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GROWTH RESPONSES, PIGMENT AND STOMATAL BEHAVIOUR OF MAIZE TO FOLIAR APPLICATION OF NAA

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ABSTRACT

An investigation was conducted to find out the responses of two varieties of maize BHM- 7 (V_1) and BHM-9 (V_2) to Naphthalene acetic acid (NAA) on the growth, dry matter, pigment and stomatal behaviour. In case of V_1, plant height was found to decrease due to all the treatments at all the ages except at 7 DAS due to T_3 treatment. In V_2, increase in plant height was recorded following T_1 and T_3 treatments at all the ages except at 21 DAS. In both the varieties at all the ages, maximum number of leaves per plant were obtained from T_3 treatment. In case of V_1 number of leaves per plant increased only due to T_3 treatment in comparison to control whereas, in V_2 number of leaves per plant increased following all the treatments. In case of V_1, dry weight of shoot increased due to T_3 treatment at 8 and 15 DAS. But in case of V_2, there was increasing tendency in dry weight of shoot due to all treatments at all ages except at 8 DAS due to T_2 treatment. In both the varieties dry weight of root increased due to T_3 treatment at all ages. Chl. a was more than chl. b at all the stages in both the varieties and both chl. a and chl. b were recorded lowest at the flowering stage. In case of V_1, amount of chl. b was remarkably higher at the tillering stage whereas, in case of V_2, amount of chl. a and carotenoid were remarkably higher at the grain filling stage. In both the varieties, the amount of chl. a was found to increase and decrease due to all the treatments at all the stages. Chlorophyll b both increased and decreased in V_1 and the maximum increase was due to T_3 at the tillering and grain filling stages. In case of V_2, chl. b increased at the tillering stage following all the treatments and maximum increase was also due to T_3. However, in V_1 carotenoid contents of leaf increased following all the treatments at the tillering stage and decreased following all the treatments at flowering and grain filling stages. Number of stomata increased except due to T_2 in V_1, whereas, number of epidermal cell decreased due to T_0 and T_2 in V_1. Stomatal density in V_2 was found relatively higher due to NAA application. It was also found that NAA increased the stomatal density with the increase of concentration except 50 ppm (T_2) in V_2. Stomatal index was found to decrease due to T_1 inV_1 and due to T_0 in V_2.

Key words: Maize, Growth, Pigment, Stomatal behaviour, Foliar application, NAA

INTRODUCTION

Maize (*Zea mays* L.) is an important cereal crop ranking third after rice and wheat in the world and also in Bangladesh (Haque 2003). It is one of the most important crops

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both for human and animal consumption. In acreage it is next to wheat and in productivity it is next to rice. It has been proved as a profitable grain because of its high productivity and low production cost. With the changing climate and rainfall patterns crop yields in Bangladesh are projected to decline. There is almost no scope for bringing new land under cultivation. In 2014 – 15 the total maize production was approximately 7.0 metric tons per hectare (BBS 2015). This yield level is poor in comparison to that of other countries. The demand of maize is also increasing day by day in Bangladesh. So its production per unit area should be increased.

There are quite a number of methods which may help to increase the crop production. The use of growth regulators is considered as one of the ways for such an attempt. In many countries of the world, experiments have been carried out to investigate effects of NAA, a synthetic auxin on the yield and quality of cereal crops including maize (Chaudhury *et al*. 1980, Sing and Gill 1985, Grewal and Gill 1986, Muthukumar *et al*. 2005). But, in Bangladesh very limited research has been done on growth and yield aspects on cereal crops with NAA *viz.* rice (Adam and Jahan 2011), wheat (Jahan and Adam 2013, Islam and Jahan 2016) and only a preliminary work was reported regarding the effect of NAA on maize (Akter 2010).

