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Effect of Salicylic Acid and Calcium Chloride on Growth of Corn Under Water Stress Condition

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ABSTRACT

Purpose: *Oxidation stress occurs when plants are exposed to water stress and it limits the plant growth and yield. The application of salicylic acid (SA) and $CaCl_2$ is expected to alleviate this stress and induce plant growth and yield in corn.*

Research Method: *Corn was planted in two main plots, normal and drought irrigation regimes (three- and four-day water withholding, respectively), and each main plot was divided into three sub plots, that were no-application, application with 0.01 SA and 0.01 SA + 45 mM $CaCl_2$ sections. A plant growth regulator was sprayed on the corn two times on days 20 (before water withholding begin) and 50. The corn height, shoot fresh weight and corn yield (ear weight, ear diameter, ear length, number of rows/ear and seeds/row) were measured on day 65. Statistically significant differences between treatments were analyzed with a two-way ANOVA.*

Findings: *Between days 25 – 60, the shoot length of the corn growing in the drought irrigation plot was higher than that in either normal irrigation plot (receiving plant growth regulator or not). On day 65, only the corn sprayed with SA shown similar shoot lengths between the different irrigation regimes. The ear diameter and weight of corn grown in the drought irrigation were better than under normal irrigation. SA application could induce shoot fresh weight and ear growth under normal irrigation, while the SA + $CaCl_2$ application induced the ear diameter and ear weight in the drought irrigated plot.*

Originality/ Value: *With these results, it is possible to use SA and $CaCl_2$ to promote plant growth and yield in acidic soil.*

Keywords: *Acidic soil; Calcium; Corn; Irrigation; Salicylic acid*

INTRODUCTION

Water stress, due to both water logging and water deficiency, is the most important problem for agriculture worldwide. Water logging reduces oxygen supply to the root and induces the plant to generate aerenchyma and adventitious roots (Sauter, 2013). Moreover, the metabolism is adapted for survival under hypoxia conditions that induce radical oxygen species, such as hydrogen peroxide, which limits plant growth and yield (Shalygo et al., 2012). On the other hand, when there is not enough water for plant growth, this can damage the plant with photosynthesis inhibition and formation of radical oxygen species (Abbaspour and Ehsanpour, 2016; Chen *et al*. 2014). This shows that oxidative stress is a critical problem for crop cultivation when plants are exposed to water stress.

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Plant growth regulators can affect plant defence systems, and are expected to alleviate oxidation stress and increase crop yield. Salicylic acid (SA) is a plant growth regulator that has been reported to induce plant adaptation to several environmental stresses, such as cold (Mutlu *et al*. 2016), salinity (Liu *et al*. 2019) and drought. For example, a 0.5 mM SA application via foliar spray could protect the mustard (*Brassica juncea* L.) cultivar Pusa Jai Kisan growing in pots when receiving water at 50% field capacity (Nazar *et al*. 2015). SA alleviated drought stress to the plants by increasing proline production and restricting ethylene formation in the plant tissue (Nazar *et al*. 2015). Pre-treatment with 1 µM SA increased corn tolerance to drought and delayed leaf rolling (Saruhan *et al*. 2012). In addition, 0.5 mM salicylic application via foliar spray could protect winter wheat growing in pots when receiving water at 30% field capacity via several mechanisms, such as alleviating photosynthesis, maintaining the membrane permeability, increasing stress protein synthesis and the activity of antioxidant enzymes (Khalvandi *et al*. 2021).

Calcium (Ca) can also alleviate plant damage from drought stress (Khushboo *et al*. 2018). Calcium is an important second messenger in plants and it has several roles in plant cells, such as stabilization of cell membranes, enhancement of cytokinins to delay plant senescence and functions of cyclic AMP and calmodulin (Hepler, 2005; Medvedev, 2005). Calcium ions are involved with plant responses to abiotic stress, including drought. Increased calcium uptake can be induced by stress and it changes the gene expression and causes responses to occur (Tuteja and Mahajan, 2007). Exogenous application of calcium chloride could induce plant growth under drought stress. For example, the application of 10 mM $CaCl_2$ to wheat genotypes HD 2733 (drought sensitive) and HD 2987 (drought tolerant) grown in Hoagland solution + 100-200 mM mannitol could increase proline, antioxidant enzymes and soluble sugar in the plant tissue (Khushboo *et al*. 2018). In addition, the net photosynthesis rate, chloroplast functions and photosynthetic pigment content of tobacco grown in nutrient solution + 25% PEG-6000 were maintained and alleviated when receiving 10 mM $CaCl_2$ (Hu *et al*. 2018).

