ab}	1.87^{ab}	3^{a}	12^{a}	1.8^{a}
	100	13.84^{ab}	2.18^{ab}	3.33^{a}	9.66^{ab}	1.91^{a}
	150	15.82^{a}	2.46^{a}	3.33^{a}	10.66^{ab}	1.83^{a}
Methionine	0	14.67^{a}	2.41^{a}	2.33^{a}	7.33^{a}	1.67^{a}
	50	13.79^{ab}	2.06^{ab}	2.66^{a}	8.33^{a}	1.67^{a}
	100	12.92^{ab}	2.02^{ab}	2.33^{a}	6^{ab}	1.53^{a}
	150	10.7^{b}	1.88^{b}	2.33^{a}	4.66^{b}	1.43^{a}

According to Tukey's test, there isn't a significant difference ($P \leq 0.05$) between means that have the same letter (s) within a column.

Pigments content

As shown in Table 4, foliar spray application of GB treatments led to a significant increase in chlorophyll a and b content. Data in Figure 1 show that GB foliar application increased chlorophyll by 25.96% compared to control plants. Also, chlorophyll b at 150 mg l^{-1} concentration was 32.83% more than the control, but there was no significant carotenoid content. GB can enhance the biosynthesis of chlorophyll or prevent it from breaking down or slowing down its decomposition. It seems that increasing the concentration of glycine betaine has caused an internal increase of the choline precursor in the leaf and has prevented the destruction of chlorophyll and the activity of the chlorophyllase enzyme; therefore, it has increased the amount of chlorophyll a, and b is added.

Data presented in Table 4 and Figure 2 show that chlorophyll a and b in control was significantly higher, while the lowest was related to the 150 mg L^{-1} Met treatment. Moreover, the foliar Met application did not change carotenoid content significantly.

The application of GB externally increased chlorophyll contents and subsequently improved the drought tolerance in wheat plants (Raza et al., 2015). Chlorophyll a and b concentrations in the leaf tissues treated with 200 mM of GB significantly degraded by 43.67% and 45.19%, respectively, compared to those without foliar spray treatment (Cha-um & Kirdmanee, 2010). An exogenous application of GB concentrations appears to increase internal precursor choline in leaves prevent chlorophyll degradation and inhibit the activity of the chlorophyllase enzyme. Therefore, chlorophyll concentration increased in the leaf (Miri & Mohammad, 2013). Hence, applying GB in different concentrations resulted in the accumulation of internal GB, thus improving the chlorophyll b content. The role of GB is preserving and regulating osmotic, maintaining the integrity of the plasma membrane, and maintaining the fourth building of protein by increasing the accumulation of chlorophylls and the absorption of carbon dioxide, facilitating the electron transfer and protecting the activity of proteins and fatty mucous membrane of thylakoid in the photosystem II can be as one from physiological factors to stresses (Murata et al., 1992).

By the findings of Seifolahzadeh et al. (2013), it has been proven that the chlorophyll and carotenoid content of the basil plant decreased as Met was applied. Also, data showed that the highest chlorophyll values in snap beans were found with Met (200 mg L^{-1}) in the initial season and by Met (100 mg L^{-1}) in the second season. The highest values of chlorophyll b were found in the control treatment in the first season and by Met at a low concentration (100 mg L^{-1}) in the second one (El-Awadi et al., 2011). Moreover, Met at the concentration of 200 mg L^{-1} showed the highest chlorophyll content in Broccoli seedlings (Shekari & Javanmardi, 2017). Also, the amino acid Met increased the efficiency of the photosynthesis process by increasing the leaf pigments (chlorophyll a, b, and carotenoids) in the Gladiolus plant (Khattab et al., 2016).

Table 4. Mean squares for the influence of glycine betaine and methionine on pigment content factors in basil.

Treatment	Variation source	df	Chlorophyll a	Chlorophyll b	Carotenoid content
Glycine betaine	Treatment	3	0.03^{**}	0.02^{**}	0.001^{ns}
	Error	8	0.003	0.003	0.001
	CV (%)	11	5.23	7.41	6.35
Methionine	Treatment	3	0.038^{**}	0.01^{*}	0.0006^{ns}
	Error	8	0.004	0.002	0.001
	CV (%)	11	7.14	8.05	7.69

ns = non-significant, * and ** represent significance at the 5% and 1% probability levels, respectively.