Stomatal mechanism encapsulates much of plant physiology – this is valid for the perception of irradiance, some photosynthetic reactions, membrane transport, cell water reactions and many other processes. Effects of growth regulator on the stomatal behaviour particularly stomatal density and stomatal index were not studied in detail. Only one report is available regarding the effect of NAA on the number of stomata of maize (Akter 2010).

Therefore, the present investigation was undertaken to study the effect of NAA on growth, dry matter, pigment and stomatal behaviour of two varieties of maize.

MATERIALS AND METHODS

The experiment was conducted in the experimental plot of the Department of Botany, University of Dhaka from April to June 2014 with two hybrid varieties of maize, BHM- 7 and BHM-9. Fertilizers were applied at the rate of 500, 210, 260 and 200 kg per hectare of urea, gypsum, triple super phosphate and muriate of potash respectively (Fertilizer Recommendation Guide 2012) as basal dose during land preparation. The experiment was laid out in a randomized complete block design (RCBD) with four replications. Seeds collected from BARI, Joydebpur, Gazipur were sterilized with 0.5% $Ca(OCl)_2$ solution for five minutes and repeatedly washed in distilled water to remove any trace of $Ca(OCl)_2$. Seeds were sown on April 2, 2014 directly in rows. Row to row distances were 20 cm. Thinning was done at the age of 10 days after emergence (DAE) of

the seedlings in such a way that healthy seedlings of uniform size and vigor were allowed to grow. In each row, the distance from one seedling to another was 20 cm. Watering was done as per necessity. Weeding was done at the age of 30 DAE. There were four treatments as follows- T_0 = distilled water (control), T_1 = 25 ppm NAA, T_2 = 50 ppm NAA and T_3 = 75 ppm NAA. Treatments were applied as foliar spray at 32 DAE. Data on plant height and number of branches per plant were recorded at an interval of one week starting from 7 days after spray (DAS) up to 42 DAS. Dry weight of shoot and root were recorded from the age of 1 DAS at an interval of one week up to 15 DAS. Leaf pigments were determined at three stages *viz*. tillering, flowering and grain filling stages. Chlorophyll a and b contents were determined following Mckinney (1940) and Machlachalan and Zalik (1963). The amount of carotenoids was determined by the equation of von Wettstein (1957). Stomatal density and stomatal index were determined from the abaxial surface of the leaves. Number of stomata and epidermal cells were counted from temporary mounts of epidermal peels by a precalibrated microscope. Density of stomata was determined as the total number of stomata per unit area and was expressed per mm^2 and stomatal index was calculated from the formula of Pandeya *et al*. (1968). Data were analyzed statistically (Steel and Torrie 1960) and treatment means were compared by LSD test at 5% level of significance.

RESULTS AND DISCUSSION

Marked variation on the growth was noted between the varieties. Plants of BHM- 7 (V_1) showed vigorous vegetative growth (Table 1) and the number of leaves were more (Table 2). The results presented in Table 1 showed that significant variations in plant height were observed only at 14 DAS in V_2. In case of V_1, plant height was found to decrease due to all the treatments at all the ages except at 7 DAS due to T_3 treatment.

Table 1. Effect of NAA on plant height (cm) of two varieties of maize at different days after spray (DAS).

Treatments	Age of plants in days after spray (DAS)									
	MHM-7 (V_1)					MHM-9 (V_2)				
	7	14	21	35	42	7	14	21	35	42
T_0	78.58	96.12	152.78	175.24	178.79	73.79	84.81 b	142.84	154.46	159.40
T_1	74.97	81.07	150.67	173.78	175.51	75.69	86.46 b	139.11	155.95	161.39
T_2	71.55	82.98	151.14	174.86	175.24	64.30	79.70 b	126.40	147.78	153.50
T_3	83.71	91.79	146.86	172.21	173.90	79.08	100.65 a	139.67	156.53	167.70
CV (%)	22.21	21.49	20.21	19.72	20.17	13.52	16.22	12.81	15.60	11.87
LSD (0.05)	NS	NS	NS	NS	NS	NS	12.87	NS	NS	NS

In V_2, plant height decreased due to all the treatments only at 21 DAS, whereas, decreased at all the ages due to T_2 treatment. However, increase in plant height was recorded following T_1 and T_3 treatments at all other ages. Tallest plants were recorded following T_3 and was significant only at 14 DAS. Akter (2010) reported both increase and decrease in plant height of maize following NAA application. Similar results of increases and decreases in plant height due to NAA application were also reported in rice (Adam and Jahan 2011) and in wheat (Jahan and Adam 2013).