Due to these reasons, the use of salicylic acid and calcium chloride to increase growth and yield of economic crops is probable. However, the application of SA alone or in combination with calcium chloride in the field under water stress in acidic soil has not been clearly studied. Thus, the effect of SA and calcium chloride application to stimulate corn growth under different irrigation regimes in acidic soil was studied. This will be helpful for agriculture under difficult water management regimes.

MATERIALS AND METHODS

Analysis of Soil Characteristic

Corn was planted in a field in Saentung Sub-district, Khaosaming District, Trat Province, Thailand. Soil from each main plot was sampled from nine points per main plot. The soil samples from each main plot were air-dried at 28-31^{0}C for at least 24 h to a constant weight and sent to the Central Laboratory (Thailand) Co. Ltd., Bangkok, Thailand for chemical and physical characterization. The soil in this area was acidic soil and the other chemical and physical characteristics of the soil are shown in Table 01.

Experimental Design

The experiment was performed between December 2020 and February 2021. The seeds of sweet corn (commercial seeds of Chia Tai Group Co., Bangkok, Thailand) were planted in soil and they germinated on December 18 – 19. The experimental plot size was 225 m^2. There were six rows of corn planted in one sub-plot and there were 10 corn plants per row. There was a 75 cm space between rows and a 25 cm space between each corn plant. The experimental design was a split plot (2x3 factors) in RCBD in triplicate. The main plots had two different irrigation regimes: 1) normal irrigation watering and withholding every three days and 2) drought irrigation watering and withholding every four days.

Table 01: Chemical and physical characteristics of soil

Characteristic	Plot 1: Drought irrigation	Plot 2: Normal irrigation	Method
pH	5.58	4.78	A handbook of soil analysis (Chemical and physical method) 1/2553.
Available phosphorus (P)	24.31 mg kg^{-1} soil	8.42 mg kg^{-1} soil	A handbook of soil analysis (Chemical and physical method) 1/2553.
Total nitrogen (N)	0.26%	0.30%	A handbook of soil analysis (Chemical and physical method) 1/2553.
Total potassium (K)	0.14%	0.13%	Manual of fertilizer analysis APSRDO. DOA; 4/2551
Organic matter	0.26%	0.20%	A handbook of soil analysis (Chemical and physical method) 1/2553.
Electrical conductivity (EC)	0.07 ds/m	0.09 ds/m	A handbook of soil analysis (Chemical and physical method) 1/2553.
Soil texture Sand Silt Clay	Clay 30.73% 14.02% 55.25%	Clay 21.31% 9.33% 69.36%	Mechanical analysis; Pipette method

The corn was irrigated by sprinklers for 40 minutes/application. Sub-plots had three different plant growth regulator applications: 1) untreated, 2) application of 0.01 mM salicylic acid and 3) application of 0.01 mM salicylic acid + 45 mM $CaCl_2$. Corn was sprayed with the plant growth regulators twice, on days 20 and 50.

Corn Cultivation

Weed and pest control was performed, as necessary. Organic fertilizer mixed with chemical fertilizer formula (N-P-K) 16-16-16 was provided to the corn at 3362.5 kg/ha and fertilizer was applied three times. These methods were standard for all experimental plots. Water management as well as weed and pest control on the farmer's field was performed by the farmer.

Ten corn plants per sub-plot were randomly selected from the middle row of the plot to measure their height weekly. The height of the sweet corn was measured from the soil surface to the top of the first fully developed leaf using a measuring tape. The corn yield measures, ear weight, ear diameter, ear length, number of rows/ear and seeds/row, were recorded randomly when the corn was harvested on day 65.

Statistical Analysis

A two-way ANOVA was used to test for statistically significant differences between treatments followed by a LSD test. The data are shown as mean ± SE, and the statistical differences are shown as $P \leq 0.05$.