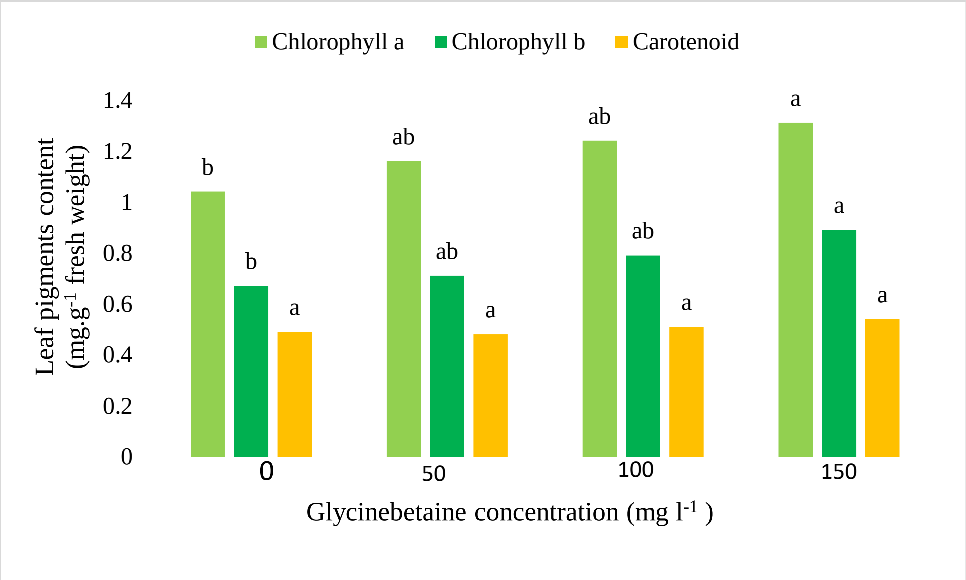

Fig. 1. Evaluating the impact of multiple levels of glycine betaine on the content of leaf pigments in basil.

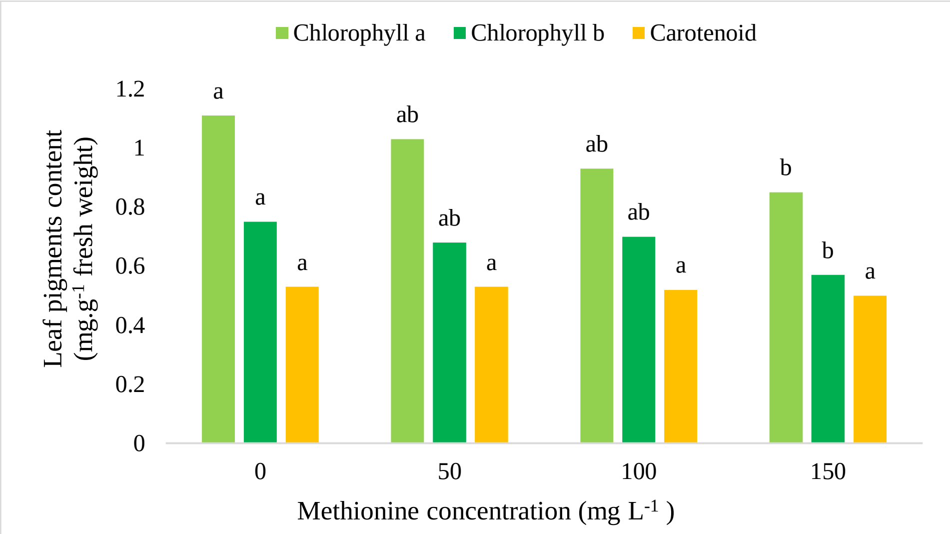

Fig. 2. The impact of varying methionine levels on basil leaf pigment content.

Nutrients content

Based on the results of Table 5, GB levels significantly affected the N and Ca content of the leaf. Thus, the highest N and Ca content in the leaf occurred at the 150 mg L^{-1} GB application level. Also, GB applications significantly affected the content of P in the leaf. Therefore, plants treated with a 50 mg L^{-1} GB usage level demonstrated the most significant P content in the leaf, while the minimum related to the 150 mg L^{-1} GB was 0.31% (Fig. 3).

The effect of Met foliar spray on nutrient content is presented in Table 5. The amino acid of methionine (100 mg L^{-1} Met) also significantly increased the leaf P content of the basil, which, averaged across rates, was 25.80% surpassing the control value. However, the result showed no significant effects of the Met application on leaf N and Ca content (Fig. 4).

The use of exogenous GB significantly improved the uptake of N, P, and Ca in plant wheat (Raza et al., 2015). This result agrees with those observed by Abd El-Aal et al. (2010), who showed that the chemical properties (P) of squash fruits were influenced by the foliar spraying of amino acid compounds during the seasons of 2006 and 2007. As well as, treatment with Met raises the absorption of nitrogen, phosphorus, and potassium in corn (Chen et al., 2005). Because Met is involved in auxin synthesis and because auxin promotes root initiation, it can help the plant absorb more nutrients (Shekari & Javanmardi, 2017).

