



Foliar Application of Folic Acid on Cabbage Seedlings Grown under Restricted Irrigation Conditions Can Alleviate the Negative Effects

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ABSTRACT

Drought, a critical abiotic stress worsened by climate change, poses a substantial threat to crop production and global food security. White cabbage is classified as a moderately drought-sensitive crop. The function of folic acid, otherwise known as folate, in the plant's response to drought conditions is not yet fully understood. The aim of the study was to evaluate the potential of folic acid in enhancing certain growth parameters and physiological traits of cabbage seedlings under limited irrigation conditions. In this investigation, the effects of FA as a foliar application at 0, 100, and 200 μM (FA0, FA1, and FA2, respectively) were examined on white cabbage seedlings grown under full-irrigation (I0) and restricted irrigation (I1), set to 50% of full capacity irrigation scheme (I0). Drought stress adversely affected the plant growth properties of cabbage seedlings, whereas FA treatments mitigated the adverse effects of drought stress on the plant growth properties of cabbage seedlings. Under restricted irrigation, plants treated with 100 μM FA (FA1) had higher plant height, stem diameter and leaf area, while plants treated with 200 μM FA (FA2) had higher plant dry weight and plant dry matter content. Plant fresh weight increased with FA treatments under restricted irrigation, but no significant difference was observed between doses. On the other hand, leaf relative water content (LRWC), which decreased under restricted irrigation conditions, increased with FA applications regardless of the dose, while electrical conductivity (EC), which increased under the same conditions, decreased with 100 μM FA (FA1) application. Leaf number, root fresh weight, root dry weight, root dry matter content and chlorophyll value (SPAD) were not affected by FA treatments under both restricted and full irrigation conditions. In conclusion, foliar spray of folic acid in cabbage can be recommended as a potential application to alleviate drought stress.

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Introduction

Increasing environmental stresses caused by of climate change trigger various responses in plants, including alterations in growth rate, productivity, cellular metabolism, and gene expression (Muhammad et al., 2024). One of the adverse effects of climate change is soil water deficit, which leads to drought stress (Shinde et al., 2024). Rosa et al (2022) in their review of global agricultural landscapes for 130 primary crops; they estimated that exposure to water scarcity is highly dependent on geographical location and month of the year, with 76% of global agricultural land facing water scarcity for at least one month per year and another 42% of these lands facing five months per year.

Drought conditions trigger a large number of important events at the morphological, physiological and biochemical levels that can cause serious dysfunctions in plant metabolism (Anjum et al., 2011; Sanchez-Reinoso et al., 2018). As Yildirim et al. (2021b) posited, insufficient hydration in plants has been demonstrated to engender a

reduction in photosynthesis, which consequently results in a decrease in vegetative growth. Moreover, the stem and leaves of plants exhibit greater sensitivity to water stress compared to the roots. In addition to alterations in metabolism, such as cell division and cell elongation, drought causes loss of turgor, deterioration in enzyme activities, and decreased photosynthesis efficiency (Bhargava and Sawant, 2013; Osakabe et al., 2014). The effect of drought is known to vary according to the severity and duration of the stress experienced, as well as the response of the plants (Farooq et al., 2009).

Cabbage (*Brassica oleracea* var. *capitata* L.), belonging to the Brassicaceae family, is predominantly distributed in the northern extratropical region of the world and originated from wild species growing on the European coasts of the Atlantic and Mediterranean (Leike, 1989). Brassica vegetables, including cabbage, comprise many economically important species cultivated worldwide (Pavlović et al., 2018). The development of Brassica crops,

which are commercially cultivated especially in Mediterranean, semi-arid and arid regions, and consequently the yield and quality of crops can be greatly affected by drought (Zhang et al., 2014). As drought is projected to increase gradually but steadily in much of the Mediterranean region, future projections indicate that the situation will worsen (Daliakopoulos et al., 2014). In fact, environmental conditions significantly affect cabbage production. Studies have shown that the growth and yield of white cabbage are negatively affected by drought and that this crop is moderately sensitive to drought (Maggio et al., 2005; Seidel et al., 2017). Despite this sensitivity, white cabbage has been shown to tolerate limited water availability to a certain extent (Pavlović et al., 2018), although the exact resilience mechanisms are not yet fully understood.