Table 2 showed that number of leaves per plant were significantly influenced at 35 and 42 DAS in V_1 and only at 35 DAS in V_2. Maximum number of leaves per plant of both the varieties were obtained from T_3 treatment at all the ages. In case of V_1 at all the ages number of leaves per plant increased only due to T_3 treatment and also due to T_2 at 14 DAS. However, in V_2 number of leaves per plant increased following all the treatments. The positive effect of NAA in the number of leaves per plant in rice (Chaudhury *et al*. 1980) and in wheat and barley (Harshan and Gill 1985) has been previously reported. Number of leaves per plant were found to increase and decrease following NAA application in different plants by different workers (Harshan and Gill 1985, Adam and Jahan 2011, Islam and Jahan 2016).

Table 2. Effect of NAA on number of leaves per plant of two varieties of maize at different days after spray (DAS).

Treatments	Age of plants in days after spray (DAS)									
	MHM-7 (V_1)					MHM-9 (V_2)				
	7	14	21	35	42	7	14	21	35	42
T_0	10.58	11.58	13.33	15.50 a	16.17 b	9.21	10.25	11.58	12.67 b	14.00
T_1	10.00	10.83	13.17	15.33 a	16.00 b	9.91	10.34	11.99	13.00 b	14.58
T_2	9.83	11.75	13.00	14.33 b	15.25 c	9.99	10.67	12.25	13.67 a	14.67
T_3	10.83	11.92	13.67	15.67 a	16.84 a	10.50	11.00	12.84	14.00 a	14.75
CV (%)	8.24	7.92	7.63	17.16	18.39	18.39	6.91	6.23	7.24	12.11
LSD (0.05)	NS	NS	NS	0.684	0.311	NS	NS	NS	0.785	NS

Significant influence on dry weight of shoot was observed only at 15 DAS in V_1 (Table 3). Dry weight of shoot was found to increase and decrease in both the varieties due to different NAA treatments. In case of V_1, dry weight of shoot decreased at all ages due to T_1 and T_2 treatments and also at 1 DAS due to T_3. The two increases were due to T_3 treatments at 8 DAS and 15 DAS. But in case of V_2, there was increasing tendency in dry weight of shoot due to all treatments at all ages except at 8 DAS due to T_2 treatment. Significant increase in dry weight of shoot of maize at harvest was also reported due to different NAA treatments (Akter 2010). Increased dry weight of other cereal plants due to

NAA application has also been reported by different investigators on different plants *viz*. rice (Jahan and Adam 2011) and wheat (Jahan and Adam 2013). Both increase and decrease in dry matter of various other plants following NAA application were also reported by several workers *viz*. Karim and Fattah (2007) on chickpea and Ullah *et al*. (2007) on cowpea.

Table 3. Effect of NAA on dry weight of shoot (g) of two varieties of maize at different days after spray (DAS).

Treatments	Age of plants in days after spray (DAS)					
	BHM-7 (V_1)			BHN-9 (V_2)		
	1	8	15	1	8	15
T_0	14.842	16.534	26.477 a	16.065	21.047	30.628
T_1	11.931	13.169	19.898 b	16.742	23.129	31.463
T_2	10.466	12.848	21.514 b	17.566	18.251	30.839
T_3	13.267	17.228	26.682 a	19.435	22.400	32.525
CV (%)	18.60	18.66	15.34	16.20	19.29	3.46
LSD (0.05)	NS	NS	4.94	NS	NS	NS

Table 4. Effect of NAA on dry weight of root (g) of two varieties of maize at different days after spray (DAS).