Table 5. Mean squares for the influence of glycine betaine and methionine on leaf nutrient factors in basil.

Treatment	Source of variation	df	Leaf nitrogen content	Leaf phosphorous content	Leaf calcium content
Glycine betaine	Treatment	3	0.36**	0.003**	0.03*
	Error	8	0.04	0.0004	0.008
	CV (%)	11	6.14	5.83	8.88
Methionine	Treatment	3	0.04ns	0.003**	0.004ns
	Error	8	0.02	0.0003	0.008
	CV (%)	11	5.15	5.48	8.68

ns = non-significant, * and ** represent significance at the 5% and 1% probability levels, respectively.

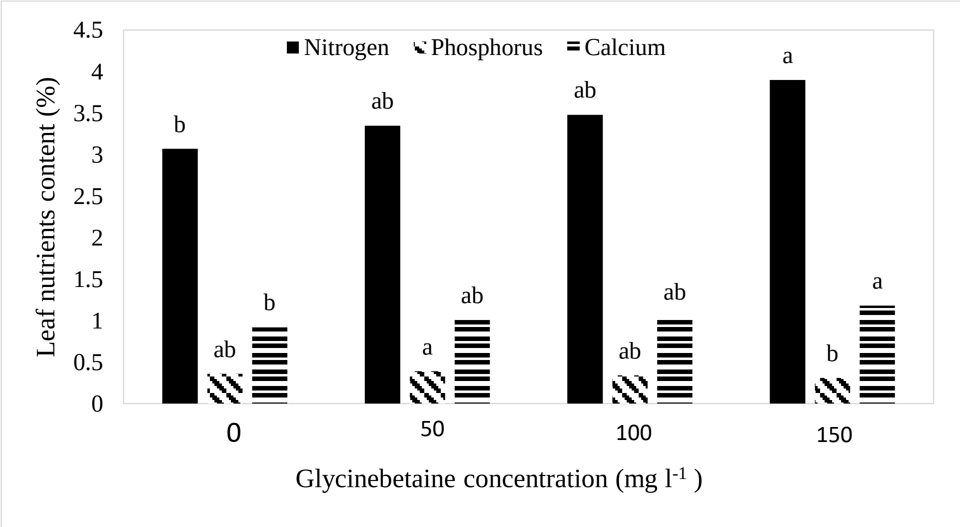

Fig. 3. The impact of various levels of glycine betaine on the nutrient content of basil leaves.

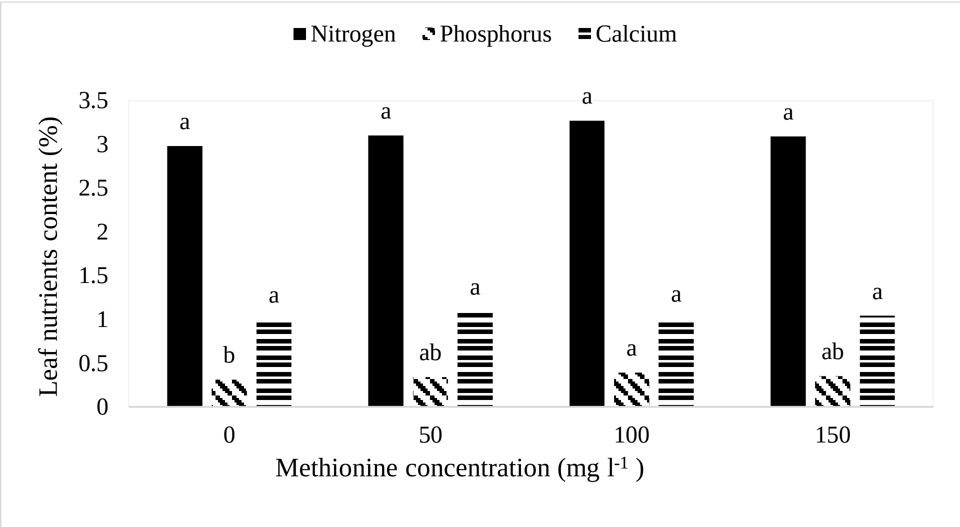

Fig. 4. Investigating the correlation between different methionine concentrations and basil leaf nutrient levels.

Total phenol and antioxidants

Data in Table 6 shows that total phenol and antioxidants are significantly affected by GB and Met applications. GB applications beyond the 50 mg L^{-1} GB treatment increased the total phenol compared to the control (Fig. 5). Also, treatments with 50 mg L^{-1} GB resulted in higher antioxidants than the control (Fig. 6). As shown in Figures 7 and 8, the highest total phenol and antioxidants were obtained when Met applied at 100 mg L^{-1} to the plants.