Vitamin B9 (folic acid; FA) and its derivatives (folates) are significant contributors to the metabolism of both carbohydrates and nitrogen (Stakhova et al., 2000). Folic acid serves as a crucial cofactor in plant one-carbon metabolism, aiding in the production of nucleic acids and amino acids (Alsamadany et al., 2022). Numerous studies have shown that foliar application of folic acid can boost vegetative growth in many plant species, including wheat (Mohamed, 2013), potato (Ibrahim et al., 2015), and broad bean (Dawood and El-Metwally, 2018). Research has also indicated that folic acid can mitigate oxidative stress caused by environmental factors through its antioxidant properties in plants (Cui et al., 2018; Gliszczynska-Święto, 2007). The external application of folic acid has been demonstrated to regulate plant responses to various environmental stressors, including drought, salt, and metal exposure. In this regard, exogenous folic acid plays a significant role in reducing the negative impacts of abiotic stresses such as drought (Poudineh et al., 2015; Ibrahim et al., 2021; Alsamadany et al., 2022; Khan et al., 2022), salinity (Kilic et al., 2016; Alsamadany et al., 2022; Al-Elwany et al., 2022), and metal toxicity (Sahito et al., 2024). A review of the literature on foliar applications of folic acid revealed a lack of research on its use to enhance drought tolerance in cabbage. To fill this knowledge gap, the current study investigated the effects of foliar applied folic acid on specific developmental and physiological characteristics of cabbage seedlings grown under water-limited conditions. The results of this research are expected to provide valuable insights for future investigations in this area.

Materials and Methods

The study was conducted in the greenhouses of Atatürk University, Plant Production Application and Research Centre, and in the laboratories of the Department of Horticulture of the Faculty of Agriculture. Seeds of the commercial standard white head cabbage variety "Yalova 1" treated with thiram to prevent possible fungal diseases, were used as plant material in this study.

The study was conducted as a pot experiment under controlled greenhouse conditions (average temperature 25°C and humidity 60%). Cabbage seeds were sown in multi-well trays containing a mixture of peat and perlite (1:1, v/v) and seedlings were transplanted after 30 days into 1.5 L pots with a 2:1 (v:v) soil:peat mixture. The study was designed as a completely randomized factorial design with

two factors; two irrigation level treatments (I0 and I1) and three doses of folic acid (FA0, FA1 and FA2). There were three replications and 5 pots per replication.

Seedlings of homogeneous size and morphology, aged two weeks, were segregated into two primary groups. One group received irrigation equivalent to field capacity for the control treatment (fully irrigated; I0), while the other group was subjected to restricted irrigation conditions, receiving 50% of the I0 treatment (I1). The volumetric determination of irrigation water application and soil moisture content in the pots was conducted utilizing a portable moisture meter. Within each major group, pots were subdivided into three subgroups to administer folic acid (C₁₉H₁₉N₇O₆) at concentrations of 0 (FA0), 100 (FA1), and 200 μM (FA2) (Alsamadany et al., 2022). Seedlings were subjected to foliar application of FA five times at 15, 20, 25, 30, and 35 days post-transplantation. The plants in the control group were treated with foliar rainwater. The volume of applied solution was 10 mL per plant per application. Tween-20, a non-ionic surfactant, was incorporated at 0.05% (v/v) in all foliar treatments.

To evaluate the impacts of the irrigation levels and FA applications on cabbage seedlings, some measurements, weighing and observations were carried out. In the study, plant height, leaf number, stem diameter, leaf area, plant fresh and dry weight, root fresh and dry weight, plant and root dry matter content, leaf relative water content (LRWC), SPAD values, and electrical conductivity (EC) were determined. SPAD value is measured with portable SPAD-502 chlorophyll meters, which express the relative chlorophyll content. The total leaf area of each plant was determined by the leaf area meter (CID Bio-Science, USA).

The determination of electrical conductivity (EC) was carried out as described by Kaya et al. (2003). Discs (1 cm diameter) were taken from fully opened true leaves of two randomly selected plants and transferred to glass bottles containing 30 ml of distilled water. The samples were shaken in a shaker for 24 hours. The electrical conductivity of the shaken water was then measured and the permeability (degree of damage) of the cell membranes determined (EC1). The samples were then autoclaved at 121°C for 20 min and the second electrical conductivity measurement was carried out in the sample water at room temperature (EC2) and the final EC value was obtained by calculating the percentage of EC1/EC2.

