Journal of Ecology of Health & Environment
An International Journal

http://dx.doi.org/10.18576/jehe/040105

Improvement of Maize Crop Yield (*Zea mays* L.) by using of Nitrogen Fertilization and Foliar Spray of Some Activators

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Received: 20 Oct. 2015, Revised: 19 Dec. 2015, Accepted: 25 Dec. 2015.
Published online: 1 Jan. 2016.

Abstract: Two field experiments in split-split plot design were carried out at the Agriculture Research Center, Shandweel Research Station, Sohag Governorate, during the summer seasons of 2010 and 2011 for 120 days to evaluate the effect of salicylic acid spraying (SA: 100 mg/L at 30 and 45 days from sowing) under three levels of nitrogen fertilizer (N: 0, 90, and 120 Kg N/feddan) on the vegetative growth and grain yield of two maize hybrids (Single cross Pioneer 30K09 and Three way cross-310). The results indicated that, there were gradual and significant increases in all growth parameters and grain yield resulted from foliar spray by SA and raising N-level from 0 to 90 and 120 kg N/fed. in both seasons. The S.C Pioneer 30K09 maize hybrid treated with 120 N/Fed. and sprayed by SA gave the maximum values of plant height, leaf area (LA), leaf area index (LAI), net assimilation rate (NAR), crop growth rate (CGR), total carbohydrates, proline and grain yield in both seasons. Photosynthetic rate (A), transpiration rate (E), stomatal conductivity (GS) and intracellular CO_2 concentration (Ci) were influenced by application of SA along with N.Generally, growing of S.C. Pioneer 30K09 fertilized with 90 or 120 N/fed. and applied 100 mg/L of SA recommended to get the maximum growth parameters and grain yield (ardab/feddan).

Keywords: Chlorophyll, Foliar spray, Leaves, Maize hybrids, Nitrogen fertilizer, Salicylic acid.

1 Introduction

Maize (*Zea mays* L.) is the third most important cereal crops in the world after wheat and rice and known as "King of grain crops" (Tollenaar and Dwyer, 1999). It is grown principally during the summer season in Egypt and considered as one of the most important cereal crops used in human consumption, animal feeding, starch industry and oil production (Amin *et al.*, 2007). Maize hybrids differed in its productivity as well as its response to nitrogen fertilization, also, growth parameters were affected by maize hybrids (Chaudhry *et al.*, 2005 and Sharifi and Taghizadeh, 2009). S.C-10 hybrid surpassed the T.W.C-310 hybrid in plant height, number of green leaves/plant and ear leaf area/plant (Moharram, 2011). In contrast, T.W.C-310 was superior S.C-10 hybrid in most growth characters (Mansour, 2009). There are differential response of maize hybrids regarding to leaf area index, leaf area duration, net assimilation rate and crop growth rate (Liu *et al.*, 2004; Luque *et al.*, 2006 and Azadgoleh and Kazmi, 2007). Maize varieties were differed in their grain yield (Ahmed, 2011). S.C. Pioneer 30K09 hybrid surpassed the T.W.C-310 hybrid in maize grain yield (El-Sheikh, 1998; Oraby *et al.*, 2005). In contrast, T.W.C-310 hybrid was superior to the S.C.-10 hybrid in grain yield (Abd El-Maksoud and Sarhan, 2008). There are no variation (Ding *et al.*, 2005 and Forrai *et al.*, 2012) in stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) between species and genera (species of *Tilia*, *Acer* and *Fraxinus* genera). While, all cultivars exhibited large seasonal variations of gas exchange parameters.

Nitrogen fertilizer greatly effect on vegetative growth, maize yield as well as grain quality (Ahmed, 1998). Nitrogen application had pronounced effect in increasing vegetative growth of crop plants (Khan *et al.*, 1999). Application of 120 kg/fed gave significant increases in number of leaves/plant, plant height and ear leaf area/plant (Bamuaafa, 2012). Nitrogen application (Namakka *et al.*, 2012) increased growth analysis such as crop growth rate and leaf area index. Photosynthetic pigments in maize plants was improved by nitrogen application, however, chlorophyll a/b ratio was decreased (Akram, 2014). Chemical constituents of corn grains as carbohydrate and oil concentrations are significant increase by nitrogen application (Ibrahim and Kandil, 2007) also, enhanced the amino acid formation and proline content (Ali *et al.*, 1999). Nitrogen application improved maize yield (Mohamed *et al.*, 2000; Shirazi *et al.*, 2011 and El-Mekser *et*

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al., 2015).

Continuous use of synthetic nitrogenous fertilizers is not only polluting the water resource rather is toxic to human as well as for animal life (Cheema *et al.,* 2010). Moreover, sole use of chemical fertilizers is causing deterioration in soil physico-chemical and biological properties. The applied N is not all taken by crop plant; a large proportion is lost due to ammonia volatilization, denitrification and leaching (Zhang *et al.,* 2009). There are various approaches to be used to enhance the crop productivity, one of them is exogenous application of plant growth promoting substances (PGPS) *vis*; salicylic acid (Farahat *et al.,* 2007).

Salicylic acid (2-hydroxybenzoic acid) as a natural plant hormone (Khan *et al.,* 2010) is an internal regulator of phenolic nature and act as a potential non enzymatic antioxidant (Faheed, 2012). The effectiveness of foliar spray of SA on the physiological processes is variable depending on its concentration, plant species, developmental stages and environmental conditions (Bidabadi *et al.,* 2012). Salicylic acid improves plant performance through modulation of its growth and yield of maize (Chattha *et al.,* 2015). An exogenous application of SA improves germination (Khan *et al.,* 2003 and Hayat *et al.,* 2010) enhance plant growth (Khan *et al.,* 2012; Mahdi *et al.,* 2013). Vegetative growth (Ligia Acatrinei, 2010) leaf area index (Kvet *et al.,* 1971) dry weight/plant (Khan *et al.,* 2003 and Tufail *et al.,* 2013) leaf area duration, crop growth rate and net assimilation rate (Clawson *et al.,* 1986) were affected by foliar spray of SA in the maize genotypes. SA enhanced triggered chlorophyll biosynthesis (Radwan and Soltan, 2012 and Chattha *et al.,* 2015), but it also decreases the amount of chlorophyll pigments and chlorophyll a/b in wheat and mung plant (Moharekar *et al.,* 2003). Exogenous applications of SA participate in regulation of several physiological processes in plants, such as stomatal closure, ion uptake and transport, and transpiration in crops (Khan *et al.,* 2012), also enhances photosynthesis and photosynthetic rate (Noreen and Ashraf, 2008). Also, it is meliorates the growth of crop and nutrient content in maize plants were enhanced (Khan *et al.,* 2010) and played positive role in increasing the yield of many cereals including wheat and maize at lower concentration (Khan *et al.,* 2003 and Mahdi *et al.,* 2013). Several studies considered in aspect of leaf gas exchange. Foliar spray of SA may regulate stomatal openings (Najafian *et al.,* 2009) enhanced photosynthetic rate, transpiration rate, stomatal conductance and sub-stomatal CO_2 concentration (Tufail *et al.,* 2013). Biochemical analysis showed that foliar spray of different growth substances enhanced total sugar contents (Farahat *et al.,* 2007) total free amino acids and proline in shoots of maize plants (El-Khallal, *et al.,* 2009) and seeds oil concentration (Metwally *et al.,* 2003).

