Australian Journal of Crop Science AJCS

AJCS 13(03):412-417 (2019) ISSN:1835-2707
doi: 10.21475/ajcs.19.13.03.p1335

The morphological, physiological and biochemical responses of sweet corn to foliar application of amino acids biostimulants sprayed at three growth stages

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Abstract

Corn (*Zea mays* L.) is one of the most important cereal crops in many countries due to its high nutritive value and a raw material for many industrial products. This research was conducted to determine the optimum concentration of the foliar application of amino acids biostimulants at appropriate growth stage for achieving more efficient use of the foliar application on sweet corn (*Zea mays saccharata)* var. merkur. The foliar application used in this study was the commercial product (Perfectose^{TM}, liquid) which is a well known product and commonly used for different crops in Jordan. The experiment was conducted at the greenhouse during the summer of 2015. Corn plants were sprayed with four different concentrations: 1, 2, 3 and 4ml/L at three different growth stages: 7th leaf, tasseling and milk stage, while the control plants were sprayed with distilled water only. The morphological characteristics (plant height, root length and ear length), physiological characteristics (leaf relative water content and leaf water potential), and biochemical characteristics (chlorophyll content, nitrogen, phosphorus, potassium and protein content) were recorded during the experiment. The results indicated that the corn plant height, leaf water potential, chlorophyll content, nitrogen, phosphorus, potassium and protein content were significantly affected by the foliar application treatments, while the rest of the parameters studied were not significant. The treatments 4ml/L at milk stage and 1ml/L and 2ml/L at 7th leaf stage were most effective in the majority of parameters studied. It can be concluded that using foliar application of amino acids biostimulants could positively enhance sweet corn morphological, physiological and biochemical characteristics.

Keywords: *Zea mays saccharata*; foliar fertilization; amino acids; biostimulants.
Abbreviations: µg_microgram; Conc._concentration of the foliar application of amino acids biostimulants; g_gram; LSD_least significant difference; mg_milligram; ml/L_milliliter per litter; MPa_mega pascal; NS_not significant; P≤0.05_probability value is equal or less than 0.05; SAS_ Statistical Analysis System.

Introduction

Corn (*Zea mays* L.) is an important cereal crops grown in many countries and used mainly for human food, livestock consumption, food processing and raw material for many industrial products (Jimenez-Lopez, 2012). Like other plants, corn growth and development is greatly associated to the availability of different nutrients. Therefore, providing plants with amino acids and nutrients elements (macro and micro) is very important due to their roles in the whole plants activity and vital processes (Ibrahim et al., 2010; Zlatev and Lidon, 2012).
Amino acids are organic nitrogenous compounds and involve in the synthesis of other organic compounds considered an important component for plant growth stimulation and development (Goss, 1973; Ibrahim et al., 2010). Furthermore, amino acids as well as nutrients elements (e.g. potassium) act as osmolytes that plays a vital role in increasing the concentration of cellular osmotic components. Also, maintaining water cellular balance and increase chlorophyll content, antioxidants activity, cell membrane integrity and the absorption of different nutrients by roots (Rai, 2002; El-Ghamry et al., 2009; Ardebili et al., 2012; Hammad and Ali, 2014).
The foliar application of amino acids and nutrient elements as a mixture was found to have a positive effects on one or more plant morphological, physiological and biochemical characteristics (Fageria et al., 2009; Abdel-Mawgoud et al., 2011; Fawzy et al., 2012; Stiegler et al., 2013; Hammad and Ali, 2014; Bernhard, 2016). Habibi et al. (2015) showed that the foliar biostimulants containing amino acids and nutrient elements significantly increase corn plants height, nitrogen content, and chlorophyll content. Also, Hammad and Ali (2014) found that using the foliar application of amino acids on wheat grown under irrigation and drought conditions significantly increased wheat plants height, leaves number, shoot and root dry weight, number of tillers per plant, leaf relative water content, membrane integrity, and the uptake of nitrogen, phosphorus and potassium.
The plant response to the nutrients applied, either in a form of foliar or soil application, can be optimized at the right time, right source, right rate and right place, which are known as 4R approaches (Bruulsema et al., 2012; Bernhard, 2016). Therefore, this research was designed to insight these approaches. In addition, researches which studied the effect of the foliar application of amino acids biostimulants