RESULTS AND DISCUSSION

Corn Growth and Flowering

An increased corn shoot length could be clearly seen with the naked eye. The corn under normal irrigation was significantly shorter ($P<0.05$) than that grown under drought irrigation for all measurement times (Figure 02 and 03A) and seemed to be slimmer (Figure 01, 02). However, the patterns of flowering and fruiting were the same between the two plots. Corn started to flower on day 45 and the flowers bloomed on about day 48. After that, young ears were seen. The corn seed appeared on the ear about day 53 and ripe ears were found from day 58. The corn was harvested on day 65 of the experiment.

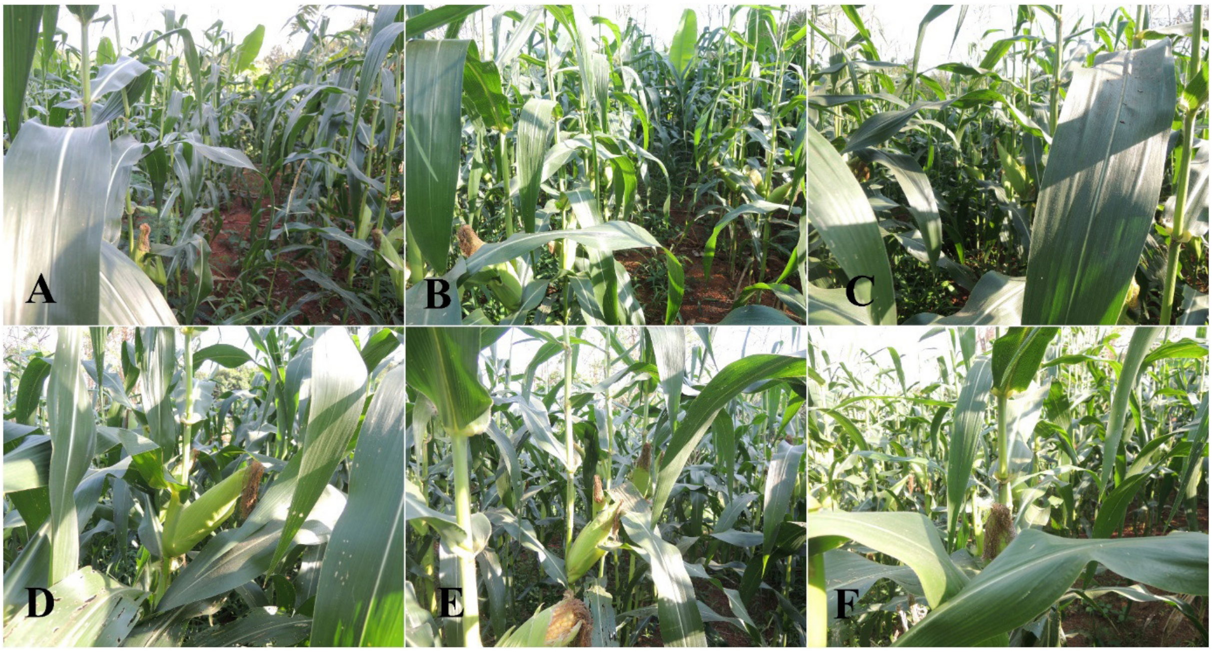

Figure 01: **Corn in each plot before harvesting, normally irrigated plot: untreated (A), SA application (B), SA + $CaCl_2$ application (C); drought irrigated plot: untreated (D), SA application (E), SA + $CaCl_2$ application (F).**

Figure 02: **Shoots of corn after harvesting on day 65 of experiment, normally irrigated plot: untreated (A), SA application (B), SA + $CaCl_2$ application (C); drought irrigated plot: untreated (D), SA application (E), SA + $CaCl_2$ application (F).**

The corn planted in the drought irrigation plot had better growth than that growing in the normal irrigation plot. This may be caused by the nature of corn that does not prefer soil saturated with water (Chaisrisuriyos *et al*. 2019). Moreover, the cytokinin content in corn leaves grown under 50–55% water of the field capacity was high via delivery from the roots to the leaves, which then increased the net photosynthesis (Wang *et al*. 2020). However, the pH of acidic soil in the normally irrigated plot was lower than that in the drought irrigated plot, and this may delay the corn growth. This point should be studied further.