Therefore, the present investigation was undertaken to study the impact of foliar spraying of salicylic acid under three levels of nitrogen on some morphological criteria, physiological activities, some biochemical constituents as well as yield of two maize hybrids.

2 Materials and Methods

2.1 Growth conditions and experimental design

A field experiment was carried out during summer seasons of 2010 and 2011 at Shandweel Research Station, Agriculture Research Center, Egypt. The experiments laid out in split-split plot design with three replications having 72 unit plots of 10 m^2 size. Two maize hybrids were planted (Single cross Pioneer 30K09 and Three way cross-310) and treated with chemical fertilizer (NPK). Nitrogen fertilizer added in the soil as urea at a rate of 0, 90 and 120 Kg N/fed. in two equal proportions, the 1st half at 30 and the 2nd at 45 days after sowing. Phosphorus fertilizer was added as superphosphate at the rate of 15 Kg P_2O_5/feddan before sowing. And potassium as potassium sulfate K_2SO_4 was added to the soil before sowing at the rate of 24 Kg K_2O/feddan. Plants were manually sprayed with salicylic acid at the rate of 100 mg/l at two growing stage, 30 and 45 days after sowing. While, control sprayed by water. Vegetative growth parameters and photosynthetic pigments were recorded two times at 60th and 80th days from sowing, while, gaseous exchange parameters were recorded at 60th days only. Leaves of plants at 60th days from sowing were dried, to determine the biochemical analysis.

2.2 Soil analysis

Surface soil samples (0-30 cm) were collected before planting from the experimental sites in both seasons for physical and chemical characterizations:-

A. Physical analysis: For mechanical analysis, Particles size distribution was determined according to the International Pipette Method A. (Piper, 1950).

B. Chemical analysis: Calcium carbonate was determined by the calcimeter method according to Williams (1949). Soil pH was measured in a 1: 2.5 (soil:water) suspension. Organic matter content was determined following the Walkely and Black's rapid titration method, modified by Walkely (Jackson, 1958). Total nitrogen, was determined in the soil samples using micro-kjeldahl method (Jackson, 1967). Available phosphorus, was extracted by 0.5 M $NaHCO_3$ (Olsen *et al.,* 1954) and determined by the stannous chloride phosphomolybdic acid method (Jackson, 1958). Available potassium was determined flamephotometrically in 1 N ammonium acetate extract according to Jackson (1958). Soil physical and chemical analyses of the experimental sites for the two seasons are presented in table (1).

2.3 Plant materials Analysis:

- **Vegetative growth**

. Plant height (cm)/plant, was determined on the basis of

Table (1): Soil characterization for the experimental sites.

Seasons	*Texture*	$CaCO_3$ %	Soil pH	O.M%	Available nutrients in soil (ppm)		
					N	P	K
2010	Sandy loom	7.50	7.9	0.6	13	18	12
2011	Sandy loom	7.55	7.7	0.8	14	17	13

the average of 5 plants from root separation point to the tip of the plant.

. Total dry weight (DW)/plant (g), was measured after drying the plant material to constant weight at 65°C for 48 h**.**

. Leaf area (LA)/plant (cm^2), individual leaf area= Leaf $_L$ X Leaf $_W$ X 0.75 **(**McKee, 1964).

. Leaf area duration, LAD (dyes), was determined by the formula given by Hunt (1978).

$$LAD = (LAI_1 + LAI_2) \times (t_2-t_1) \times 0.5$$

. Crop growth rate (g m^2 day^{-1}), calculated using the following equation (Radford, 1967).

$$CGR = W_2-W_1/T_2-T_1$$

Where, W_1= Total dry matter of plant at time T_1, T_1=First time observation

W_2= Total dry matter of plant at time T_2, T_2= Final time observation

. Net assimilation rate (g m^2 day^{-1}), was determined as described by Radford, (1967). $NAR= (W_2 - W_1)(Log_e L_2 – Log_e L_1) / (L_2 – L_1)(T_2 – T_1)$

. Estimation of photosynthetic pigments; the mean of randomly chosen five plants were recorded using the portable chlorophyll meter (SPAD-502, Minolta, Japan). Chlorophyll content was determined as SPAD unit as described by Minolta (1989).

. Estimation of photosynthesis rate and gaseous exchange parameters, using an Infra Red Analyzer (Li-COR Company Lincoln, NE). Photosynthetic rate, transpiration rate, stomatal conductivity and intracellular CO_2 concentration were measured and calculated using LCi portable systems on field on non damaging plant.

- **Biochemical analysis**

Leaves of plants at 60 days from planting were rapidly washed in the tap water and dried in an oven at 70 ºC to determine to carry out the biochemical analysis. At full maturity, seed samples were taken for biochemical analysis. Total carbohydrates were determined by anthrone sulphuric acid method was used according to Fales (1951). Seed oil content determined by using soxhlet apparatus and petroleum ether as solvent according to A.O.A.C. (1975). Estimation of total free amino acids were extracted from the plant tissues and determined according to the method of Moore and Stein (1948). Proline content carried out calorimetrically according to Bates *et al.,* (1973).

C. Harvest characteristics

At full maturity, Harvest carried out manually after 120 days from sowing, seed yields (ard.fed^{-1}.) were determined. Seed samples were taken for biochemical analysis (total carbohydrates, oil, total free amino acid and proline).

2.4 Statistical analysis

The data were statically analyzed according to Gomez and Gomez (1984), using the computer MSTAT-C statistical analysis package (Freed *et al.,* 1989). Using the least significant difference (L.S.D.) at 5% and 1% for comparison between means of the two maize hybrids.

3 Results

The obtained data are including plant vegetative growth, some physiological activities and some chemical constituents of plant leaves and seeds as well as yield production of two maize hybrids (Single cross Pioneer 30K09 and Tree way cross-310), affected by foliar spray of 100 mg/L salicylic acid along with three levels of nitrogen fertilizer at two growing seasons in 2010 and 2011. The results obtained that (Table 2) vegetative growth parameters were significantly affected by the studied hybrids. It could be noticed that S.C. Pioneer 30K09 hybrid surpassed T.W.C-310 hybrid with respect to plant height. This finding was completely true, at two sampling dates, 60 days (246.97 and 249.52 cm) in 2010 and 2011, respectively, and at 80 days (280.18 and 288.96 cm) in 2010 and 2011, respectively. On the other hand, there are non significant differences between two maize hybrids in the criteria of leaves number/plant, total dry weight/plant and leaf area/plant (Table 2) this findings are true at two sampling dates, in both seasons, except at 80 days in 2010 season, S.C. Pioneer 30K09 hybrid surpassed T.W.C-310 hybrid with respect to total dry weight/plant (293.28 g). Also, it could be detected from the results presented in table (2) that S.C. Pioneer 30K09 hybrid gave the highest value of leaf area index (6.29) at 60 days from planting in 2010 season only, and no significant differences between two hybrids at sampling date of 80 days. Also, we found that S.C. Pioneer 30K09 hybrid gave the highest values of Chl. (46.39 SPAD unit) at 60 days after planting, in 2010, and 48.49 SPAD unit at 80 days after planting, in 2011 (Table 2).

Table (2): Comparison between 2 maize hybrids and 2 sampling dates (60 and 80 days) on vegetative growth parameters, in 2 successive seasons.