Under the condition of high salinity stress, it has been reported that GB has shown an increase in antioxidant enzyme activities in rice, maize, and mung beans (Hasanuzzaman et al., 2014; Hossain & Fujita, 2010; Nawaz & Ashraf, 2010). Shams et al. (2016) reported that the total phenolic content in the lettuce plants treated with 25 mM GB was significantly increased.

A similar result was also described by Seifolahzadeh et al. (2013), who showed that using Met has an inhibitory effect on the antioxidant enzyme activity, including polyphenol oxidase and peroxidase. Also, Met leaf treatment increased the phenolic compounds in the basil plant.

Table 6. Mean squares for the impact of glycine betaine and methionine on various biochemical parameters in basil.

Treatment	Variation source	df	Total antioxidants	Total phenol
Glycine betaine	Treatment	3	18.96**	28.38*
	Error	8	1.33	4.67
	CV (%)	11	3.25	4.86
Methionine	Treatment	3	8.26**	16.61*
	Error	8	0.38	3.59
	CV (%)	11	1.75	4.23

ns = non-significant, * and ** represent significance at the 5% and 1% probability levels, respectively.

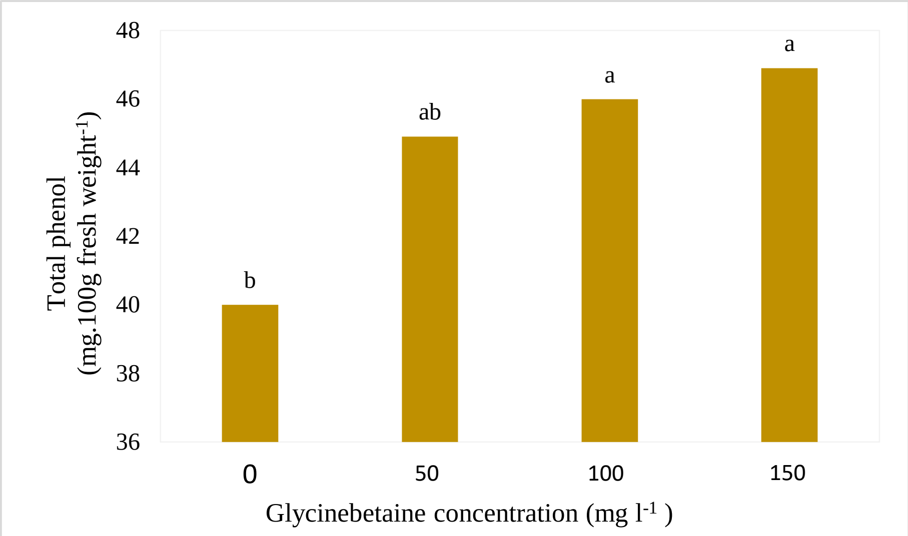

Fig. 5. Impact of various glycine betaine levels on the total phenolic compounds found in basil.

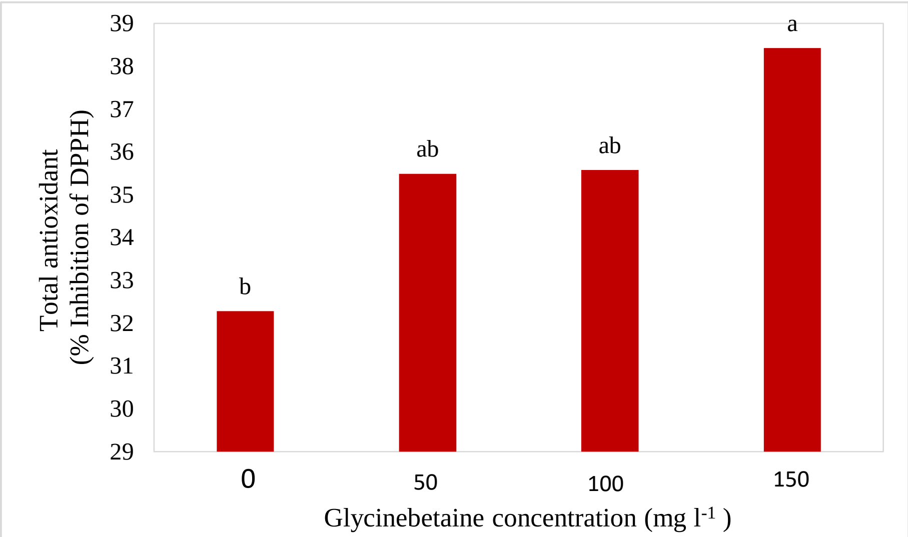

Fig. 6. The influence of various concentrations of glycine betaine on the total antioxidants found in basil.

