ENHANCEMENT OF GROWTH AND YIELD COMPONENTS THROUGH FOLIAR APPLICATION OF NAPHTHALENE ACETIC ACID (NAA) AND BEZYLAMINOPURINE (BAP) IN SPINACH

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ABSTRACT

The present research work was conducted at the Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad to evaluate the effect of growth regulators (NAA and BAP) on growth and yield components of spinach (Spinacia oleracea L.) during 2006. Different combinations and concentrations of NAA and BAP were tested to evaluate different vegetative parameters. The data were recorded at 40 and 60 days after sowing. Highest data for plant height at 40 DAS was recorded for the combined affect of NAA and BAP at a concentration of 10^{-3}M (each). BAP at 10^{-4} M concentration showed maximum plant height at 60 DAS. Maximum number of leaves was shown by NAA10^{-5}M both at 40 and 60 DAS. Increase in leaf length was observed for NAA10^{-5}M and BAP10^{-4}M both at 40 and 60 DAS. Significant variations were found for root length. Maximum root length was recorded for BAP (10^{-3}M) at 40 DAS. At 60 DAS maximum root length was observed for BAP (10^{-5}M) and NAA (10^{-3}M). Mean values for shoot fresh weight indicated significant results. Shoot fresh weight was observed maximum for NAA (10^{-3}M) and BAP (10^{-3}M). Maximum shoot dry weight was observed for the combined effect of NAA and BAP at a concentration of (10^{-3}M each). Significant variations were found in root dry weight. Highest data for root dry weight was noted for NAA at 10^{-3}M.

Key Words: *Growth regulators (NAA and BAP), growth and yield components, Spinach*

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INTRODUCTION

Spinach (*Spinacia oleracea* L.) is an annual herb of the family Chenopodiaceae. It is native to West Asia, and at present, is widely cultivated in the world as a popular vegetable. Among fresh leafy greens, spinach is an important source of nutrients in the diet ranking 2nd behind kale in total carotenoids and folate (Holden *et al*., 1999; USDA, 2003). Spinach is also a rich source of Vitamin A, Vitamin C and several vital antioxidants. Fresh leaves of Spinach are a rich source of vitamin A. Recently, opioid peptides called rubiscolins have also been found in spinach. It is a source of folic acid, and this vitamin was first purified from spinach (Cardwell, 2005). The shelf life of spinach is less than 14 days after harvest (Kader, 2002). However, strategies to increase shelf life include reducing physical damage during processing and storing at lower temperatures in modified atmospheres (Price and Floros, 1993).

Foliar application entails the applications, via spraying of nutrients to plants leaves and stems and their absorption at those sites. It is observed that foliar application result in increase yield, resistance to diseases and insect pests, improved drought tolerance, and enhanced crop quality. The growth regulators or hormones stimulate and promote growth phenomenon in plants (Salisbury and Ross, 1985). At present, for example, foliar sprays are commonly recommended to correct zinc deficiencies in grapes (Williams and Williams, 1986), to control bitter pit and cork spot in apples (Greene *et al.*, 1995) and for general supplementary nutrition in strawberries (Deremiens, 1995). Very dilute solutions of nutrient formulations are suggested. Sometimes as little as one cup to two quarts per acre of an active ingredient is all that is required to obtain the desired response (Anonymous, 1985).

Aim and Objective of Present Study

The present research work was carried out to evaluate the effect of growth regulators on growth and yield components, as plant height, number of leaves, leaf length, shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight.

MATERIALS AND METHODS

A series of experiments were designed and conducted at the Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad during 2006. The research work was carried out to study the effects of foliar application of Naphthalene acetic acid (NAA) and 6-Benzylaminopurine (BAP) on growth and yield of leaves, stem and roots in Spinach (*Spinacia oleracea* L.). Healthy seeds of *Spinacia oleracea* L. var. Local were selected for sowing. Initially six seeds per pot were sown. The plants were thinned to three seedlings per pot. The seeds were sown on 5th February, 2006. Soil was collected from the university campus. Stones and pebbles were removed from the soil. The pots were watered by using a rose can so as to avoid packing of the soil and washing away of seeds. The pots were irrigated three times a week. The pots were constantly cleaned up to make them free form weeds. For foliar application 20-25 ml of hormonal solution of varying strengths was sprayed per pot. . First foliar spray of hormones of different combinations and concentrations was performed 20 days after mean germination. Second foliar spray was made 40 days after sowing. The third foliar spray was carried out 60 days after sowing. The data was recorded a week after the spray was given to the plants. The plants were harvested 85-90 days after sowing in the first week of May, 2006.