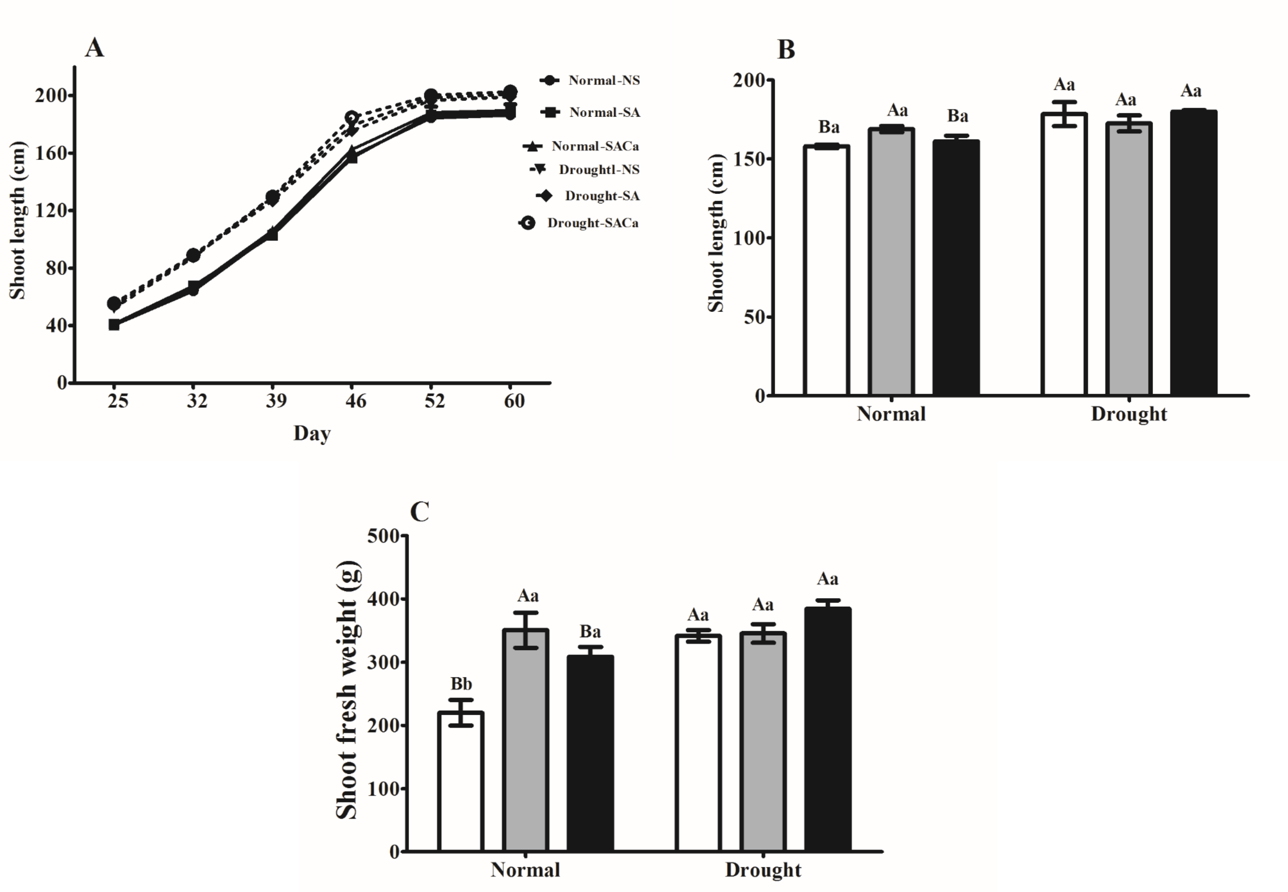

Figure 03: **Corn growth for 65 days of experiment (A), shoot length (B) and shoot fresh weight (C) of corn receiving different plant growth regulators and irrigation regimes in acidic soil. Symbols: untreated, SA application, SA + $CaCl_2$ application**

Effect of SA and Calcium on Corn Shoot Length and Fresh Weight

SA and SA + $CaCl_2$ did not clearly affect the shoot growth of corn (Figure 03B). Under normal irrigation, SA and SA + $CaCl_2$ application did not increase the corn shoot length but did increase the shoot fresh weight of the corn so it was 308.74 - 350.39 g, while the shoot fresh weight of untreated corn was 219.81 g (Figure 03C). Under the drought irrigation regime, SA and SA + $CaCl_2$ application did not increase the shoot length and shoot fresh weight when compared with the untreated corn. However, the shoot length and fresh weight of corn under the normal irrigation regime were lower than those for the corn grown under the drought irrigation regime, except for the corn with SA applied, under which the shoot length and shoot fresh weight were not significantly different between the plots.