sprayed at different growth stages of sweet corn were a few. So, the objectives of this research was to (1) study the effect of the foliar application of amino acids biostimulants on the morphological, physiological and biochemical characteristics of sweet corn (*Zea mays saccharata*) var. merkur; (2) determine the optimum foliar application concentration at appropriate growth stage of application for achieving more efficient use of the foliar application on corn.

Results and Discussion

Growth characteristics

The different foliar application concentrations sprayed at different growth stages affecting corn plant height presented in Fig 1. All over the treatments, the results were significantly noticed by plants treated with 2ml/L at 7^{th} leaf stage (109.41cm) followed by 1ml/L at the same stage (108.75cm) and 4ml/L at milk stage (108.75cm) compared to the tasseling stage were plants treated with 2ml/L (100.19cm) that scored the lowest plant height. While, leaves number, root length, root/shoot length ratio and ear length measurements were not significantly different between treatments. In this matter, El-Ghareib et al. (2014) reported that foliar amino acids treatments increased corn plants height compared to the control and the trend of increase were higher when the foliar amino acids mixed with nutrients. Similarly, Hammad and Ali (2014) showed that the majority of treated wheat plants (*Triticum aestivum* L.) with foliar application of amino acids under irrigation and drought conditions had higher plant height comparing to the control in both seasons. The positive effect of amino acids on plant can be attributed to the observable increase in the chlorophyll content (Table 3), thus the photosynthetic rate increased which improves plant metabolism and protein assimilation which are necessary for cell formation and consequently increase fresh and dry mater (Basha and El-Aila, 2015; Sadak et al., 2015).

Physiological parameters

The different foliar application concentrations sprayed at different growth stages on corn showed no significant effect on the leaf relative water content. However, plants treated with 2ml/L at 7^{th} leaf and 4ml/L at milk stage showed a slight increase in the leaf relative water content compared to other treated plants including the control.
The results, presented in Fig 2, showed that the foliar application at different growth stages was significantly affected the leaf water potential. The results showed that all treated plants with the foliar application had higher (more negative) leaf water potential compared to the control except those treated at 7th leaf stage. However, the plants treated with 4ml/L at milk stage scored the highest leaf water potential value. These results can be explained based on a well known factor that the accumulation of solutes in the plant cells causes an increase in their leaf water potential (become more negative) and leading to an increment in osmotic potential which consequently attracts water into the cell and tends to maintain turgor pressure (Zlatev and Lidon, 2012).

Biochemical analyais

Results in Table 1 revealed that the majority of treated plants with the foliar application had higher chlorophyll content compared to the control with superiority to 4ml/L at milk stage (5.018mg) and 1ml/L at 7th leaf stage (4.350mg) for chlorophyll a and 4ml/L at tasseling and milk stage (14.063 and 12.695mg) respectively for chlorophyll b. Our findings coincide with the earlier findings of Habibi et al. (2015) on corn and Dromantienė et al. (2015) on winter wheat. The increment in chlorophyll contents might be due to the availability of higher rates of amino acids in the treated plants as amino acids help to increase the chlorophyll content and consequently increase the photosynthetic activity (Awad et al., 2007). Also, photosynthetic pigments are also affected by minerals such as iron, copper and manganese (Kirkby and Römheld, 2004) that were existed in the foliar application used in this study.
The plant minerals content (Table 2) was variable all over the foliar applications treatments and did not follow a specific pattern for the comparison since each stage had its special status. The results showed that the nitrogen content was significantly increased in plants treated with 4ml/L at milk and 2ml/L at 7^{th} leaf stage while the lowest nitrogen content was recorded by plants treated with 2ml/L at tasseling and milk stage respectively. The phosphorus content increased significantly in treated plants under 4ml/L at tasseling followed by 1ml/L at 7^{th} leaf and 4ml/L at milk stage respectively. The potassium content was higher in the majority of treated plants at different stages of application and the plants treated with 3ml/L at milk scored the highest value compared to the other treatments.
The increase of the mineral content (nitrogen, phosphorus and potassium) of treated corn plants during the experiment may be due to the positive effect of the foliar application by providing the plant cells a readily source of nutrient elements as well as increasing their uptake by roots and their accumulation and translocation in leaves (Rai, 2002; Liu et al., 2007). Thom et al. (1981) pointed out that nitrogen provided by amino acids is more available and a rapid source for plant cells to take than inorganic nitrogen.
The foliar application treatments showed a significant effect on protein content in corn plants, presented in Table 2. The protein content ranged from 4.45 to 6.94% and the maximum protein content was observed when plants treated with 2ml/L at 7th leaf and 4ml/L at milk stage respectively. The lowest protein content was observed when plants treated with 2ml/L at tasseling stage. A study of El-Ghareib et al. (2014) reported that treated corn plants under irrigation with foliar amino acids showed significantly higher protein content than the control. Hammad and Ali (2014) presented similar responses.