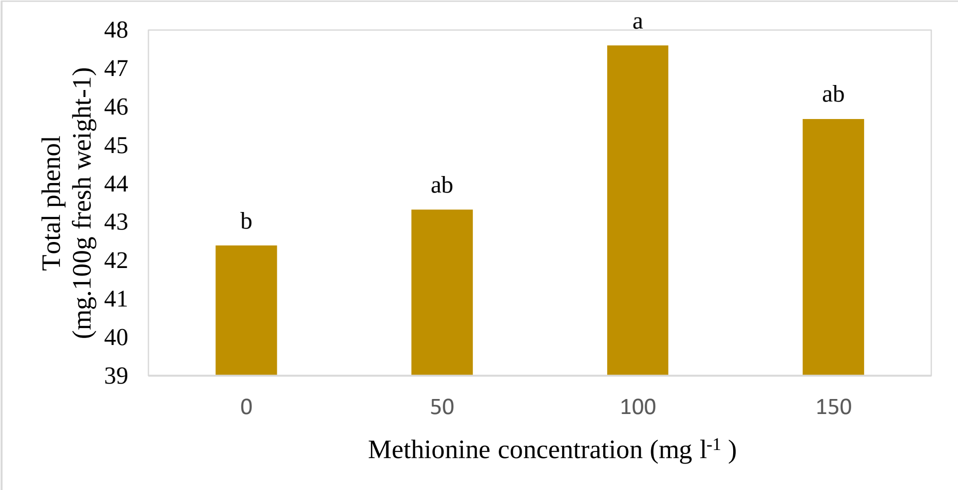

Fig. 7. The impact of different methionine levels on basil total phenolic compounds.

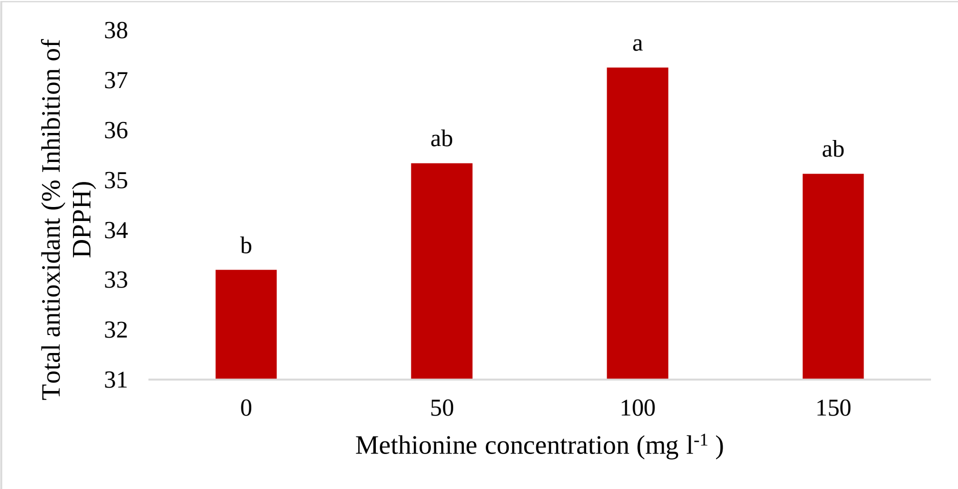

Fig. 8. The impact of different methionine levels on basil's total antioxidant content.

CONCLUSION

GB at a concentration of 150 mg.L^{-1} significantly affected the biochemical and morphological traits (stem's dry weight, root's fresh weight, leaf's fresh and dry weight, content of nitrogen and calcium in leaves, pigments chlorophyll a and b, and total antioxidants). The positive influence of amino acids can be attributed to several reasons. Initially, amino acids maintain the structure of proteins required for cell division. Then, amino acids help the division and growth of plant cells by entering the hormonal structures and, finally, the ability of amino acids to convert into polyamines that function in cell division, differentiation, and growth. GB is a quaternary amine, and it is made as a compatible substance and osmolyte in some plants and has a unique protective effect on the organs, biological membranes, proteins, and cellular enzymes. However, some plants are not able to synthesize this material under stress and non-stress conditions, so researchers are trying, or using genetic manipulations, to enable these plants to synthesize GB or exogenously use this material on plants, particularly on plants that cannot synthesize GB, it improves growth. Previous studies have proven that amino acids can directly or indirectly affect plant physiological activities.

Conflict of interest
The authors declare that they have no conflict of interest.

REFERENCES

Abd El-Aal, F. S., Shaheen, A. M., Ahmed, A. A., & Mahmoud, A. R. (2010). Effect of foliar application of urea and amino acids mixtures as antioxidants on growth, yield, and characteristics of squash. *Research Journal of Agriculture and Biological Sciences, 6*(5), 583-588.