Treatments	Age of plants in days after spray (DAS)					
	BHM-7 (V_1)			BHN-9 (V_2)		
	1	8	15	1	8	15
T_0	0.981	2.463 b	3.506	1.730 b	2.218 b	3.427 c
T_1	0.927	3.538 a	3.618	1.599 c	2.366 a	3.794 b
T_2	0.836	2.009 b	2.943	1.936 a	2.044 c	3.891 b
T_3	1.014	2.572 b	3.841	1.747 b	2.395 a	4.102 a
CV (%)	11.48	24.46	9.96	7.84	6.47	6.92
LSD (0.05)	NS	0.602	NS	0.126	0.044	0.134

Dry weight of root of V_1 was decreased due to T_2 treatment but increased due to T_3 treatment at all ages (Table 4). In case of V_2, dry weight of root was increased following all the NAA treatments at all the ages except at 1 DAS due to T_1 and 8 DAS due to T_2 treatment. Similar results of increase and decrease in dry weight of root following NAA application have also been reported in other plants (Al-Wahaibi *et al*. 2012). Dry matter of any crop is the output of net photosynthesis. It is mostly depended on the size of the photosynthetic system or its activity as well as the length of the growth period during which photosynthesis continues. In both the varieties, at 15 DAS dry weight of root was

highest due to T_3 and this provides a clear evidence of a shift of allocation in growth resources in favour of root development.

Table 5. Effect of NAA on pigment content of leaf (mg/g) of BHM-7 (V_1) at three different stages.

Treatments	Tillering			Flowering			Grain filling		
	Chl.a	Chl.b	Carotenoids	Chl.a	Chl.b	Carotenoids	Chl.a	Chl.b	Carotenoids
T_0	0.736 b	0.473	6.113	0.530 a	0.182	4.826 a	0.822	0.271	5.622
T_1	0.729 b	0.401	6.400	0.515 a	0.241	4.437 a	0.535	0.301	5.995
T_2	1.035 a	0.528	6.142	0.171 b	0.151	3.199 b	0.756	0.235	6.519
T_3	0.675 b	0.554	6.162	0.583 a	0.221	4.983 a	0.657	0.318	5.541
CV (%)	51.90	14.67	12.04	39.15	2.62	19.86	11.84	17.02	7.02
LSD (0.05)	0.501	NS	NS	0.126	NS	0.978	NS	NS	NS

Table 6. Effect of NAA on pigment content of leaf (mg/g) of BHM-9 (V_2) at three different stages.

Treatments	Tillering			Flowering			Grain filling		
	Chl.a	Chl.b	Carotenoids	Chl.a	Chl.b	Carotenoids	Chl.a	Chl.b	Carotenoids
T_0	0.389 b	0.180	4.278 b	0.223 c	0.146 a	4.290 a	0.647 a	0.245 a	6.018
T_1	0.523 a	0.245	5.615 a	0.415 a	0.156 a	3.813 b	0.537 c	0.298 a	5.832
T_2	0.312 c	0.282	4.285 b	0.273 b	0.077 b	2.037 c	0.516 c	0.167 b	4.897
T_3	0.360 b	0.299	3.942 b	0.167 d	0.080 b	1.403 d	0.793 a	0.281 a	5.177
CV (%)	21.86	4.38	17.90		36.13	43.7	18.7	21.3	11.8
LSD (0.05)	0.051	NS	1.14	0.048	0.023	0.322	0.044	0.056	NS