Hormonal Concentrations Used

The following concentrations of auxin (NAA) and cytokinin (BAP) were prepared and applied as foliar application to observe their effect on growth and yield in Spinach.

1st concentration = 10^{-3} M
2nd concentration = 10^{-4} M
3rd concentration = 10^{-5} M

Table I ***Combinations and concentrations of NAA and BAP used for foliar application on spinach***

Treatment number	Hormones & Combination	Concentration of Phytohormones Used	
T_0	Control	--	--
T_1	NAA	10^{-3} M	--
T_2	NAA	10^{-4} M	--
T_3	NAA	10^{-5} M	--
T_4	BAP	10^{-3} M	--
T_5	BAP	10^{-4} M	--
T_6	BAP	10^{-5} M	--
T_7	NAA + BAP	10^{-3} M	10^{-3} M
T_8	NAA + BAP	10^{-3} M	10^{-4} M
T_9	NAA + BAP	10^{-3} M	10^{-5} M
T_{10}	BAP + NAA	10^{-3} M	10^{-4} M
T_{11}	BAP + NAA	10^{-3} M	10^{-5} M
T_{12}	NAA + BAP	10^{-4} M	10^{-4} M
T_{13}	NAA + BAP	10^{-4} M	10^{-5} M
T_{14}	BAP + NAA	10^{-4} M	10^{-5} M
T_{15}	BAP + NAA	10^{-5} M	10^{-5} M

Preparation of Stock Solutions

Different concentrations of auxin (NAA) and Cytokinins (BAP) were prepared by calculating the weight of each hormone by following formula. Calculated weight of each hormone was dissolved in few drops of dilute sodium hydroxide (NaOH) and final volume was made up to the volume required. First 10^{-3} M solution was prepared and solutions of 10^{-4} M and 10^{-5} M were prepared by dilution method.

$$\text{Weight required} = \frac{\text{Molarity x Molecular weight x Volume required}}{1000}$$

Parameters Recorded and Studied

Plant Height

Plant height at two different stages (40 and 60 DAS) was measured with the help of an inch tap in centimeters.

Root Length and Leaf Length

Root length and leaf length were measured with the help of an inch tap in centimeters. Data for root length and leaf length was measured and recorded at 40 and 60 DAS.

Number of Leaves

Number of leaves per plant were counted and recorded from each treatment. The data was taken at 40 and 60 DAS.

Fresh Weight and Dry Weight

Fresh weight was calculated by removing the plants from the soil. Different parts of the plants i.e. stem and roots were first separated and then fresh weights were taken by electrical weight balance in grams. When fresh weight data was recorded, the plants (roots and shoots) were kept in an oven at 40^{o}C for 72 hours to get the dry weight. The data for fresh and dry weight was collected at 60 DAS.

Statistical Analysis

The experiment was conducted in randomized complete block design with three replicates per treatment. The data were recorded and analyzed by applying Analysis of Variance (ANOVA) and Least Significant Difference (LSD) Test (Steel and Torrie, 1984).

RESULTS AND DISCUSSION

The present research work was conducted to evaluate the effect of growth regulators (NAA and BAP) on growth and yield of leaves, stems and roots in spinach (*Spinacia oleracea* L.). The results obtained from the present study indicate that growth and morphological components were affected by the applications of different combinations and concentrations of hormones.

Plant Height

The plant height was significantly affected by the treatments both at 40 and 60 DAS. Maximum plant height was recorded at 60 DAS where (10^{-4}M) solution of BAP was applied. Minimum plant height at 60 DAS was shown by NAA keeping the concentration at (10^{-5}M). It is found that applying cytokinin (BAP) alone to plants is very much effective to enhance plant height. Increase in shoot elongation was found in Citrus plants by the application of BAP, as stated by Bhatt *et al.,* (1992) which is in confirmation to our results.