Akram, N. A., Ashraf, M., Ashraf, M., & Sadiq, M. (2020). Exogenous application of L-methionine mitigates the drought-induced oddities in biochemical and anatomical responses of bitter gourd (*Momordica charantia* L.). *Scientia Horticulturae, 267*, 109333. https://doi.org/10.1016/j.scienta.2020.109333

Arafa, A. A., Khafagy, M. A., & El-banna, M. F. (2007). The effect of glycine betaine or ascorbic acid on the salt-stress induced damages in sorghum plant cells. *International Journal of Botany, 3*, 251-259. https://doi.org/10.3923/ijb.2007.251.259

Ashraf, M., & Foolad, M. R. (2007). Role of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany, 59*(2), 206-216. https://doi.org/10.1016/j.envexpbot.2005.12.006

Ashraf, M., & Harris, P. (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Science, 166*(1), 3–16. https://doi.org/10.1016/j.plantsci.2003.10.024

Aur, A. H., Yang, S. F., Pratt, H. K., & Biale, J. B. (1971). Ethylene biosynthesis in fruit tissues. *Plant Physiology, 47*(5), 696-699. https://doi.org/10.1104/pp.47.5.696

Azarmi-Atajan, F., Sayyari-Zohan, M. H., & Mirzaei, A. (2023). Evaluation of the growth and status of some nutrients in pistachio seedlings treated with phosphorus under different levels of irrigation water salinity. *Journal of Horticulture and Postharvest Research, 6*(3), 261-270. https://doi.org/10.22077/jhpr.2023.6271.1314

Bakhshi, D., & Arakawa, O. (2006). Induction of phenolic compounds biosynthesis with light irradiation in the flesh of red and yellow apples. *Journal of Applied Horticulture, 8*(2), 101-104. https://doi.org/10.37855/jah.2006.v08i02.23

Bohnert, H., & Jensen, R. (1996). Metabolic engineering for increased salt tolerance-the next step. *Australian Journal of Plant Physiology, 23*(5), 661–666. https://doi.org/10.1071/pp9960661

Burg, S. P., & Clagett, C. (1967). Conversion of methionine to ethylene in vegetative tissue and fruits. *Biochemical and Biophysical Research Communications. 27*(2), 125-130. https://doi.org/10.1016/s0006-291x(67)80050-0

Cha-um, S., & Kirdmanee, C. (2010). Effect of glycine betaine on proline, water use, and photosynthetic efficiencies, and the growth of rice seedlings under salt stress. *Journal of Agriculture and Forestry, 34*(6), 517-527. https://doi.org/10.3906/tar-0906-34

Cha-um, S., Rai, V., & Takabe, T. (2019). Proline, glycine betaine, and trehalose uptake and inter-organ transport in plants under stress. *Osmoprotectant-Mediated Abiotic Stress Tolerance in Plants*, 201-223. https://doi.org/10.1007/978-3-030-27423-8_9

Chen, M., Cheng, B., Zhang, Q., Ding, Y., & Yang, Z. (2005). Effects of applying L-methionine, L-phenylalanine, and L-tryptophan on *Zea mays* growth and it is nutrient uptake. *The Journal of Applied Ecology, 16*(6), 1033-1037.

Crowe, J., Hoekstra, F., & Crowe, L. (1992). Anhydrobiosis. *Annual Review of Physiology, 54*, 579–599. https://doi.org/10.1146/annurev.ph.54.030192.003051

Dorostkar, M., Moradinezhad, F., & Ansarifar, E. (2022). Influence of active modified atmosphere packaging pre-treatment on shelf life and quality attributes of cold stored apricot fruit. *International Journal of Fruit Science, 22*(1), 402-413. https://doi.org/10.1080/15538362.2022.2047137

El-Awadi, M. E., El-Bassiony, A. M., Fawzy, Z. F., & El-Nemr, M. A. (2011). The response of snap bean (*Phaseolus vulgaris* L) plants to nitrogen fertilizer and foliar application with methionine and tryptophan. *Nature and Science, 9*(5), 87-94.

Fani, E. (2023). Response of some physiological and biochemical traits of Pursalane (*Portulaca Oleracea*) to silica fertilizer under salinity stress. *Journal of Horticulture Postharvest Research, 6*(2), 157-168. https://doi.org/10.22077/jhpr.2023.5689.1291.

Hamoody, A.-H. M., Abood, J. N., & Dheeb, B. I. (2020). The synergistic effect of fungus filter *Aspergillus terreus* and aqueous extract of *Fucus vesiculosus* on some growth characteristics of the *Ocimum basilicum* and its content of active substances. *EurAsian Journal of BioSciences*, *14*(1), 161-166.