Traits / Hybrids	Plant height (cm)		Leaves No.		Total Dry weight (g)		Leaf area (cm^2)		Leaf area index		Chl. (SPAD unit)	
Season	2010											
Sampling date	60	80	60	80	60	80	60	80	60	80	60	80
S.C. Pioneer 30K09	246.97*	280.18**	14.63	15.32	171.86	293.28**	635.15	702.38	6.23*	7.22	46.39*	47.63
T.W.C-310	239.84	265.53	14.72	15.47	168.09	258.20	567.58	693.88	5.61	7.20	45.49	47.38
LSD 0.05	6.36	3.97	NS	NS	NS	5.02	NS	NS	0.51	NS	0.72	NS
LSD 0.01	NS	9.16	NS	NS	NS	11.58	NS	NS	NS	NS	NS	NS
Season	2011											
Sampling date	60	80	60	80	60	80	60	80	60	80	60	80
S.C. Pioneer 30K09	249.52*	288.96*	15.02	15.99	177.36	312.41	559.61	634.14	5.67	6.89	46.80	48.49*
T.W.C-310	237.78	272.98	15.52	15.89	168.02	302.31	566.01	621.20	5.92	6.65	43.59	46.38
LSD 0.05	10.97	6.65	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.54
LSD 0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

***, *, ns: Significant at 1 and 5 % probability levels and non significant, respectively.*

Table (3): Comparison between 2 maize hybrids on growth analysis (60 and 80 days) and crop yield, in 2 successive seasons.

Season	2010				2011			
Traits / Hybrids	Leaf area duration (days)	Net assimilation rate (g m^2 d^{-1})	Crop growth rate (g m^2 d^{-1})	Yield (ard.fed^{-1}.)	Leaf area duration (days)	Net assimilation rate (g m^2 d^{-1})	Crop growth rate (g m^2 d^{-1})	Yield (ard.fed^{-1}.)
S.C. Pioneer 30K09	134.44**	3.45	6.07**	20.79**	125.49	3.88**	6.75	21.56**
T.W.C-310	128.01	3.32	4.51	18.05	125.69	3.71	6.72	18.56
LSD 0.05	0.38	NS	0.32	1.43	NS	0.07	NS	0.71
LSD 0.01	0.87	NS	0.75	2.30	NS	0.15	NS	1.65

***, *, ns: Significant at 1 and 5 % probability levels and non significant, respectively.*

Table (4): Comparison between 2 maize hybrids on gas exchange parameters.

Traits / Hybrids	Photosynthetic rate (P_N) (µmol(CO_2)m^2s^{-1})	Stomatal conductance (g_s) (mol (H_2O)$m^{-2}s^{-1}$)	Transpiration rate (E) (m mol $m^{-2}s^{-1}$)	Intercellular CO_2 Concentration (C_i) (µmol mol^{-1})
S.C. Pioneer 30K09	17.08*	0.184	5.55*	130.90
T.W.C-310	14.85	0.154	4.83	131.56
LSD 0.05	1.30	NS	0.41	NS
LSD 0.01	NS	NS	NS	NS

***, *, ns: Significant at 1 and 5 % probability levels and non-significant, respectively.*

For the growth analysis LAD, NAR and CGR of maize crop as affected by various maize hybrids are depicted in table (3) and showed that, LAD (60-80 DAS) was significantly higher in S.C. Pioneer 30K09 hybrid than T.W.C-310 hybrid with corresponding values of 134.44 and 128.01 days, for two hybrids, respectively in 2010 season and no significant differences between two hybrids in 2011 season. Net Assimilation Rate (60-80 DAS) exhibit highly significant variation among maize hybrids in 2011 season only. S.C. Pioneer 30K09 hybrid highly significantly produced more NAR (3.88 g m^{-2} day^{-1}), in 2011 season, also it was recorded highest significant value (6.07 g m^{-2} day^{-1}) in respect to CGR, in 2010 season only. Moreover, there are significant differences among those two maize hybrids for yield

(ard.fed^{-1}.) in both growing seasons. S.C. Pioneer 30K09 hybrid significantly surpassed T.W.C-310 hybrid by the values 20.79 and 21.56 ard.fed^{-1}. in the 1st and 2nd seasons, respectively.

Data presented in table (4) showed the significant increase in photosynthetic rate (17.08 µmol $m^{-2}s^{-1}$) and transpiration rate (5.55 mol $m^{-2}s^{-1}$) for S.C. Pioneer 30K09 hybrid, and there are no significant differences between two hybrids in respect to stomatal conductance and intercellular CO_2 concentration. Variations in biochemical analysis for maize seeds and leaves showed in table (5), there are no significant differences between two hybrids in seed oil contents in both seasons. The results showed that T.W.C-310 hybrid exhibit significant increase in seed total carbohydrate contents (63.05 and 61.84 %), in 2010 and 2011 seasons, respectively. While, seed proline contents were higher in S.C. Pioneer 30K09 hybrid (2.73 and 2.19 mg g dw^{-1}), in both seasons, respectively. Biochemical analysis of leaves showed that, there are no significant differences between two hybrids in respect to total free amino acid and proline contents in 2010 season only. While, T.W.C-310 hybrid exhibit higher contents of total carbohydrate than S.C. Pioneer 30K09 hybrid (60.34 and 59.70 %), in both seasons, respectively.

Concerning the effect of foliar spray of salicylic acid and their interaction with nitrogen fertilizer levels on vegetative growth parameters, data tabulated in table (6 and 7) showed that growth parameters of maize plant highly significant affected by all treatments of foliar sprayed of SA under N fertilizer compared with control, in two sampling dates, in both seasons. Also, it could be noticed that the highest values for plant height, leaf area, leaf area index and dry weight were recorded in S.C. Pioneer 30K09 hybrid with application of SA under recommended dose of N fertilizer with no significant differences with SA under 3/4 recommended dose of N fertilizer, at two sampling dates, in both seasons. In respect to total chlorophyll content (Table 6 and 7), the highest values found in S.C. Pioneer 30K09 hybrid sprayed with SA under recommended dose of N fertilizer (51.80 and 52.97 SPAD unit) with no significant differences with SA under 3/4 recommended dose of N fertilizer (49.47 and 51.43 SPAD unit) at two sampling date, respectively, in 2010 season, while, in 2011 season, the values were 50.97 and 53.13 SPAD unit, given by treatment of SA under recommended dose of N fertilizer with no significant differences with SA under 3/4 recommended dose of N fertilizer (49.97 and 52.17 SPAD unit) at two sampling dates, respectively. Also, there are highly significant differences between treatment of SA under recommended dose of N fertilizer and SA under 3/4 recommended dose of N fertilizer compared with control in respect to leaves number/ plant, at 60 days in both seasons, and there are no significant differences between application of SA under recommended dose of N fertilizer and SA under 3/4 recommended dose of N fertilizer, in both hybrids, at two 80 days, in both seasons.

For the growth analysis of maize crop, LAD, NAR and CGR were measured as affected by two maize hybrids, foliar spray of SA and nitrogen fertilizer levels are depicted in table (8). Experiment indicated a highly significant and gradual increase in leaf area duration (LAD), net assimilation rate (NAR) and crop growth rate (CGR) in two maize hybrids by SA foliar application under N fertilizer levels compared with control. Maximum LAD recorded by treatment of SA under recommended dose of N fertilizer with no significant differences with SA under 3/4 recommended dose of N fertilizer, in 2010 season only. Maximum NAR found in S.C. Pioneer 30K09 hybrid foliar sprayed by SA under recommended dose of N fertilizer and 3/4 recommended dose of N fertilizer, in both seasons. While, highest values of CGR were observed in both hybrids when treated by SA under recommended dose of N fertilizer or 3/4 recommended dose of N fertilizer, in both seasons. Periodic data on gas exchange parameters are presented in table (9). Exogenous application of SA under recommended dose of N fertilizer enhanced photosynthetic rate (23.65 µmol (CO_2) m^2s^{-1}), transpiration rate (7.64 mmol $m^{-2}s^{-1}$), stomatal conductance (0.270 mol(H_2O)$m^{-2}s^{-1}$) and sub-stomatal CO_2 concentration (143.98 µmol mol^{-1}) in S.C. Pioneer 30K09 hybrid.