Table II ***Effect of foliar application of NAA and BAP on plant height at 40 and 60 DAS***

Treatments	Plant height (cm) (40 DAS) - Mean ± SE	LSD Rank	Plant height (cm) (60 DAS) - Mean ± SE	LSD Rank
T0 (control)	10.36 ± 0.18	h	29.06 ± 0.41	i
T1	13.33 ± 0.28	bc	31.36 ± 0.49	cde
T2	14.1 ± 0.21	a	30.9 ± 0.46	def
T3	12.97 ± 0.18	cd	28.66 ± 0. 57	i
T4	11.8 ± 0.15	efg	31.63 ± 0. 20	cd
T5	12.4 ± 0.15	def	33.4 ± 0.15	a
T6	11.5 ± 0.36	g	30.3 ± 0.25	fgh
T7	14.3 ± 0.21	a	32.3 ± 0.06	bc
T8	13.83 ± 0.18	ab	30.43 ± 0.23	efg
T9	10.4 ± 0.21	h	32.96 ± 0.45	ab
T10	12.73 ± 0.09	cd	30.3 ± 0.26	fgh
T11	12.50 ± 0.23	de	29.26 ± 0.18	hi
T12	11.86 ± 0.63	efg	31.06 ± 0.69	def
T13	12.93 ± 0.32	cd	32.96 ± 0.43	ab
T14	11.60 ± 0.09	fg	29.43 ± 0.09	ghi
T15	10.33 ± 0.33	h	30.33 ± 0.33	efg
***LSD Value**	**0.754102**		**1.052266**	

Number of Leaves

There were non-significant difference in the number of leaves at 40 DAS but still maximum number of leaves at 40 DAS was shown by NAA (10^{-5}M) which was (6.33). Significant variation at 60 DAS was achieved. Maximum number of leaves (11.3) at 60 DAS was given by NAA alone at (10^{-5}M). The results indicated that the Phytohormone NAA has increased the number of leaves and is effective at a concentration of (10^{-5}M). Earlier experts have also concluded that plant growth regulator, like Auxin, can accelerate number and leaf area as observed

by Tuomine *et al.* (1997) and Awan *et al.* (1999). Effects of combined application of nutrients and hormones on soybean yield were positively observed by Kalpana and Krishnarajan (2003) which is in confirmation to our studies.

Table III ***Effect of foliar application of NAA and BAP on number of leaves at 40 and 60 DAS***

Treatments	Number of Leaves (40 DAS) - Mean ± SE	LSD Rank	Number of Leaves (60 DAS) - Mean ± SE	LSD Rank
T0 (control)	4.66 ± 0.33	cd	7.66 ± 0.33	g
T1	6.0 ± 0.58	ab	10.33 ± 0.67	abc
T2	5.0 ± 0.58	bcd	10.66 ± 0.67	ab
T3	6.33 ± 0.33	a	11.3 ± 0.33	a
T4	6.0 ± 0.58	ab	9.33 ± 0.33	bcdef
T5	5.0 ± 0.00	bcd	9.66 ± 0.88	bcde
T6	5.6 ± 0.33	abc	10.0 ± 0.00	abcd
T7	4.33 ± 0.33	d	8.66 ± 0.33	defg
T8	5.33 ± 0.33	abcd	9.0 ± 0.00	cdefg
T9	5.66 ± 0.33	abc	7.66 ± 0.33	g
T10	4.66 ± 0.33	cd	8.33 ± 0.33	efg
T11	5.33 ± 0.33	abcd	9.33 ± 0.33	bcdef
T12	6.0 ± 0.58	ab	10.0 ± 0.58	abcd
T13	4.33 ± 0.67	d	8.33 ± 0.88	efg
T14	5.6 ± 0.33	abc	9.0 ± 0.58	cdefg
T15	5.0 ± 0.58	bcd	8.0 ± 0.00	fg
***LSD Value**	**1.301895**		**1.412828**	

Leaf Length

All the treatments significantly affected leaf length both at 40 and 60 DAS. Maximum leaf length was observed at 60 DAS. The highest value at 40 DAS was (8.53cm), while at 60 DAS leaf length was (17.63cm), where NAA (10^{-5}M) and BAP (10^{-4}M) were applied in combination. Enhancing role in leaf elongation was recorded when NAA and BAP were applied in combination. The treatments in which NAA was applied as single dose yielded better results. Similar observations were being reported by Ulskov *et al.*, (1992).