Materials and methods

Plant material

The seeds used in this study are hybrid sweet corn (*Zea mays saccharata*) var. merkur that produced by Monsanto Company. According to the producer, this variety is suitable

Table 1. Chlorophyll a and b of corn plants treated with the foliar application at three growth stages (7th leaf, tasseling and milk).

Conc.	Chlorophyll a (mg/g fresh weight)			Chlorophyll b (mg/g fresh weight)		
	7th leaf	Tasseling	Milk	7th leaf	Tasseling	Milk
Control	2.825^{cd}	2.825^{cd}	2.825^{cd}	9.653^{c-e}	9.653^{c-e}	9.653^{c-e}
1 ml/L	4.350^{ab}	3.783^{a-c}	3.028^{b-d}	11.079^{b-d}	11.542^{a-d}	11.964^{a-c}
2 ml/L	3.239^{b-d}	3.699^{a-d}	3.750^{a-c}	10.663^{b-e}	11.264^{b-d}	10.969^{b-d}
3 ml/L	3.121^{b-d}	2.803^{cd}	3.368^{b-d}	9.548^{c-e}	8.952^{de}	10.556^{b-e}
4 ml/L	2.357^{d}	4.275^{ab}	5.018^{a}	8.199^{e}	12.965^{ab}	14.063^{a}
LSD	1.356			2.645		

Means followed by the same letters do not differ by the LSD test, at P ≤0.05.

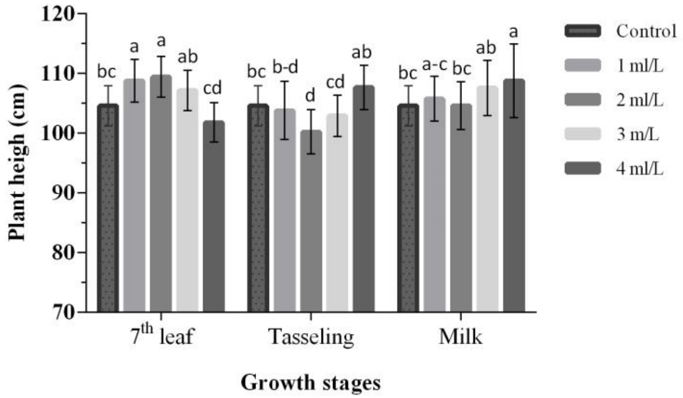

Fig 1. Corn plants height treated with the foliar application of amino acids biostimulants at three growth stages. Columns with similar letters do not differ by the LSD test, at P ≤0.05.

Table 2. Minerals content of corn plants after treatment with the foliar application at three growth stages (7th leaf, tasseling and milk).