Hasanuzzaman, M., Alam, Md., Rahman, A., Hasanuzzaman, Md., Nahar, K., & Fujita, M. (2014). Exogenous proline and glycine betaine mediated upregulation of antioxidant defense and glyoxalase systems provide better protection against salt-induced oxidative stress in two rice (*Oryza sativa* L.) varieties. *BioMed Research International, 2014*, 1-17. https://doi.org/10.1155/2014/757219

Hossain, M., & Fujita, M. (2010). Evidence for a role of exogenous glycine betaine and proline in antioxidant defense and methylglyoxal detoxification systems in mung bean seedlings under salt stress. *Physiology and Molecular Biology of Plants, 16*(1), 19–29. https://doi.org/10.1007/s12298-010-0003-0

Hosseini, A., Moradinezhad, F., Khayyat, M., & Aminifard, M. H. (2021). Influence of foliar application of calcium nitrate and potassium nitrate on qualitative and quantitative traits of seedless barberry *(Berberis vulgaris* L.). *Erwerbs-Obstbau*, *63*(2), 151-161. https://doi.org/10.1007/s10341-021-00553-x

Jones, J. E. (2001). *Laboratory guide for conducting soil tests and plant analysis*. Boca Raton: CRC Press. https://doi.org/10.1201/9781420025293

Khattab, M., Shehata, A., El-Saadat, E. A., & Al-Hasni, K. (2016). Effect of glycine, methionine, and tryptophan on vegetative growth, flowering, and corms production of gladiolus plant. *Alexandria Science Exchange Journal*, *37*, 647-959. https://doi.org/10.21608/asejaiqjsae.2016.2543

Kim, J., & Leustek, T. (2000). Repression of cystathionine -synthase in *Arabidopsis thaliana* produces partial methionine auxotrophy and developmental abnormalities. *Plant Science*, *151*(1), 9-18. https://doi.org/10.1016/s0168-9452(99)00188-0

Lieberman, M. (1979). Biosynthesis and action of ethylene. *Annual Review of Plant Physiology*, *30*(1), 533-591. https://doi.org/10.1146/annurev.pp.30.060179.002533

Lieberman, M., Kunishi, A., Mapson, L. W., & Wardale, D. A. (1966). Stimulation of ethylene production in apple tissue slices by methionine. *Plant Physiology*, *41*(3), 376-382. https://doi.org/10.1104/pp.41.3.376

Makela, P., Jokinen, K., Kontturi, M., Peltonen-Sainio, P., Pehu, E., & Somersalo, S. (1998). Foliar application of glycine betaine- a novel product from sugar beet as an approach to increase tomato yield. *Industrial Crops and Products*, *7*(2-3), 139-148. https://doi.org/10.1016/s0926-6690(97)00042-3

Mapson, L. W., March, J. F., Rhodes, M. J. C., & Wooltorton, L. S. C. (1970). A comparative study of the ability of methionine or linolenic acid to act as precursors of ethylene in plant tissues. *Biochemical Journal*, *117*(3), 473-479. https://doi.org/10.1042/bj1170473

Minguez-Mosquera, M. I., & Prez-Galvez, A. (1998). Color quality in paprika oleoresins. *Journal of Agricultural and Food Chemistry*, *46*(12), 5124-5127. https://doi.org/10.1021/jf980728n

Miri, H. R., & Mohammad, A. (2013). The interaction effect of drought and exogenous application of glycine betaine on corn (*Zea mays* L.). *European Journal of Experimental Biology*, *3*, 197-206.

Murata, N., Mohanty, P. S., Hayashi, H., & Papageorgiou, G. C. (1992). Glycine betaine stabilizes the association of extrinsic proteins with the photosynthetic oxygen-evolving complex. *FEBS Letters, 296*(2), 187–189. https://doi.org/10.1016/0014-5793(92)80376-r

Nawaz, K., & Ashraf, M. (2010). Exogenous application of glycine betaine modulates the activities of antioxidants in maize plants subjected to salt stress. *Journal of Agronomy and Crop Science*, *196*(1), 28–37. https://doi.org/10.1111/j.1439-037x.2009.00385.x

Pandey, A. K., Singh, P., & Tripathi, N. N. (2014). Chemistry and bioactivities of essential oils of some Ocimum species: an overview. *Asian Pacific Journal of Tropical Biomedicine*, *4*(9), 682-694. https://doi.org/10.12980/APJTB.4.2014C77

Rahman, M., Miyake, H., & Takeoka, Y. (2002). Effects of exogenous glycine betaine on growth and ultrastructure of salt-stressed rice seedlings (*Oryza sativa* L.). *Plant Production Science*, *5*(1), 33–44. https://doi.org/10.1626/pps.5.33

Raza, S., Aown, M., Saleem, M. F., & Khan, I. H. (2015). Combined application of glycine betaine and potassium on the nutrient uptake performance of wheat under drought stress. *Pakistan Journal of Agricultural Sciences*, *52*(1), 19-26.

