STIMULATORY EFFECT OF NAA (NAPHTHALENE ACETIC ACID) AND BAP (BENZYL AMINOPURINE)ON FLOWERS, SEEDS, CHLOROPHYLL AND PROTEIN-CONTENT IN SPINACH *(SPINACIA OLERACEA* L.*)*

FARAN DURRANI*, MUHAMMAD SUBHAN*, SULTAN MEHMOOD*,
SAIRA ABBAS*, ASIM MUHAMMAD* and FAYYAZ CHAUDHARY**

* *Departments of Botany and Biotechnology, University of Science and Technology, Bannu.*
** *Department of Microbiology, Quaid-I-Azam University, Islamabad*

ABSTRACT

The current research work was conducted at the Faculty of Biological sciences, Quaid-i-Azam University, Islamabad during the year 2006. The study was based on to apply the growth regulators (NAA and BAP) in preflowering and flowering stages to estimate their effects on seed formation, seed setting, chlorophyll and protein contents in spinach (Spinacia oleracea L.). Highly significant value was concluded for number of bolts per plant through NAA at 10^{-3}M concentration. Non-significant result was obtained in case of treatment for seed yield although maximum seed yield was recorded with concentraion of BAP (10^{-3}M) and NAA (10^{-4}M). At the same time, seed weight was noted as significantly increased; whereas maximum seed weight was achieved for NAA 10^{-5}M. The present research work indicated that chlorophyll content was not affected by the application of BAP and NAA at different combinations and concentrations. For protein analysis the treatments in which NAA and BAP were applied in combination showed maximum variability in seed protein electrophoratic banding profile.

Key Words: **Foliar application, Growth regulators, NAA, BAP, flowers, seed, Chlorophyll and protein contents, Spinach (*Spinacia oleracea* L.)**

Citation: Durrani,. F, M. Subhan, S. Mehmood, S. Abbas, A. Muhammad and F. Chaudhary. 2010. Stimulatory effect of NAA (Naphthalene acetic acid) and bap (benzyl aminopurine) on flowers, seeds, chlorophyll and protein-content in spinach (spinacia oleracea l). Sarhad J. Agric. 26(4): 519-526

INTRODUCTION

Spinach (*Spinacia oleracea* L.) belongs to the family Chenopodiaceae. It is an annual herb. The flowers are inconspicuous, greenish- white, and are borne in clusters on a spike. The female flowers develop into seed like fruits. Male plants usually bolt earlier comparatively to female, which are wind pollinated. Spinach becomes reproductive in response to the day length and temperature conditions. As day length reaches 15 hours, the seed stalk develops which is accelerated in 40-50^{o}F temperatures. Spinach is widely cultivated in the world as it is one of the most popular vegetables. Spinach is highly nutritious and it is second to kale in total carotenoids and folate in diet ranking (Holden *et al*., 1999 and USDA, 2003).

Application of nutrients through foliar spray enhances crop nutrition in both conventional and alternative production systems. Observed effects of foliar fertilization have included yield increases, resistance to diseases and insect pests, improved drought tolerance, and enhanced crop quality **(**Kalpana, and Krishnarajan, 2003). Plant reaction is dependent on species, fertilizer form, concentration, and frequency of application, as well as the stage of plant growth. Foliar applications are used to help plants in recovery from transplant shock, hail damage and weather extremes. They are also used to control bitter pit and cork spot in apples (Greene *et al.,* 1995) and also for general supplementary nutrition in strawberries (Deremiens, 1995). Foliar application of hormones is being used on a wide variety of crops i.e. horticultural and agronomic crops. But it is highly used for horticultural crops because horticultural crops are of higher value and their nutrient status is more carefully examined. Foliar sprays are also commonly suggested to correct zinc deficiencies in grapes (Williams and Williams, 1986).

Spinach is an important vegetable crop of the world. It is the crop of poor people. It has high amount of nutrients. It contains vitamin A, C, K, and large amount of proteins and iron. Therefore, it is the best source of protein, iron, and vitamin. The present research work was based on to know the effect of growth regulators on flowers, seeds, chlorophyll and protein content of spinach.