For the measurement of leaf relative water content (LRWC), discs (1 cm diameter) were taken from fully opened true leaves of two randomly selected plants and their fresh weight (FW) was determined by rapid weighing on a high precision balance. The discs were then placed in a glass beaker containing pure water, so that the underside of the leaf was in contact with the water, and kept in the dark for 24 hours. The excess water on the discs was then gently removed with blotting paper and the turgor weights (TW) of the discs were again weighed on a precision balance and recorded. The discs were then dried in an oven at 72°C for 48 hours and weighed by same balance again to determine the dry weight (DW). LRWC values were calculated based on the following formula (equation 1) (Shams et al., 2019):

$$\text{LRWC} = [(\text{FW} - \text{DW}) / (\text{TW} - \text{DW})] \times 100 \quad (1)$$

The data were analyzed by using the SPSS 27 package program, and the difference between the means was determined by Duncan multiple range tests.

Results and Discussion

Figure 1, Table 1, and Table 2 illustrate the effects of folic acid on the growth and physiological characteristics of cabbage seedlings subjected to water deficit.

The effects of folic acid doses and irrigation levels on plant height, stem diameter, number of leaves and leaf area were found to be significant at the $p < 0.05$ level, except for FA treatments for stem diameter. Cabbage seedlings exposed to water deficit showed a significant decrease in plant height, stem diameter, number of leaves and leaf area by 6.19%, 19.49%, 21.17% and 31.56% compared to the control plants under well-irrigated conditions. As reported by Fathi and Tari (2016), drought stress during the vegetative period has multifaceted effects on plant development, including leaf and stem development, photosynthesis and accumulation of plant components. The study findings were consistent with the results of Jang et al. (2024), who reported drought stress-induced decrease in leaf area, fresh weight, and leaf water content of Chinese

cabbage seedlings. Samancioğlu et al. (2016) also confirmed that low irrigation levels reduce the growth parameters of cabbage seedlings. However, foliar application of folic acid has been shown to mitigate the adverse effects of deficient irrigation on the growth characteristics of cabbage seedlings (Figure 2). In plants experiencing water stress and treated with folic acid, FA1 and FA2, significant increases in plant height (11.02 and 5.43%), stem diameter (12.79 and 1.83%), and leaf area (21.02 and 11.74%) were observed compared to the control (F0). Folic acid treatments had no statistically significant impact on cabbage seedlings' leaf number, regardless of watering conditions (Figure 1). Gorelova et al. (2017) documented the significant regulatory role of folates in plant growth and development processes. In the study by Aljuaid et al. (2022), the application of folic acid was found to significantly improve the shoot fresh-weight of plants subjected to stress, particularly at concentrations of 0.1 and 0.2 mM, in comparison to untreated plants. A similar outcome was reported in a study conducted in coriander, where foliar FA application was found to enhance plant growth under conditions of deficient irrigation (Khan et al., 2022).