Rezaei, M. A., Jokar, I., Ghorbanli, M., Kaviani, B., & Kharabian Masouleh, A. (2012). Morpho-physiological improving effects of exogenous glycine betaine on tomato (*Lycopersicum esculentum* Mill.) cv. PS under drought stress conditions. *Plant Omics*, *5*(2), 79–86.

Roy, T. S., Imtiaz, N., Chakraborty, R., Kundu, B. C., & Chakraborty, E. (2022). Applying biochar and different form of nitrogen: be a good agricultural practice for better yield and processing quality of potato. *Journal of Horticulture Postharvest Research*, *5*(2), 187-196. https://doi.org/10.22077/jhpr.2022.4551.1232.

Sakamoto, A., & Murata, N. (2002). The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. *Plant, Cell and Environment*, *25*(2), 163–171. https://doi.org/10.1046/j.0016-8025.2001.00790.x

Sakamoto, A., & Murata, N. (2000). Genetic engineering of glycine betaine synthesis in plants: current status and implications for enhancement of stress tolerance. *Journal of Experimental Botany*, *51*(342), 81-88. https://doi.org/10.1093/jexbot/51.342.81

Sanchez-Moreno, C., Larrauri, J. A., & Saura-Calixto, F. (1999). A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food and Agriculture*, *76*(2), 270-276. https://doi.org/10.1002/(SICI)1097-0010(199802)76:2

Seifolahzadeh, S., Oraghi Ardebili, Z., & Motalebi, E. (2013). *The Effects of Methionine and Selenium on Basil Plants*, Islamic Azad University Garmsar Branch.

Shams, M., Yildirim, E., Ekinci, M., Turan, M., Dursun, A., Parlakova, F., & Kul, R. (2016). Exogenously applied glycine betaine regulates some chemical characteristics and antioxidative defence systems in lettuce under salt stress. *Horticulture, Environment, Biotechnology*, *57*(3), 225-231. https://doi.org/10.1007/s13580-016-0021-0

Shekari, G., & Javanmardi, J. (2017). Effects of foliar application pure amino acid and amino acid containing fertilizer on broccoli (*Brassica oleracea* L. var. Italica) transplants. *Advances in Crop Science and Technology*, *5*(3), 1-4. https://doi.org/10.4172/2329-8863.1000280

Singh, S., Lal, R., Maurya, R., & Chanotiya, C. (2018). Genetic diversity and chemotype selection in genus Ocimum. *Journal of Applied Research on Medicinal Aromatic Plants, 9*, 19-25. https://doi.org/10.1016/j.jarmap.2017.11.004

Smirnoff, N., & Cumbes, O. (1989). Hydroxyl radical scavenging activities of compatible solutes. *Phytochemistry, 28*(4), 1057–1060. https://doi.org/10.1016/0031-9422(89)80182-7

Tavarini, S., Degl-Innocenti, E., Remorini, D., Massai, R., & Guidi, L. (2008). Antioxidant capacity, ascorbic acid, total phenol, and carotenoids change during harvest and after storage of Hayward kiwifruit. *Food Chemistry, 107*(1), 282-288. https://doi.org/10.1016/j.foodchem.2007.08.015

Wang, G., Zhang, X., Li, F., Luo, Y., & Wang, W. (2010). Over-accumulation of glycine betaine enhances tolerance to drought and heat stress in wheat leaves in the protection of photosynthesis. *Photosynthetica*, *48*(1), 117–126. https://doi.org/10.1007/s11099-010-0016-5

WHO (2007). *Protein and amino acid, requirements in human nutrition*. Report of a Joint WHO/FAO/UNU expert conclusion. Geneva: World Health Organization, Press, http://whqlibdoc.who.int/trs/WHO_ TRS_935_eng.pdf

Zangeneh, M. M., Zangeneh, A., Salmani, S., Jamshidpour, R., & Kosari, F. J. (2019). Protection of phenylhydrazine-induced hematotoxicity by aqueous extract of *Ocimum basilicum* in Wistar male rats. *Comparative Clinical Pathology, 28*(2), 331-338. https://doi.org/10.1007/s00580-018-2845-8

Zeraatgar, H., Davarynejad, G. H., Moradinezhad, F., & Abedi, B. (2019). Preharvest application effect of salicylic acid and calcium nitrate on physicochemical characteristics of fresh jujube fruit (*Ziziphus jujuba*. Mill) during storage. *Erwerbs-Obstbau, 61*(2). https://doi.org/10.1007/s10341-018-0408-4