SA is an important plant growth regulator that induces plant growth under stress. In this experiment, 0.01 mM SA increased only the shoot fresh weight of corn under normal irrigation. Differentiation of corn plants was seen between the irrigation plots. However, SA was found to increase corn yield under normal irrigation. There have been several reports showing that exogenous SA application could increase plant growth under several stresses. Foliar application of 2 mM SA to *Ammi visnaga* L. in combination with drought irrigation increased the polyphenol content and antioxidant activity in plants. In addition, exogenous salicylic acid priming to *Leymus chinensis* seeds enhanced the distribution of endogenous abscisic acid, gibberellin and indole-3-acetic acid in the germinating seeds under salt-alkali conditions (Chen *et al*. 2021). Foliar pretreatment with 0.5 mM SA to zoysiagrass (*Zoysia japonica* Steud.) before exposure to drought stress enhanced the net photosynthesis, anti-oxidant enzyme activity and decreased the lipid peroxidation in plants (Chen *et al*. 2014). Increased plant yields after application of SA have also been reported in tomato. Foliar application of 150 mg L^{-1} SA to grape tomato grown under 75% and 100% water field capacities produced their highest yields at the same level and 100 mg/l SA could induce grape tomato grown under 100% water field capacity to produce the highest yield (Chakma *et al*. 2021). These may involve SA inducing antioxidant systems in the corn that have been reported in corn pre-treated with SA and then exposed to drought stress (withholding water for 17 days) (Saruhan *et al*. 2012). When corn was not stressed with any radicals, the yield reductions in the corn were alleviated.

Effect of SA and Calcium on Ears

SA and $CaCl_2$ clearly increase the quality of the corn ears. Under the normal irrigation regime, application of SA or SA + $CaCl_2$ increased the ear weight with coat, ear weight without coat, ear length, ear diameter and number of seeds per row when compared with corn without any applications. Ear weight with coat, ear weight without coat, ear length, ear diameter and number of seeds per row of corn with SA and SA+ $CaCl_2$ applied were 352.60 – 356.32 g, 276.67 – 306.94 g, 24.42 – 25.44 cm, 6.02 – 6.11 cm and 41.88 – 42.07 seeds, respectively, while the ear weight with coat, ear weight without coat, ear length, ear diameter and number of seeds per row for untreated corn were 265.37 g, 230.0 g, 20.16 cm, 5.46 cm and 38.07 seeds, respectively (Table 02). However, under the drought irrigation regime, ear weight without coat, ear length and number of seeds per row for treated and untreated corn were not significantly different. Only applying SA + $CaCl_2$ could increase the ear diameter to 6.76 cm, which was significantly greater than under other treatments, and could increase the ear weight with coat to 456.53 g, which was higher than the untreated corn (Table 02). When compared between plots, ear weight with coat, ear weight without coat and ear diameter of corn under the drought irrigation regime were higher than for corn under the normal irrigation regime. SA application only decreased the ear length of corn under the drought irrigation regime when compared with the normal irrigation regime. Seeds per row for untreated corn ears grown under normal irrigation were lower than seeds per row for untreated corn ears under the drought irrigation regime (Table 02).

Table 02: Ear yield of corn grown under different irrigation regimes and sprayed with different treatments

Treatment	Ear length with coat (cm)	Ear diameter with coat (cm)	Ear weight with coat (g)	Ear weight without coat (g)	Rows per ear	Seeds per row
Normal						
NS	20.16 ± 0.64Ab	5.46 ± 0.07Bb	265.37 ± 4.70Bb	230.00 ± 9.43Bb	15.13 ± 0.33Aa	38.07 ± 1.79Bb
SA	25.44 ± 0.51Aa	6.02 ± 0.07Ba	352.60 ± 16.35Ba	306.94 ± 17.60Ba	16.44 ± 0.33Aa	41.88 ± 0.65Aa
SA + Ca	24.42 ± 1.10Aa	6.11 ± 0.03Ba	356.32 ± 15.66Ba	276.67 ± 9.81Ba	15.47 ± 0.30Aa	42.07 ± 0.63Aa
Drought						
NS	21.14 ± 0.22Aa	6.48 ± 0.02Ab	402.09 ± 6.03Ab	375.33 ± 2.37Aa	15.67 ± 0.22Aa	43.87 ± 0.48Aa
SA	21.55 ± 0.14Ba	6.55 ± 0.06Ab	418.52 ± 14.42Aab	393.33 ± 11.52Aa	16.20 ± 0.41Aa	43.13 ± 0.55Aa
SA + Ca	22.43 ± 0.28Aa	6.76 ± 0.02Aa	456.53 ± 6.44Aa	405.33 ± 7.20Aa	15.73 ± 0.22Aa	44.33 ± 0.71Aa
Irrigation	*	**	**	**	ns	**
PGR	**	**	**	**	ns	ns
Irrigation*PGR	*	**	ns	ns	ns	ns