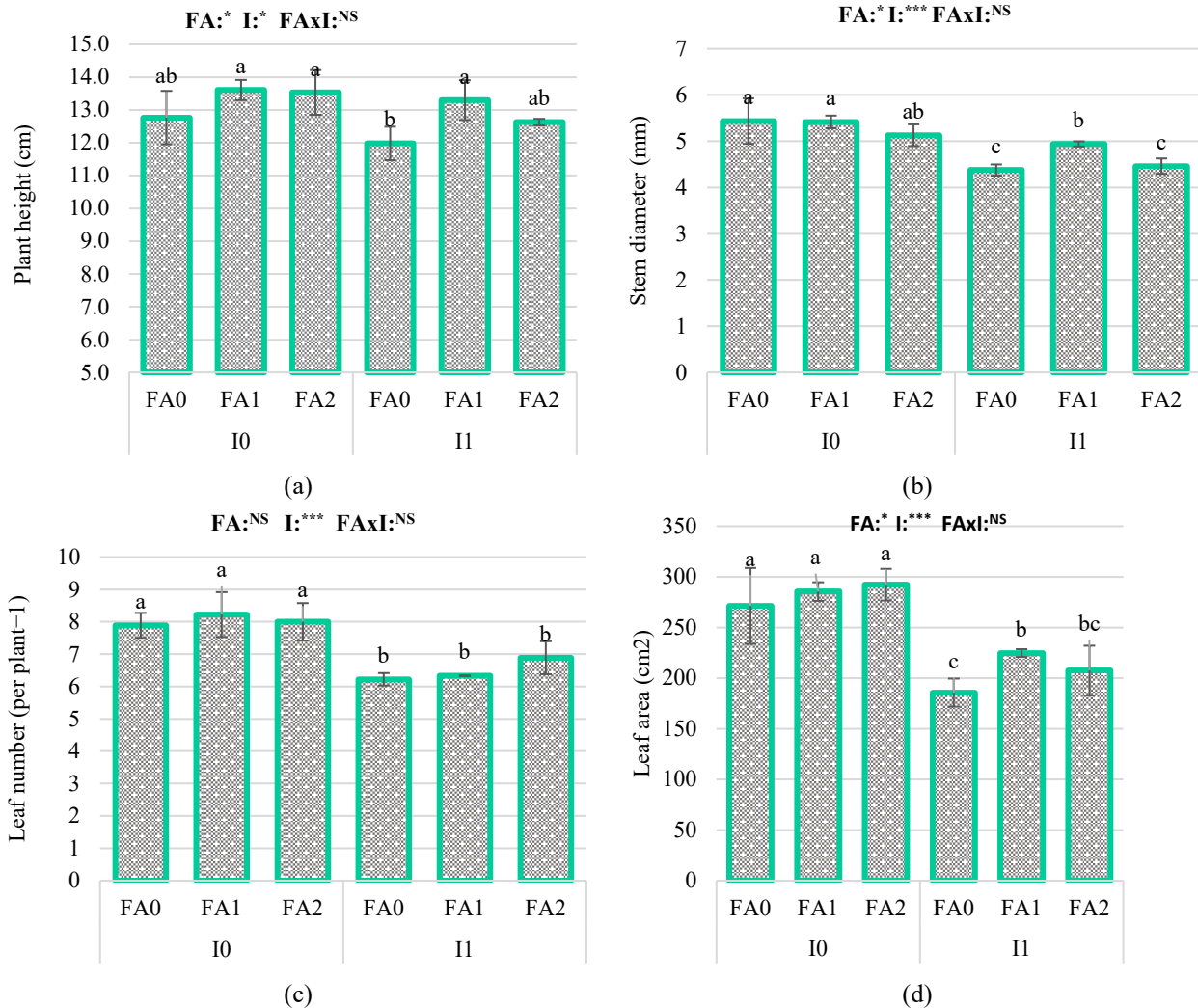


Figure 1. Effect of foliar application of 0 (FA0), 100 (FA1), and 200 μM (FA2) on (a) plant height (cm), (b) stem diameter, (c) leaf number, (d) leaf area in I0 and I1.

Graph values are the mean ± SE of three replicates. Bars exhibited with different letters indicated a significant difference between means ($p \leq 0.05$).