Results given in tables (8) revealed that, foliar spray of SA under N fertilizer levels was most effective to give better yield for S.C. Pioneer 30K09 hybrid and T.W.C-310 hybrids. There are gradual increase in crop yield and highly significant variation between all treatments compared with control. The maximum crop yield found in S.C. Pioneer 30K09 hybrid sprayed with SA under recommended dose of N fertilizer (27.51 and 29.10 ard.fed^{-1}.), in 2010 and 2011 seasons, respectively, with no significant differences with treatments of SA under 3/4 recommended dose of N fertilizer, for both hybrids, in 2010 season only.

All criteria of biochemical analysis *vis;* leaf and seed total carbohydrate contents (Fig. 1, 2), seeds oil contents (Fig. 3), total free amino acid contents in leaf (Fig. 4) as well as leaf and seed proline contents (Fig. 5, 6) exhibit highly significant differences with foliar spray of salicylic acid under nitrogen fertilizer levels compared with the control treatment. T.W.C-310 hybrid foliar sprayed with SA under recommended dose of N fertilizer significantly increased leaf and seed total carbohydrate contents and leaf proline contents, in both seasons. While, proline contents in seed and total free amino acids in leaf were increased by SA application under recommended dose N fertilizer in S.C. Pioneer 30K09 hybrid, in both seasons, with no significant

Table (5): Comparison between 2 maize hybrids on biochemical analysis of seeds and leaves at 60 days, in 2 successive seasons.

Season	2010			2011		
	Seeds					
Traits / Hybrids	Oil contents	Total carbohydrate contents	Proline contents	Oil contents	Total carbohydrate contents	Proline contents
S.C. Pioneer 30K09	5.99	54.50	2.73**	6.57	54.94	2.19**
T.W.C-310	6.31	63.05*	1.40	6.74	61.84**	1.32
LSD 0.05	NS	4.85	0.08	NS	2.88	0.13
LSD 0.01	NS	NS	0.18	NS	6.64	0.30
	Leaves					
Traits / Hybrids	Total free amino acid contents	Total carbohydrate contents	Proline contents	Total free amino acid contents	Total carbohydrate contents	Proline contents
S.C. Pioneer 30K09	2.74	51.67	2.26	3.41	54.65	2.30
T.W.C-310	2.54	60.34*	2.31	4.52*	59.70*	2.52**
LSD 0.05	NS	5.24	NS	0.91	3.69	0.08
LSD 0.01	NS	NS	NS	NS	NS	0.19

***, *, ns: Significant at 1 and 5 % probability levels and non-significant, respectively.*

Table (6): Effect of foliar spray of 100 mg/L of SA under levels of nitrogen fertilizer, on vegetative growth parameters of 2 maize hybrids for 60 days in 2 successive seasons.

Maize hybrids	N levels kg N/fed.	Foliar spray salicylic	2010 season					
			Plant height (cm)	Leaves No.	Total dry weight (g)	Leaf area (cm^2)	Leaf area index	Chlorophyll (SPAD unit)
S.C. Pioneer 30K09	0	Without SA	228.13	13.34	133.51	535.40	4.76	40.10
		With SA	251.17	14.89	173.86	646.14	6.42	47.83
	90	Without SA	237.93	13.89	149.95	609.23	5.64	43.83
		With SA	258.33	15.56	204.42	691.31	7.17	49.47
	120	Without SA	244.62	14.11	159.90	627.84	5.90	45.30
		With SA	261.61	16.00	209.52	700.95	7.49	51.80
T.W.C-310	0	Without SA	220.98	13.56	130.25	495.20	4.47	39.87
		With SA	240.77	14.89	168.10	529.14	5.25	44.27
	90	Without SA	228.63	13.89	142.04	523.37	4.85	43.20
		With SA	254.11	15.44	198.90	632.70	6.52	50.07
	120	Without SA	233.88	14.33	166.24	571.12	5.46	44.77
		With SA	260.00	16.22	203.00	653.97	7.08	50.77
LSD 0.05			**4.50**	**0.58**	**17.75**	**73.98**	**0.78**	**3.22**
LSD 0.01			**6.10**	**0.79**	**24.05**	**100.22**	**1.06**	**4.37**
			2011 season					
S.C. Pioneer 30K09	0	Without SA	222.17	13.78	117.93	414.91	3.82	39.90
		With SA	267.89	14.89	160.01	555.05	5.52	48.03
	90	Without SA	225.17	14.56	142.74	489.88	4.74	45.10
		With SA	269.22	15.56	218.03	635.19	6.61	49.97
	120	Without SA	240.11	14.78	160.37	546.65	5.38	46.8
		With SA	272.56	16.56	265.07	715.99	7.92	50.97
T.W.C-310	0	Without SA	215.22	14.45	110.92	426.14	4.11	37.87
		With SA	250.67	15.01	168.39	578.88	5.79	41.90
	90	Without SA	218.33	14.56	139.37	507.16	4.93	43.73
		With SA	255.72	16.89	206.50	659.38	7.43	46.60
	120	Without SA	229.00	14.90	153.67	545.62	5.42	43.87
		With SA	257.72	17.34	229.26	678.87	7.85	47.60
LSD 0.05			**5.38**	**1.53**	**15.15**	**27.21**	**0.76**	**1.68**
LSD 0.01			**7.28**	**2.07**	**20.52**	**36.86**	**1.03**	**2.28**

differences with SA under 3/4 recommended dose of N fertilizer. In the present study, seed oil contents of dried seeds significantly increased with foliar application of 100 mg/L SA under recommended dose N fertilizer for two hybrids with no significant differences with 100 mg/L SA under 3/4 recommended dose of N fertilizer, in both seasons.

Table (7): Effect of foliar spray of 100 mg/L of SA under levels of nitrogen fertilizer, on vegetative growth parameters of 2 maize hybrids at 80 days in 2 successive seasons.