Conc.	Nitrogen (%)			Phosphorus (%)			Potassium (mg/g)			Proteins content (%)		
	7th leaf	Tasseling	Milk	7th leaf	Tasseling	Milk	7th leaf	Tasseling	Milk	7th leaf	Tasseling	Milk
Control	0.80^{cd}	0.80^{cd}	0.80^{cd}	5.01^{cd}	5.01^{cd}	5.01^{cd}	20.67^{b-d}	20.67^{b-d}	20.67^{b-d}	5.01^{cd}	5.01^{cd}	5.01^{cd}
1 ml/L	0.83^{b-d}	0.89^{bc}	0.79^{cd}	5.17^{b-d}	5.17^{b-d}	5.17^{b-d}	23.70^{ab}	18.86^{d-f}	22.85^{a-c}	5.17^{b-d}	5.58^{bc}	4.96^{cd}
2 ml/L	0.95^{b}	0.71^{d}	0.78^{cd}	5.92^{b}	5.92^{b}	5.92^{b}	19.67^{c-f}	16.92^{ef}	20.80^{a-d}	5.92^{b}	4.45^{d}	4.89^{cd}
3 ml/L	0.81^{cd}	0.79^{cd}	0.81^{cd}	5.03^{cd}	5.03^{cd}	5.03^{cd}	21.90^{a-d}	16.44^{f}	24.17^{a}	5.03^{cd}	4.97^{cd}	5.06^{cd}
4 ml/L	0.83^{b-d}	0.77^{cd}	1.11^{a}	5.19^{b-d}	5.19^{b-d}	5.19^{b-d}	20.02^{c-e}	20.98^{a-d}	21.85^{a-d}	5.19^{b-d}	4.83^{cd}	6.94^{a}
LSD	0.13**			0.60**			3.39*			0.84**		

Means followed by the same letters do not differ by the LSD test, at P ≤0.05.

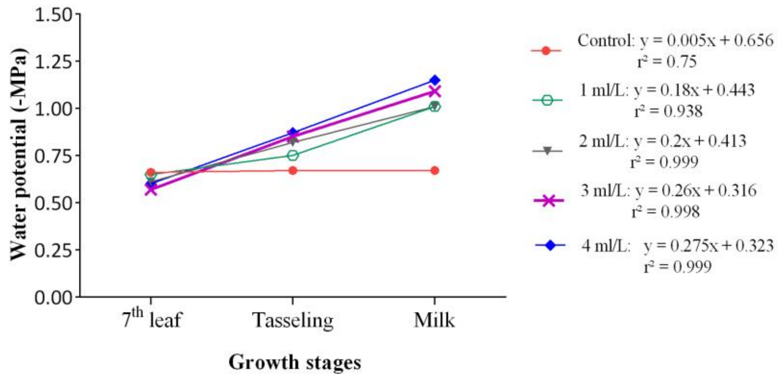

Fig 2. Leaf water potential of corn plants treated with the foliar application at three growth stages.

Table 3. The chemical analysis of the media.

Total Nitrogen (%)	Phosphorus (mg/g dry weight)	Potassium (µg/g dry weight)	pH
0.17	5.70	406.22	6.60

Table 4. The chemical composition Perfectose™.

Constituent	Value (%)	Amino acid	Value (gm/100g protein)	Amino acid	Value (gm/100g protein)
Nitrogen (N)	2 – 3	L-Alanine	4.3	L-Leucine	8.2
Magnesium (Mg)	0.50	L-Arginine	7.6	L-Lysine	6.3
Copper (Cu)	0.25	L-Aspartic Acid	11.6	L-Methionine	1.3
Manganese (Mn)	1.00	L-Cysteine	1.3	L-Phenylalanine	5.2
Iron (Fe)	0.25	L-Glutamic Acid	19.1	L-Proline	5.1
Zinc (Zn)	2.80	L-Glycine	4.2	L-Serine	5.2
Boron (B)	0.025	L-Histidine	2.6	L-Threonine	3.7
Total amino acids	27.30	L-Isoleucine	4.6	L-Tryptophan	1.4

for tropical and warm climatic conditions, has a good tolerance to diseases and can be grown on a wind range of soils. The purity of this variety is 99% and the germination is 96%.