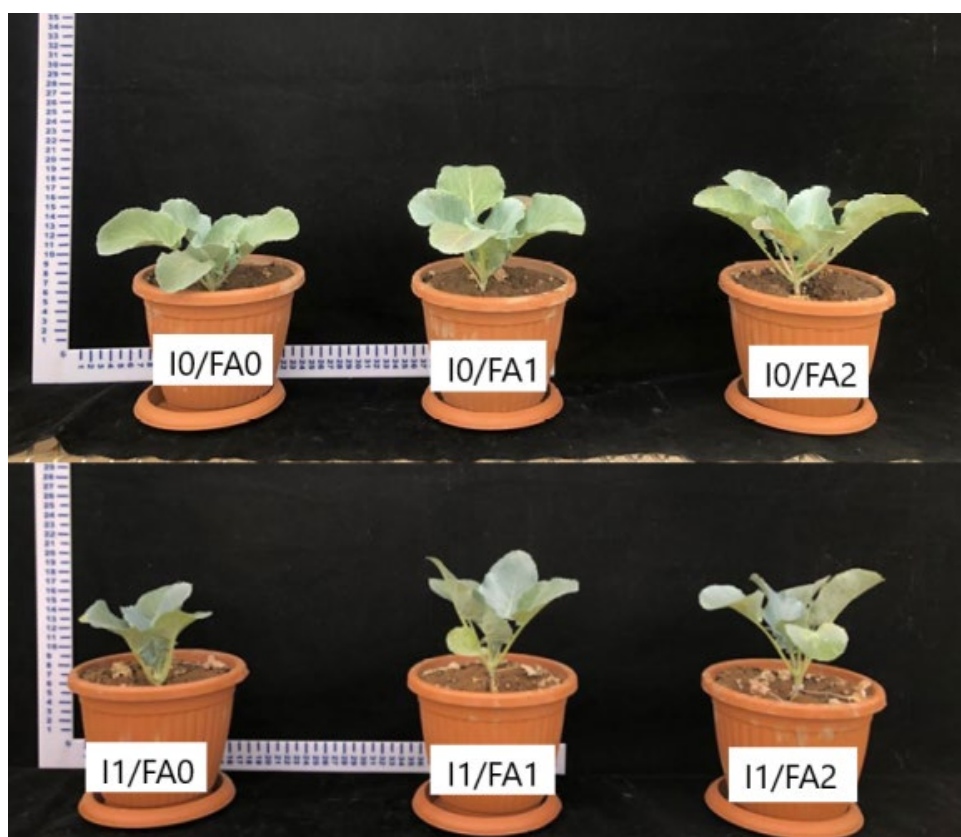


Figure 2. Response of cabbage seedlings to folic acid applications under varying irrigation conditions.
I0: 100% irrigation; I1, 50% irrigation; FA0, 0 μM FA; FA1, 100 μM FA; FA2, 200 μM FA.

Table 1. Effect of 0 (FA0), 100 (FA1) and 200 μM (FA2) foliar applications on plant fresh and dry weight, plant dry matter content, root fresh and dry weight and root dry matter content at I0 and I1.

Irrigation	Folic acid	Plant fresh weighth (g)	Plant dry weighth (g)	Plant dry matter content (%)	Root fresh weight (g plant ⁻¹)	Root dry weight (g plant ⁻¹)	Root dry matter content (%)
I0	FA0	15.37±0.56 a	3.40±0.18 a	22.18±1.13 a	4.44±0.94 a	3.45±0.09 a	14.05±1.38 b
	FA1	15.54±0.44 a	3.59±0.16 a	23.13±0.95 a	4.20±0.25 a	3.42±0.03 a	14.46±0.09 b
	FA2	15.39±1.39 a	3.41±0.20 a	22.14±1.42 a	4.81±0.40 a	2.94±0.07 a	13.08±0.64 b
I1	FA0	8.91±0.13 c	1.80±0.07 c	20.20±0.58 b	2.26±0.13 b	1.85±0.04 b	17.02±1.84 a
	FA1	9.16±0.68 b	1.97±0.08 bc	21.54±2.14 ab	2.36±0.38 b	2.05±0.05 b	17.92±1.76 a
	FA2	9.43±0.41 b	2.12±0.07 b	22.5±0.59 a	2.02±0.13 b	1.52±0.04 b	18.90±1.09 a
Falic Acid (FA)		*	*	*	NS	NS	NS
Irrigation (I)		***	***	**	***	***	***
FA×I		NS	NS	NS	NS	NS	NS

Table values are the mean \pm SE of three replicates. Means with different letters in the same column indicate a significant difference ($p \leq 0.05$).

On the other hand, a marked decline in plant fresh weight, plant dry weight and plant dry matter content was observed, amounting to 42.03%, 47.06% and 8.93%, respectively, as a consequence of the restricted irrigation. Concurrently, the fresh and dry weights of the cabbage seedlings' roots decreased by 49.10% and 46.38%, respectively, while root dry matter content exhibited an increase of 21.14%. Our results are consistent with those of the study by Şahin et al. (2018), which found that severe drought stress caused a 46.11-48.29% decrease in the relative fresh and dry weights of plant shoots. On the other hand, as a result of restricted irrigation, plant fresh weight, plant dry weight and plant dry matter content of cabbage seedling decreased significantly by 42.03%, 47.06% and 8.93%, respectively. Meanwhile, the restriction of irrigation led to a decline in the fresh and dry weights of cabbage seedlings' roots by 49.10% and 46.38%,

respectively, while root dry matter content exhibited an increase of 21.14%. However, folic acid foliar application significantly increased plant fresh weight under restricted irrigation conditions, though no differences were observed between doses. The most favourable outcomes were observed for plant dry weight and plant dry matter content of cabbage seedlings, with 17.78% and 11.39%, respectively, attained in the 200 μM FA (FA2) treatment under restricted irrigation conditions. Conversely, the impact of folic acid on root fresh weight and root dry weight of seedlings under restricted irrigation conditions was found to be insignificant. (Table 1). Khan et al. (2022) reported that foliar application of folic acid to coriander plants under IR75 and IR50 irrigation regimes resulted in higher plant fresh biomass (28% and 131%) and dry biomass (63% and 66%) in comparison to plants not treated with folic acid.