Results revealed that chl. a was more than chl. b at all the stages in both the varieties. In case of V_1 chlorophyll a varied significantly at tillering and flowering stages (Table 5) but, in V_2 it was significantly influenced by different treatments at all the three stages (Table 6). In both the varieties, the amount of chl. a was found to increase and decrease due to different treatments at all the stages. In V_2, amount of chl.a was found highest due to all the treatments at the grain filling stage. Chlorophyll b was not significantly influenced at any stage in V_1 (Table 5), but in V_2 it was significantly influenced at the flowering and grain filling stages (Table 6). Highest amount of chl. b in V_1 was obtained at the tillering stage while in V_2 chl. b was highest at the grain filling stage. Chlorophyll b both increased and decreased in V_1 and the maximum increase was due to T_3 at the tillering and grain filling stages. In case of V_2, chl. b increased at the tillering stage following all the treatments and maximum increase was also due to T_3. In both the varieties chl. a and chl. b were recorded lowest at the flowering stage. Significant variations in carotenoid contents were observed only at the flowering stage in V_1 whereas, at the tillering and flowering stages in V_2. Carotenoids were remarkably higher

in the leaves of V_1 at all the three stages (Table 5) than those of V_2 (Table 6). In case of V_1 maximum carotenoids was obtained at the tillering stage except due to T_2 and in V_2 it was maximum at the grain filling stage. However, in comparison to control carotenoid contents of leaf increased following all the treatments at the tillering stage in V_1 and decreased following all the treatments at flowering and grain filling stages in V_2. There are a number of reports on the increase in leaf pigments of different kinds of plants following NAA treatments (Chaudhury *et al*. 1980, Grewal and Gill 1986, Jahan *et al*. 1992). Jahan and Adam (2014) reported that pigment content of leaf at different stages increase in one variety of rice while decrease in another variety depending on concentration of the chemical. The decrease in pigment content may be due to increase in number of older leaves which lost photosynthetic activity. This is also because pigments breakdown after certain growth stage.

Table 7. Effect of NAA on stomatal density and stomatal index in two varieties of maize.

Variety	No. of stomata and epidermal cell before spray		Stomatal Index (SI)	Treatments	No. of stomata and epidermal cell after spray		Stomatal Index (SI)
	Stomata	Epidermal cell			Stomata	Epidermal cell	
V_1	53.5	360	0.13	T_0	55.72	286.02	0.16
				T_1	57.71	420.01	0.12
				T_2	49.03	318.0	0.13
				T_3	57.87	364.60	0.14
V_2	52.5	350	0.13	T_0	56.47	461.32	0.11
				T_1	72.55	456.97	0.14
				T_2	78.18	428.64	0.16
				T_3	82.93	468.16	0.16

The results given in Table 7 showed that before spray, number of stomata on the dorsal surfaces in V_1 and V_2 were 53.5 and 52.5 per mm^2, respectively. But after spray, the number of stomata increased except due to T_2 in V_1, whereas, number of epidermal cell decreased due to T_0 and T_2 in V_1. Stomatal density in V_2 was found relatively higher due to NAA application. It was also found that NAA increased the stomatal density with the increase of concentration except 50 ppm (T_2) in V_2. Stomatal index was found to decrease due to T_1 inV_1 and due to T_0 in V_2. Akter (2010) reported significant decrease in number of stomata in maize var. Pacific 283 with an increase in concentration of NAA. The decrease in number of stomata indicates that the loss of water through transpiration decreased. Hence, less amount of water is required for this crop. It also reduces the irrigation and labour cost. It is a positive indication for better adaptation in dry region. As maize is a C4 plant, its productivity will also be increased. All these indicate that

stomatal density and index have some influence on plant growth. However, stomatal study in relation to growth regulator warrant further study.

Thus the results obtained during this experiment indicated that NAA has effect on different physiological and biochemical parameters, but the magnitude of the effect depended on concentrations of NAA and also on the parameters observed. Of all the concentratios of NAA, 75 ppm (T_3) produced better stimulation. Moreover, two varieties of maize responded differently.

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