Preparations

Uniform and equal size sweet corn seeds were planted at a rate of three seeds per 4 liter pot containing a mixture of soil: perlite: peat moss in a volume ratio of 1:1:1. The chemical analysis of the media is shown in Table 3. The plants were maintained at field capacity (well watered) during the experiment. The weight of soil water content at field capacity was calculated as the difference between the soil weight after drainage and soil weight after oven drying at 105°C for 24 hours. Two weeks after planting, the seedlings were thinned to one seedling per pot.

Foliar application treatments

The source of amino acids biostimulants used in this study was a commercial product (Perfectose™, liquid). The Perfectose™ is a well known product which is commonly used on different crops in Jordan. The chemical constituents of the product are presented in Table 4. The spraying of the foliar application was carried out at the early morning by using a hand sprayer in order to increase the delicacy of the spraying.

This experiment includes twelve treatments in addition to control plants. Each foliar concentration was applied with four concentrations at three plant stages. Treatment 0 in which plants were sprayed with distilled water only (control). Treatments 1 to 4: plants were sprayed at 7th leaf stage with 1, 2, 3 and 4ml/L respectively. Treatments 5 to 8: plants were sprayed at tasseling stage with 1, 2, 3 and 4ml/L respectively. Treatments 9 to 12: plants were sprayed at milk stage with 1, 2, 3 and 4ml/L respectively.

Measurements and data recorded

The morphological measurement of corn plants height, root length and ear length was performed using a measuring tape (±0.1mm), while, the number of leaves was counted manually.

The leaf relative water content was determined according to the method developed by Barrs and Weatherley (1962). The leaf water potential (Ψ_w) was measured by using a pressure chamber instrument (model 1000, PMS Instrument Company) according to the method of Scholander et al. (1965) with slight modifications instructed by PMS Instrument Company for corn plants (PMS, 2008). A fully expanded and healthy leaf was taken from each replicate using a scissor to make a strait clean cut across the leaf after sliding a plastic bag over the leaf in which the tip of the leaf was slightly rolled at the bottom of the bag. Then, the bag and the leaf together were placed inside the chamber after pulling down each side of the leaf cut in which the central rib was isolated and did not exceed the maximum width of the grass seal. Readings were recorded immediately when water comes out across the cut. Leaf chlorophyll a and b were determined according to the method proposed by Arnon (1949). The determination of the whole plant total nitrogen content was carried out using the Kjeldahl method as describe by AOAC (1995). Potassium content was obtained by the dry-ashing method as describe by AOAC (1995). Total phosphorus was determined by dry-ashing procedure using spectrophotometer method as described by Homer and Pratt (1961). The protein content of the dry plant samples was calculated by multiplying the nitrogen percentage by the factor of 6.25 as described by Ferguson and Terry (1954).

Experimental design and statistical analysis

The experimental design was completely randomized block design (CRBD) with split-plot arrangement and four replications per treatment, where the growth stages assigned in the main plots and foliar application treatments were in the subplots. Data were statistically analyzed using general linear model (GLM) analysis with the statistical software package SAS version 9.0 (2002). The differences among the means of different treatments were tested using the Least Significant Differences (LSD) at probability 5% ($P \leq 0.05$).

Conclusion

This study was designed to investigate the effect of the foliar application of amino acids biostimulants on the morphological, physiological and biochemical characteristics of corn plants (*Zea mays saccharata*) var. merkur. From the results, we conclude that corn plants sprayed with the foliar application of amino acids biostimulants with 4ml/L at milk followed by 1 and 2ml/L at 7th leaf stage granted the best results for the majority of corn parameters studied in this research. We believe that providing plants with amino acids biostimulants through leaves enhances corn plants characteristics and consequently their growth and development. Further studies are needed to evaluate the foliar application of amino acids biostimulants in the cellular level for fully understanding its effects and mode of action. As well as, by focusing on the effect of foliar application on corn under other environmental conditions such as heat stress and salinity to explore the effectiveness of the foliar application under these conditions.

Acknowledgements

We would like to thank Jordan University of Science and Technology (JUST) represented by the deanship of research for funding this research.

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