Maize hybrids	N levels kg N/fed.	Foliar spray salicylic	2010 season					
			Plant height (cm)	Leaves No.	Total dry weight (g)	Leaf area (cm^2)	Leaf area index	Chlorophyll (SPAD unit)
S.C. Pioneer 30K09	0	Without SA	245.56	13.78	224.27	629.73	5.78	40.53
		With SA	287.40	15.00	289.80	706.63	7.07	48.00
	90	Without SA	260.53	14.44	248.21	658.58	6.34	45.67
		With SA	295.14	17.00	347.40	752.15	8.52	51.43
	120	Without SA	273.50	14.56	272.58	674.18	6.53	47.20
		With SA	318.93	17.12	377.44	793.03	9.05	52.97
T.W.C-310	0	Without SA	246.67	13.89	193.96	621.73	5.75	41.50
		With SA	266.47	15.45	243.67	671.31	6.91	48.13
	90	Without SA	256.27	14.12	212.28	662.56	6.23	45.60
		With SA	271.44	17.33	315.82	750.56	8.67	51.03
	120	Without SA	266.17	14.56	242.61	685.62	6.65	46.13
		With SA	286.14	17.45	340.89	771.75	8.96	51.87
LSD 0.05			**7.42**	**0.75**	**22.54**	**43.85**	**0.47**	**3.14**
LSD 0.01			**10.05**	**1.02**	**30.5**	**59.41**	**0.64**	**4.25**
			2011 season					
S.C. Pioneer 30K09	0	Without SA	251.17	14.44	193.07	472.47	4.55	41.37
		With SA	296.17	15.67	315.80	684.42	7.15	49.93
	90	Without SA	276.83	14.67	226.68	508.02	4.97	46.73
		With SA	315.00	17.78	412.52	784.93	9.30	52.17
	120	Without SA	277.17	14.89	254.79	553.34	5.49	47.63
		With SA	317.45	18.45	471.58	801.66	9.86	53.13
T.W.C-310	0	Without SA	241.83	14.67	193.65	475.06	4.64	39.07
		With SA	279.17	15.11	308.58	675.32	6.80	45.50
	90	Without SA	265.17	14.78	223.92	534.64	5.27	45.80
		With SA	293.06	17.56	397.93	724.11	8.48	50.20
	120	Without SA	273.28	15.23	252.12	582.39	5.91	46.37
		With SA	285.39	18.01	437.62	735.65	8.83	51.37
LSD 0.05			**14.51**	**1.03**	**17.12**	**25.41**	**0.54**	**3.36**
LSD 0.01			**19.66**	**1.40**	**23.19**	**34.42**	**0.73**	**4.55**

Table (8): Effect of foliar spray of 100 mg/L of SA under levels of nitrogen fertilizer on growth analysis (LAD, NAR and CGR) at 60-80 days after planting and crop yield (ard.fed^{-1}.) in 2 maize hybrids in 2 successive seasons.

Maize hybrids	N levels kg N/fed.	Foliar spray salicylic	Leaf area duration (days)		Net assimilation rate (g m^2 d^{-1})		Crop growth rate (g m^2 d^{-1})		Yield (ard.fed^{-1}.)	
			2010 season	2011 season	2010 season	2011 season	2010 season	2011 season	2010 season	2011 season
S.C. Pioneer 30K09	0	Without SA	105.45	83.61	3.39	3.72	4.55	3.76	9.39	8.65
		With SA	134.80	126.65	3.44	3.77	5.80	7.79	11.63	11.26
	90	Without SA	119.81	97.15	3.33	3.80	4.91	4.20	24.55	25.26
		With SA	156.91	159.03	3.52	3.97	7.15	9.73	26.46	27.93
	120	Without SA	124.40	108.78	3.47	3.82	5.63	4.72	25.17	27.15
		With SA	165.29	177.72	3.55	4.15	8.39	10.33	27.51	29.10
T.W.C-310	0	Without SA	102.22	87.49	3.18	3.48	3.19	4.14	7.64	8.40
		With SA	121.62	125.89	3.39	3.79	3.78	7.01	10.71	10.77
	90	Without SA	110.82	101.83	3.20	3.57	3.51	4.23	20.46	20.74
		With SA	151.86	158.95	3.39	3.80	5.85	9.57	22.28	23.81
	120	Without SA	121.10	113.22	3.38	3.59	3.82	4.92	23.06	22.75
		With SA	160.41	166.73	3.40	4.00	6.89	10.42	24.18	24.75
LSD 0.05			8.32	10.37	0.32	0.27	0.90	1.16	1.49	1.43
LSD 0.01			11.28	14.05	NS	0.36	1.22	1.57	2.07	2.26

Table (9): Effect of foliar spray of 100 mg/L of SA under levels of nitrogen fertilizer on gas exchange parameters at 60 days from planting of 2 maize hybrids.

Maize hybrids	N levels kg N/fed	Foliar spray salicylic	Gas exchange parameters			
			Photosynthetic rate (P_N) ($\mu mol(CO_2)m^2s^{-1}$)	Stomatal conductance (g_s) ($mol\ (H_2O)m^{-2}s^{-1}$)	Transpiration rate (E) ($m\ mol\ m^{-2}s^{-1}$)	Intercellular CO_2 concentration (C_i) ($\mu mol\ mol^{-1}$)
S.C. Pioneer 30K09	0	Without SA	11.00	0.110	3.62	125.13
		With SA	15.55	0.149	5.08	125.01
	90	Without SA	16.10	0.179	5.21	129.03
		With SA	18.00	0.196	5.84	130.74
	120	Without SA	18.15	0.201	5.88	131.54
		With SA	23.65	0.270	7.64	143.98
T.W.C-310	0	Without SA	9.65	0.082	3.20	125.13
		With SA	16.20	0.172	5.25	138.78
	90	Without SA	15.05	0.169	4.88	130.37
		With SA	19.60	0.228	6.38	138.85
	120	Without SA	13.90	0.133	4.49	125.88
		With SA	14.70	0.139	4.78	130.37
LSD 0.05			**0.91**	**NS**	**0.02**	**3.52**
LSD 0.01			**1.23**	**NS**	**0.02**	**4.77**

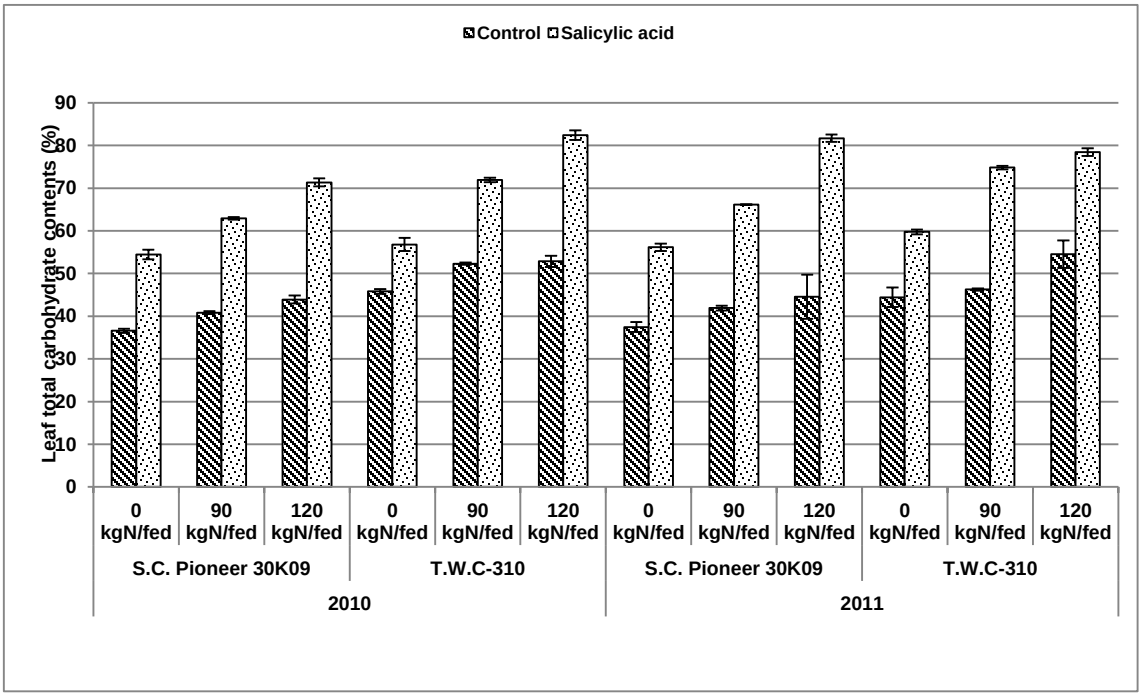

Figure (1): Total carbohydrate contents in leaves of 2 maize hybrids affected by foliar spray of 100 mg/L salicylic acid under levels of nitrogen fertilizer at 60th day from sowing in successive seasons. Values are means of three replicates ± SD.