Table IV ***Effect of foliar application of NAA and BAP on leaf length at 40 and 60 DAS***

Treatments	Leaf Length (cm) (40 DAS) - Mean ± SE	LSD Rank	Leaf Length (cm) (60 DAS) - Mean ± SE	LSD Rank
T0 (control)	6.63 ± 0.15	e	13.6 ± 0.17	fg
T1	8.4 ± 0.10	ab	16.2 ± 0.20	abcd
T2	8.43 ± 0.15	a	15.13 ± 0.47	cdef
T3	7.33 ± 0.15	d	17.5 ± 0.76	ab
T4	7.33 ± 0.29	d	13.83 ± 1.41	efg
T5	7.83 ± 0.19	c	16.53 ± 0.72	abcd
T6	8.0 ± 0.06	bc	17.2 ± 0.47	ab
T7	6.76 ± 0.07	e	14.66 ± 0.88	defg
T8	7.23 ± 0.06	d	17.03 ± 0.87	abc
T9	5.8 ± 0.26	f	12.83 ± 0.33	g
T10	8.0 ± 0.10	bc	15.6 ± 0.38	bcde
T11	7.4 ± 0.12	d	13.1 ± 0.61	g
T12	8.2 ± 0.10	abc	15.16 ± 0.44	cdef
T13	7.36 ± 0.12	d	14.66 ± 1.32	defg
T14	8.53 ± 0.09	a	17.63 ± 0.35	a
T15	5.76 ± 0.07	f	14.03 ± 0.32	efg
***LSD Value**	**0.415291**		**1.99238**	

Root Length

The values regarding root length were found highly significant both at 40 and 60 DAS. Maximum root length (9.37cm) at 40 DAS was given by BAP alone at concentration of (10^{-3}M). At 60 DAS maximum root length (16.46cm) was noted for (BAP10^{-5}M) and (NAA10^{-3}M). The present study showed that BAP alone or in combination with NAA is effective. Cleland, (1996) has already reported that kinetin and other cytokinins have the ability to enhance cell division in the presence of auxin and also promote bud and root formation which is the direct confirmation of our result. Elongation and increase in number of roots was also observed thereby in garlic due to foliar spray of IAA treatments by Baren *et al.*, 1988.

Table V ***Effect of foliar application of NAA and BAP on root length at 40 and 60 DAS***

Treatments	Root Length (cm) (40 DAS) - Mean ± SE	LSD Rank	Root Length (cm) (60 DAS) - Mean ± SE	LSD Rank
T0 (control)	7.33 ± 0.24	gh	13.46 ± 0.09	d
T1	7.9 ± 0.23	fg	16.26 ± 0.15	a
T2	8.17 ± 0.18	ef	16.33 ± 0.13	a
T3	6.66 ± 0.44	i	15.33 ± 0.20	b
T4	9.37± 0.18	a	13.63 ± 0.23	d
T5	9.1 ± 0.15	ab	13.4 ± 0.10	d
T6	9.03 ± 0.18	abc	14.3 ± 0.17	c
T7	8.46 ± 0.27	cdef	15.4 ± 0.15	b
T8	8.36 ± 0.24	def	16.23 ± 0.12	a
T9	8.83 ± 0.22	abcd	16.46 ± 0.15	a
T10	7.87 ± 0.07	fg	13.63 ± 0.18	d
T11	7.13 ± 0.19	hi	14.46 ± 0.15	c
T12	8.7 ± 0.25	bcde	14.06 ± 0.07	c
T13	8.43 ± 0.15	cdef	15.3 ± 0.17	b
T14	7.13 ± 0.09	hi	13.3 ± 0.06	d
T15	8.33 ± 0.12	def	14.23 ± 0.15	c
***LSD Value**	**0.618714**		**0.439193**	

Shoot Fresh Weight and Dry Weight

Mean values for shoot fresh weight indicated that the results were highly significant. Maximum shoot fresh weight (29.52g) was observed with combination and concentration of (NAA10^{-3}M) and (BAP10^{-3}M). The present study showed that the cytokinin (BAP) has a major role in the enhancement of biomass in case of spinach. Significant variation was also obtained in shoot dry weight. Maximum shoot dry weight (4.84g) was observed for NAA and BAP at a concentration of (10^{-3}M) each. It was also noted that auxin and cytokinin (NAA and BAP) in any suitable concentrations resulted in maximum plant dry weight or biomass. Use of BAP was found to increase yield in *Brassica* and sugar beets as reported by Yadav and Tikkoo, (2001) which fully support our present findings.