Figure 04: Ear with coat before removal from shoot of corn receiving different plant growth regulators and irrigation regimes in acidic soil for 65 days. Normally irrigated plot: untreated (A), SA application (B), SA + $CaCl_2$ application (C); drought irrigated plot: untreated (D), SA application (E), SA + $CaCl_2$ application (F).

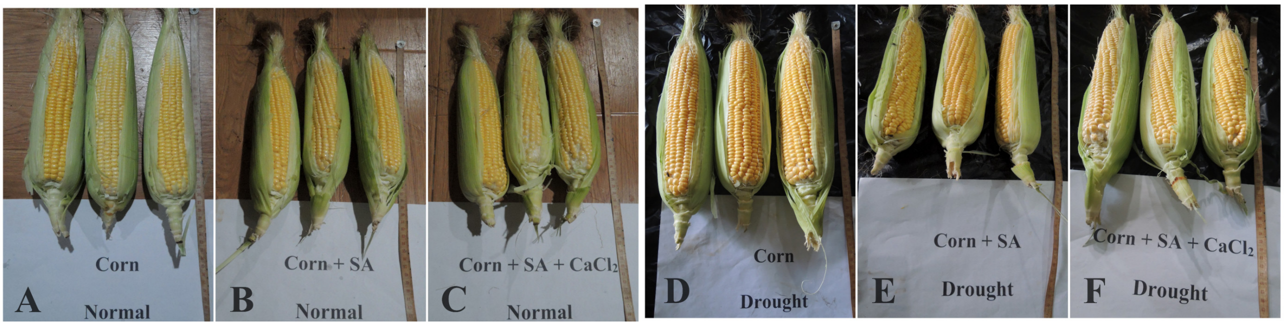

Figure 05: Ear yield of corn receiving different plant growth regulators and irrigation regimes in acidic soil for 65 days. Normally irrigated plot: untreated (A), SA application (B), SA + $CaCl_2$ application (C); drought irrigated plot: untreated (D), SA application (E), SA + $CaCl_2$ application (F).

The corn yield from the drought irrigation plot was improved only by SA + $CaCl_2$ application for ear diameter and ear weight with coat. Calcium application has been reported to increase plant yield. Foliar application of 40 mg/l Ca^{2+} improved the grain yield and quality of corn under water stress (Naeem *et al*. 2018). However, co-application of SA and $CaCl_2$ has been reported only under salt stress. SA and $CaCl_2$ application could alleviate salt stress in olive (*Olea europeae*). Barley pretreated with 50 mM $CaCl_2$ showed an increase in chlorophyll and carotenoid contents under drought stress (Kaczmarek *et al*. 2017). One year-old olives planted in sand had 200 mM NaCl applied after a foliar application of 0.5 – 1.0 mM SA when also adding 10 – 20 mM $CaCl_2$. SA (1.0 mM) pretreatment could decrease the Na+ transport to leaves while 1.0 mM SA and 10 mM $CaCl_2$ pretreatment could increase the dry weight, photosynthesis activity and non-enzymatic anioxidant activity (Methenni *et al*. 2018). There have been reports that co-application of SA and zinc to peppermint (*Mentha piperita* L.) increased the menthol and menthone yields under a moderate drought condition (Jahani *et al*. 2021).

CONCLUSIONS

Corn grew well in both normal and drought irrigation plots, but shorter corn shoots were seen in the normally irrigated plot where the soil was more acidic than the drought irrigated plot. Salicylic acid could improve the corn yield and shoot fresh weight in the normal irrigation plot. Both salicylic acid and $CaCl_2$ did not affect the shoot growth of corn but did increase the ear weight with coat and ear diameter of corn in the drought irrigation plot.

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