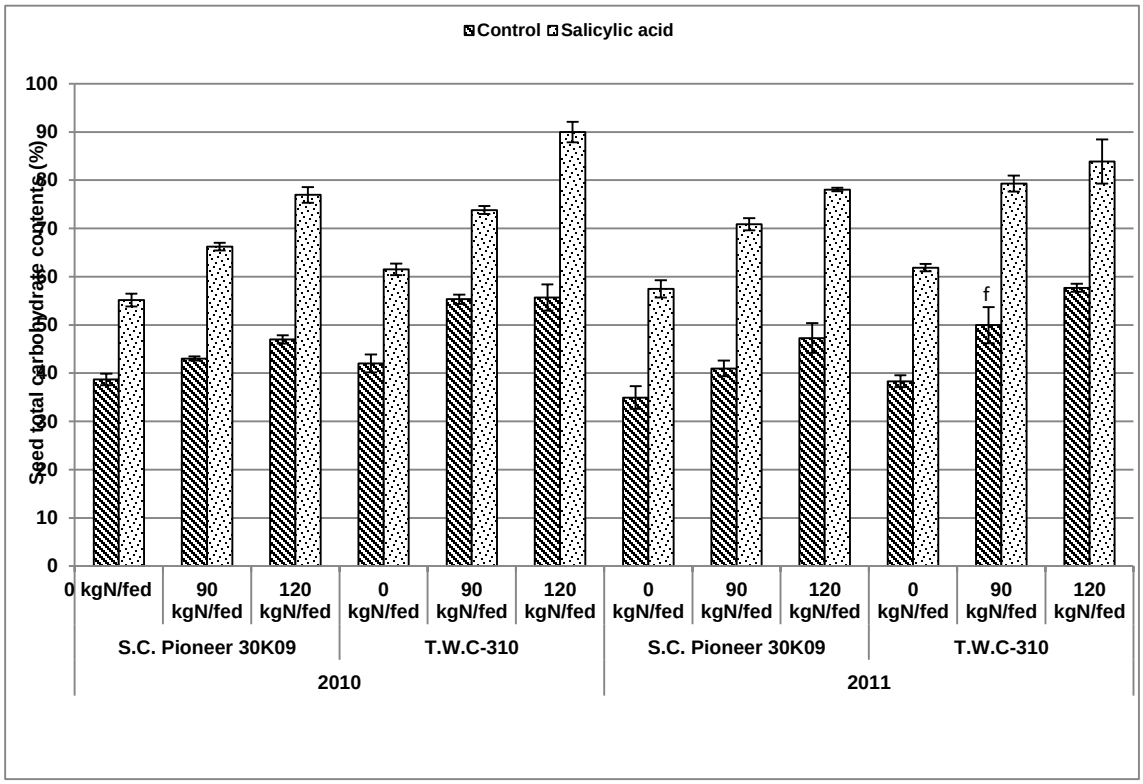

Figure (2): Total carbohydrates contents in seeds of 2 maize hybrids affected by foliar spray of 100 mg/L salicylic acid under levels of nitrogen fertilizer at 60th day from sowing in successive seasons. Values are means of three replicates ± SD.

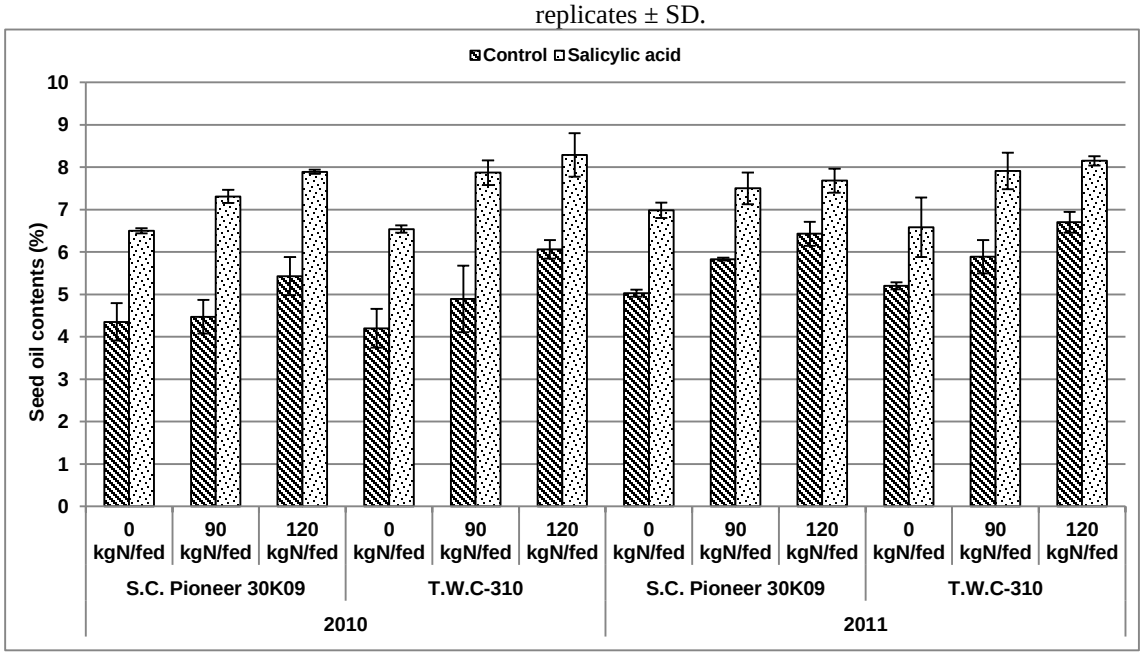

Figure (3): Seed oil contents of 2 maize hybrids affected by foliar spray of 100 mg/L salicylic acid under levels of nitrogen fertilizer at 60th day from sowing in successive seasons. Values are means of three replicates ± SD.