Table VI ***Effect of foliar application of NAA and BAP on fresh weight and dry weight***

Treatments	Shoot fresh weight (g) - Mean ± SE	LSD Rank	Shoot dry weight (g) Mean ± SE	LSD Rank
T0 (control)	23.34 ± 0.37	h	3.75 ± 0.14	de
T1	28.71 ± 0.71	ab	3.82 ± 0.04	cde
T2	27.76 ± 0.49	bc	3.77 ± 0.11	de
T3	26.55 ± 0.32	def	3.61 ± 0.08	e
T4	27.52 ± 0.28	cd	4.24 ± 0.17	b
T5	29.34 ± 0.36	a	4.0 ± 0.01	bcd
T6	28.09 ± 0.69	bc	3.76 ± 0.03	de
T7	29.52 ± 0.36	a	4.84 ± 0.04	a
T8	27.25 ± 0.30	cde	3.91 ± 0.01	cd
T9	23.32 ± 0.18	h	4.23 ± 0.13	b
T10	26.23 ± 0.46	ef	2.47 ± 0.12	g
T11	25.01 ± 0.10	g	3.81 ± 0.04	de
T12	25.58 ± 0.30	fg	3.03 ± 0.19	f
T13	27.60 ± 0.40	bcd	3.77 ± 0.09	de
T14	27.34 ± 0.39	cde	4.11 ± 0.05	bc
T15	23.56 ± 0.37	h	3.81 ± 0.02	de
***LSD Value**	**1.186536**		**0.288161**	

Root Fresh Weight and Dry Weight

The results for root fresh weight were non-significant. The results indicated that the treatments have no such impressive role in increasing root fresh weight. Maximum root fresh weight (4.0g) was recorded for BAP alone at a concentration of (10^{-4}M) solution. The results stated that BAP has affected the root length and root dry weight. Significant variation was found for root dry weight. Maximum root dry weight was observed for NAA (10^{-3}M) which was (0.61g). The minimum weight was recorded by control having no hormonal concentration. The data revealed that affect of hormonal combination and concentration on root fresh weight was not striking, but there was noted as significant effect on part of root dry weight. Kothule *et al.,* (2003) observed the enhanced affect of exogenous application of growth regulators on growth parameters which corroborate our findings.

Table VII *Effect of foliar application of NAA and BAP on fresh weight and dry weight of root*

Treatments	Root fresh weight (g) - Mean ± SE	LSD Rank	Root dry weight (g) Mean ± SE	LSD Rank
T0 (control)	2.71 ± 0.14	a	0.45 ± 0.01	h
T1	2.83 ± 0.02	a	0.62 ± 0.00	a
T2	2.91 ± 0.06	a	0.57 ± 0.01	cde
T3	3.01 ± 0.04	a	0.59 ± 0.01	bc
T4	3.81 ± 0.09	a	0.47 ± 0.01	gh
T5	4.0 ± 0.05	a	0.47 ± 0.02	gh
T6	3.78 ± 0.09	a	0.48 ± 0.02	fgh
T7	3.11 ± 0.10	a	0.54 ± 0.01	cdef
T8	2.77 ± 0.02	a	0.60 ± 0.01	ab
T9	2.99 ± 0.07	a	0.55 ± 0.01	cde
T10	2.97 ± 0.06	a	0.59 ± 0.01	bcd
T11	2.50 ± 0.13	a	0.53 ± 0.01	cdef
T12	3.28 ± 0.03	a	0.48 ± 0.01	fgh
T13	2.54 ± 0.18	a	0.50 ± 0.01	efgh
T14	2.74 ± 0.02	a	0.52 ± 0.01	defg
T15	2.98 ± 0.08	a	0.54 ± 0.02	cdef
***LSD Value**	**0.240043**		**12.16163**	

COCLUSION

The results suggested that some of the growth parameters as leaf length, root length, shoot fresh and dry weight were affected by the combined foliar application of hormones. BAP alone showed maximum plant height, while maximum number of leaves and increased root dry weight was observed by the application of NAA alone. However, further research work is needed for meaningful recommendation.

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