Table 2. Effect of 0 (FA0), 100 (FA1) and 200 μ M (FA2) foliar applications on plant fresh weight, plant dry weight, plant dry matter content, root fresh weight, root dry weight and root dry matter content at I0 and I1.

Irrigation	Folic Acid	SPAD	LRWC (%)	EC (%)
I0	FA0	50.07 \pm 0.88 c	89.27 \pm 0.65 b	43.63 \pm 1.11 d
	FA1	47.69 \pm 1.07 d	90.31 \pm 3.45 b	39.26 \pm 0.86 e
	FA2	47.43 \pm 1.09 d	94.87 \pm 2.98 a	41.31 \pm 1.4 e
I1	FA0	59.04 \pm 0.15 a	67.35 \pm 1.95 d	64.02 \pm 1.24 a
	FA1	54.62 \pm 0.59 b	74.78 \pm 1.43 c	55.26 \pm 1.19 c
	FA2	55.16 \pm 0.88 b	72.52 \pm 0.95 c	57.79 \pm 1.06 b
Folic Acid (FA)		***	***	***
Irrigation (I)		***	***	***
FA \times I		NS	**	**

Table values are the mean \pm SE of three replicates. Means with different letters in the same column indicate a significant difference ($p \leq 0.05$)

Finally, it was shown that SPAD and EC values of plants increased by 17.92% and 46.73%, respectively, under restricted irrigation conditions. In contrast, leaf relative water content (LRWC) decreased by 24.56% compared to control plants. In a study examining the effects of drought stress on cabbage seedlings, it was found that plants maintained more LRWC (69.12%) under well-watered conditions, while lower LRWC (56.06%) was recorded under stress conditions (Yildirim et al., 2021a). Xu and Leskovar (2014)'s study also reported a significant decrease in the relative water content (RWC) value under conditions of water stress. Also in the current study, regardless of dosage, FA application resulted in an increase (11.03% for FA1 and 7.68% for FA2) in LRWC, which decreased due to deficit irrigation. Furthermore, under the same conditions, a 13.68% decrease in the increased EC value was observed with the application of 100 μ M FA (FA1). Ibrahim et al. (2021) reported that foliar FA applications at restricted irrigation levels significantly improved RWC in green bean leaves compared to untreated plants in a two-season study (Ibrahim et al., 2021). As a last point, foliar FA1 and FA2 applications reduced the increased SPAD value due to similarly restricted irrigation by 7.49% and 6.57%, respectively, regardless of the dose (Table 2).

Conclusion

The study's findings indicated that a restricted irrigation treatment resulted in a decline in several pivotal parameters of cabbage seedlings' growth. The parameters that demonstrated a decline included plant height, stem diameter, leaf number, leaf area, plant fresh and dry weight, plant dry matter content and root fresh and dry weight. In addition, restricted irrigation increased root dry matter content, SPAD value and EC, while decreasing LRWC. However, folic acid application increased plant height, stem diameter, leaf area, plant fresh and dry weight and plant dry substance content in cabbage seedlings under restricted irrigation conditions. The best results in terms of plant height, stem diameter and leaf area were observed at 100 μ M FA (FA1) folic acid dose in the restricted irrigation group. A folic acid dose of 200 μ M (FA2) was found to be most effective in increasing plant dry weight and dry matter content in the drought-affected group. Furthermore, it was observed that folic acid sprays, irrespective of the dose, enhanced plant fresh weight, which had been diminished by the effects of drought. The study concluded that foliar application of folic acid in cabbage can be recommended as a possible intervention to reduce the effects of drought stress.

Declarations

This paper was presented in 6th International Conference on Food, Agriculture and Animal Sciences.

Author Contribution Statement

R.K. carried out all stages such as designing, conducting, analyzing the research, preparing pictures and tables, and writing the article.

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