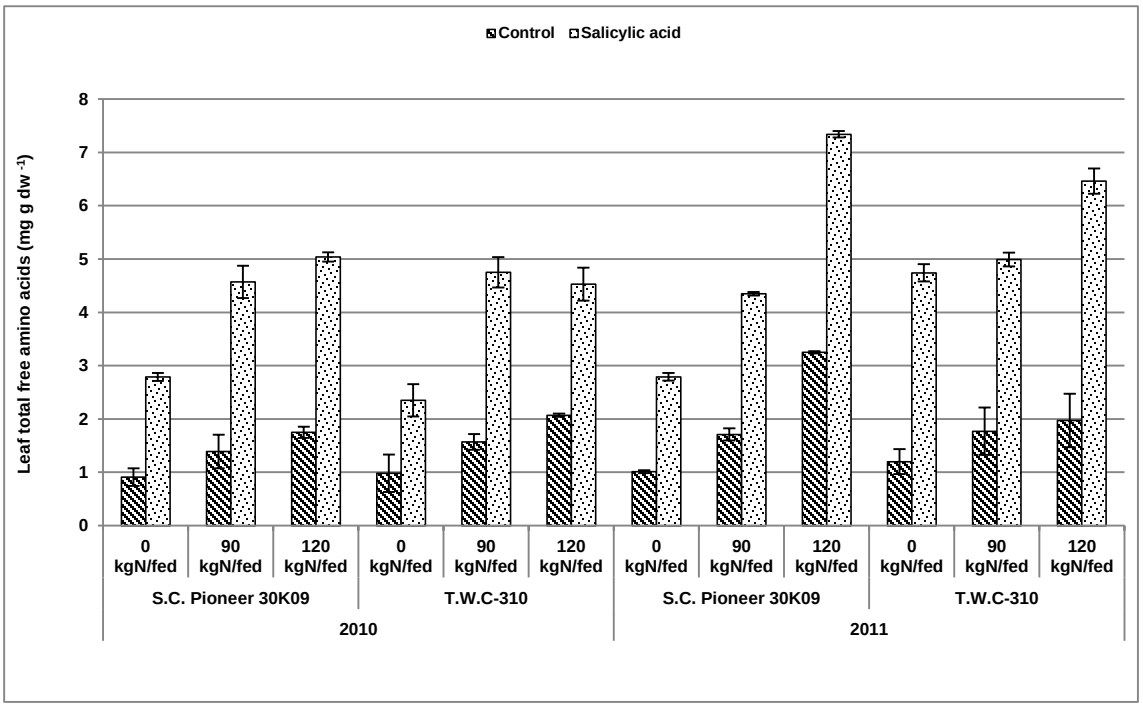

Figure (4): Total free amino acid contents in leaves of 2 maize hybrids affected by foliar spray of 100 mg/L salicylic acid under levels of nitrogen fertilizer at 60th day from sowing in successive seasons. Values are means of three replicates ± SD.

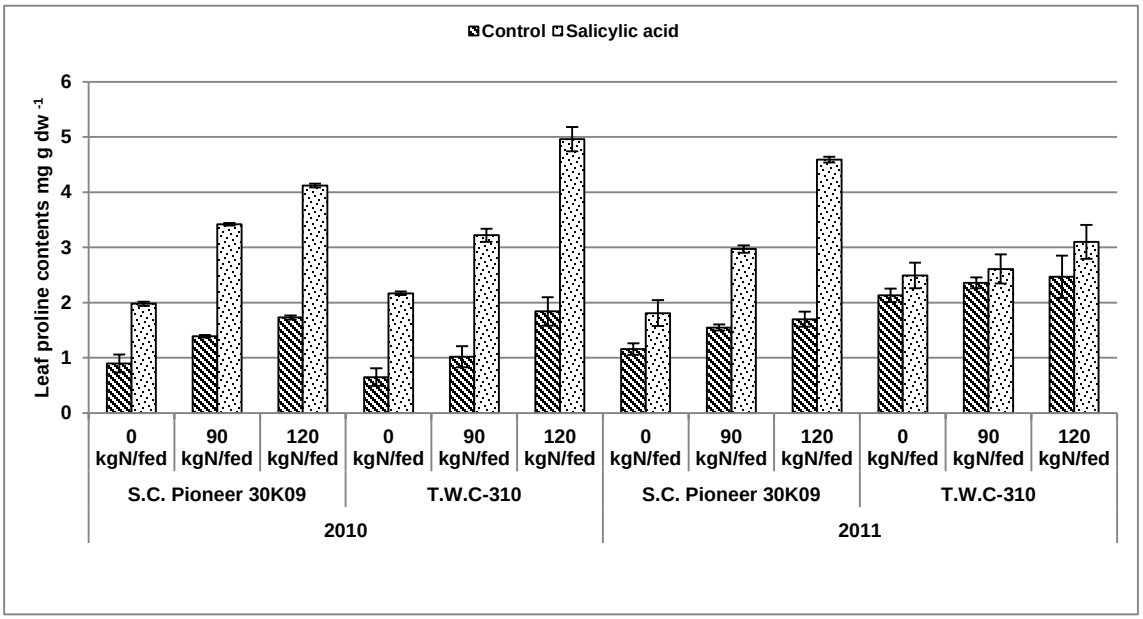

Figure (5): Proline contents in leaves of 2 maize hybrids affected by foliar spray of 100 mg/L salicylic acid under levels of nitrogen fertilizer at 60th day from sowing in successive seasons. Values are means of three replicates ± SD.

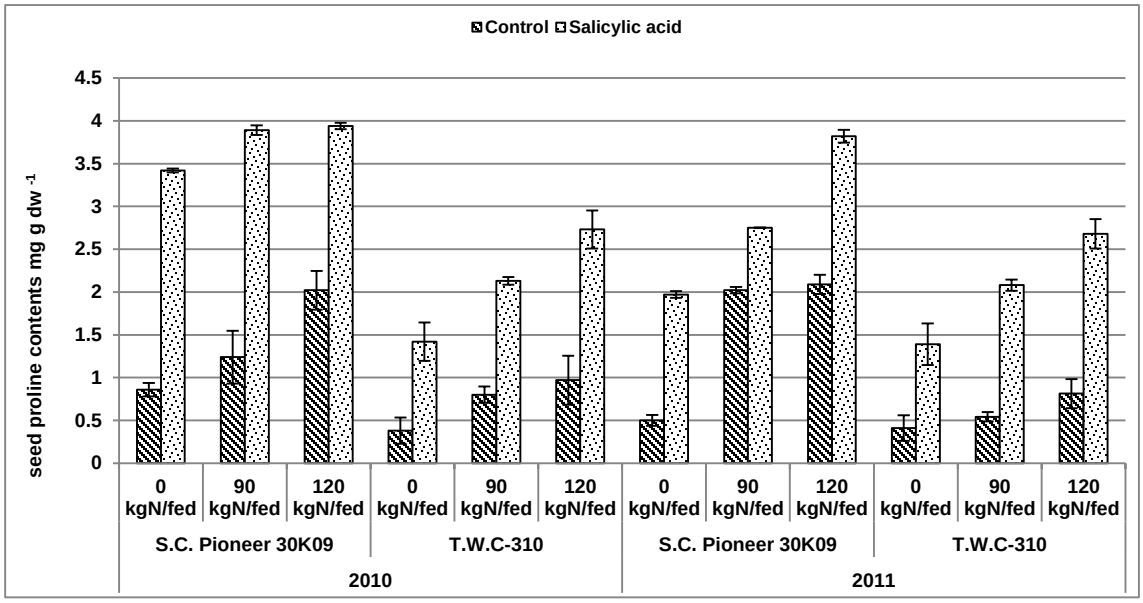

Figure (6): Proline contents in seeds of 2 maize hybrids affected by foliar spray of 100 mg/L salicylic acid under levels of nitrogen fertilizer at 60th day from sowing in successive seasons. Values are means of three replicates ± SD.

4 Discussion

In this study, we provide evidence for the ability of salicylic acid to improve plant performance through modulation of the vegetative growth, physiological process and yield of maize.

In the present study, it was observed that most of vegetative growth parameters, (Tables 2 and 3) and chlorophyll were significantly affected by maize hybrids these results are in agreement with these obtained by Sharifi and Taghizadeh (2009). Results in Tables (3) clear that the two maize hybrids differed significantly for grain yield ard.fed^{-1}. in both seasons. These results could be attributed to the genetic differences among two hybrids studied. Similar results were obtained by Ahmed (2009); Ahmed (2011); Moharram (2011) and Kandil (2013). Data achieved (Table 4) from the experiment showed a noticeable increase in photosynthetic rate, stomatal conductance, transpiration rate and sub-stomatal CO_2 concentration for S.C. Pioneer 30K09 hybrid. These results are similar to those found by Forrai *et al.*, (2012) who stated that, there are significant differences in stomatal conductance and transpiration rate between species and genera even under similar temperature which suggests again different responses to environmental conditions. Seed oil concentration was recorded no significant differences among the two hybrids, in both seasons, whereas, there were significant differences between the two hybrids under study in seed and leaf contents of carbohydrate, proline and total free amino acids (Table 5). This result is in agreement with that reported by Ahmed (2009); Ahmed (2011) and Moharam (2011) noticed significant differences among corn cultivars in most vegetative parameters.

Results given in Tables (6 and 7) cleared that vegetative growth parameters were statistically affected by foliar spray of SA under nitrogen fertilizer compared to the control plant. These effects were true in the two seasons for two various growth stages. Regardless the significant effect, the available data revealed that the most effective dose of nitrogen on vegetative growth was between 90 and 120 kg N/fed. The positive effect of N on vegetative growth is mainly due to important role of N as the most important element in building up plant organs. This result is in accordance with Ahmed (2009). The increases in growth parameters in response to foliar spray of SA might be due to increase the level of cell division within the apical meristem of seedling roots which caused an increase in plant growth (Sakhabutdinova *et al.*, 2003) and the role of SA to improve plant performance through modulation of growth of maize (Chattha *et al.*, 2015), improves germination (Hayat *et al.*, 2010) and enhance plant growth (Mahdi *et al.*, 2013).

As shown in table (6 and 7) total chlorophyll highly significantly increased in all SA treatment under N fertilizer compared with the control, the stimulatory response of SA acid might be due to its role in enhanced triggered chlorophyll biosynthesis (Farahat *et al.*, 2007) and also due to the phenomenon of antioxidant scavenging to provide protection to chloroplast and chlorophyll against degradation caused by reactive oxygen species (Gharib, 2007). This results not agreement with Molazem and Bashirzadeh (2014) who said that effect of salicylic acid on chlorophyll content was not significant. These results are in line with the findings of Khandaker (2011) and Tufail *et al.*, (2013). Concerning the effect of interaction, there are highly significant differences between SA under 3/4 recommended dose of N fertilizer (90 Kg N/fed.) and SA under recommended dose of N fertilizer (120 Kg N/fed.) compared to control in respect to total chlorophyll content, this may due to the role of nitrogen to improving mineral nutrition, increasing soil

nitrogen contents (Raut *et al.,* 2004) as well as stimulated chlorophyll synthesis through encourages pyridoxal enzymes formation, which plays an important role in α-amino levulinic acid synthetase as primary compound in chlorophyll synthesis (Ramadan *et al.,* 2003) also, nitrogen fertilizer could be accelerates formation of chlorophyll, and increases cell counts and volume per leaf (Hammad *et al.,* 2012). This results clarifying with those of Mosavifeyzabadi *et al.,* (2013) and Akram, (2014). Maize yield was highly significant increase by application of SA under nitrogen fertilizer. These results may be due to the increase of photosynthetic surface, which in turn resulted in an increase in metabolic processes and building more grain yield. Furthermore, the effect of nitrogen in increasing the vegetative growth and its components consequently increased the grain yield. These results confirmed by those reported by Shafshak *et al.,* (1994); Ahmed (2009) and Mansour (2009). Regarding to the effect of SA, Dawood *et al.,* (2012) observed that increase in kernel yield and yield components of sunflower by SA were due to the effect of physiological and biochemical processes that were led to ameliorate in vegetative growth and active assimilation translocation from source to sink.

Periodic data for the effect of the interaction of foliar spray of SA and nitrogen fertilizer on gas exchange parameters are presented in table (9), showed that exogenous application of SA under nitrogen fertilizer were increased photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration in both hybrids. The maximum values of photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration were found at foliar spray of 100 mg/L SA under of recommended dose of N fertilizer (120 Kg N/fed) in S.C. Pioneer 30K09 hybrid. These results in agreement with those obtained by (Tufail *et al.,* 2013) who found that exogenous application of SA was more effective on enhanced photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration in maize plant. And the increase in photosynthetic rates following the applications of SA could be increased the enzyme activity related to CO_2 uptake at the chloroplast level, rather than simple increases in stomatal opening (Khan *et al.,* 2003).

Biochemical analysis revealed that, foliar spray of 100 mg/L SA under N fertilizer levels significantly affected the parameters under discussion. And there are significant differences between SA treatments under N fertilizer compared with the control, in both hybrids at two growing seasons. The significant increase in total carbohydrate concentration by SA application under nitrogen fertilizer might due to that the nitrogen is essential in the structure of porphyrines, which are found in cytochrome enzymes. This increase in the cytochrome enzymes results in an increase in the rate of photosynthesis, and in a promotion of carbohydrate synthesis and accumulation, these results agreed with those obtained by Attia and Saad (2001) on *Catharanthus roseus.* The increment in oil yield might be due to the increase in vegetative growth, nutrients uptake or changes in leaf oil gland population and monoterpins biosynthesis due to application of N fertilizer (Tiwari and Banafar, 1995 and Gharib, 2007). These results were agreement with that detected by Ibrahim and Kandil, (2007). Concerning the effect of SA foliar application Ram *et al.,* (1997) reported that SA application had no effect on the herbage and essential oil yields in *Pelargonium graveolens*, *Mentha arvensis* and *Cymbopogon martini*. They concluded that either the synthesis of essential oil constituents occurs constitutively, without the intervention of SA or the amount of SA required for the induction of synthesis of essential oil constituents are already available in these plants. The increment in proline and total free amino acids due to the application of N with SA may be attributed to increasing of nitrogen in plant which is considered a main constituent of total free amino acids and proline (Ali *et al.,* 1999). SA increased proline content as compared to SA untreated plants in both hybrids; these results are in agreements with El-Tayeb (2005). Proline is a major constituent of osmoregulation in the expanded leaves of many species (Morgan, 1994). Besides osmotic adjustment, other possible functions of proline include the protection of plasma membrane integrity, the prevention of protein denaturation and acting as hydroxyl radical scavenger (Ramanjulu and Barels, 2002 and Bartels and Sunkur, 2005).

5 Conclusion

The present study showed that all treatments of 100 mg/L salicylic acid with application of nitrogen fertilizer enhanced vegetative growth, gas exchange characters and chemical constituents of leaves and seeds; total carbohydrates, total free amino acid, oil, and proline contents in both hybrids compared with the control. Also, there are no significant differences between the application of SA under 3/4 recommended dose of nitrogen fertilizer (90 Kg N/fed.) and SA under recommended dose of nitrogen fertilizer (120 Kg N/fed.) in most traits, these means that we can used the 3/4 of recommended dose of nitrogen fertilizer to decrease the harmful effect of chemical fertilizers and gave the highest maize crop yield. Also, the study concluded that S.C. Pioneer 30K09 hybrid was remained superior in attaining the high yield compare to T.W.C-310 hybrid. Finally, we concluded that growing of S.C. Pioneer 30K09 fertilized with 90 N/fed. combined with foliar sprayed by 100 mg/L of SA recommended to get the maximum vegetative growth parameters, chemical constituents of leaves and seeds as well as grain yield (ard.fed^{-1}.).

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