MATERIALS AND METHODS

A series of experiments were planned and conducted in February, 2006 at the Faculty of Biological sciences, Quaid-i-Azam University, Islamabad. The research work was carried out to study the effects of foliar application of Naphthalene acetic acid (NAA) and Benzyl aminopurine (BAP) on growth of flowers, stimulation of seeds setting and protein contents in Spinach (*Spinacia oleracea* L.). First foliar spray of hormones was performed 20 days after mean germination. Second was made 40 days after sowings (DAS) and third was applied 60 days after sowing. The pots were watered using a rose cane so as to avoid packing of the soil and washing away of seeds. The pots were irrigated three times a week and were constantly cleaned up to make them free from weeds. Floral parameters were observed and studied at two different stages (40 and 60 DAS) of the life cycle of spinach.

Data was recorded on the following parameters.

Number of Bolts per Plant

Number of bolts per plant from each treatment were counted and recorded.

Seed Yield

After harvesting the plants, seeds were obtained from each of the selected plant and were weighed on electrical weight balance in grams.

1000-Seeds Weight

Seeds from each treatment were collected and weighed in grams with the help of electrical weight balance. For 1000 seed weight, first weight of 100 seeds was recorded. It was multiplied with 10 to get 1000 seeds weight.

Chlorophyll Content

Chlorophyll content was measured at 60 DAS (days after sowing). Total amount of chlorophyll was determined by SPAD-502 chlorophyll content meter in the intact leaves of plants. This portable digital equipment was utilized for quick measurement of chlorophyll content without damaging of leaves.

Total Seed Protein Analysis

For total seed protein analysis, seeds were grounded to fine power. Protein extraction buffer (400 µl) was added to 0.01g of seed flour as extraction buffer and mixed well with Automatic Lab-Mixer DH-10. The extraction buffer contained 0.5M Tris HCl (Ph. 6.8), 2.5% SDS, 10% glycerol and 5% 2-mecraptoethanol. Bromophenol Blue (BPB) was added to the protein extraction buffer as tracking dye to watch the movement of protein in the gel. In order to check the reproducibility of the method two separate gels were run under similar electrophoretic conditions. The molecular weight of the dissociation polypoides were determined by using molecular weight protein sanders" MW-SDS-70 Kit (Sigma). The SDS-PAGE of total seed protein was carried out in the discontinuous buffer system. The SDS-PAGE is considered to be a particularly reliable method because storage proteins are largely independent of environmental fluctuations (Nisar *et al.,* 2006 and Nisar *et al.,* 2007). After staining and de-staining, the gel was dried using (Atto, Rapidery-Mini Japan) gel drier. The data were scored on the presence (+) or absence (-) of protein bands and intensity of glow was considered as major bands and low intensity as miner bands, similarity index was calculated for all possible pairs of protein types (Nisar *et al.,* 2007). To avoid taxonomic weighing, the intensity of bands was not taken into consideration, only the presence of the bands was taken as indicative. The similarity matrix thus generated was converted to a dissimilarity matrix and used to construct dendrogram by the UPGMA (Sneath and Sokal, 1973).

Concentration and Combination of Phytohormones Used

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

1^{st} concentration=10^{-3} M
2^{nd} concentration=10^{-4} M
3^{rd} 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 with the 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^{-5} M were prepared by dilution method.

$$\text{Weight Required} = \frac{\text{Molarity} \times \text{Molecular weight} \times \text{Volume Required}}{1000}$$

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 result obtained from the present study revealed that application of phytohormones affected formation of flowers and seeds in spinach.

Table-II ***Effect of foliar application of NAA and BAP on number of bolts (Flowers) per plant of*** *Spinacia oleracea L.*

Treatments	No of Bolts (Flowers) Per Plant Mean ± SE	LSD Rank
T0 (control)	8.33 ± 0.33	g
T1	12.33 ± 0.33	a
T2	12.0 ± 0.58	ab
T3	11.33 ± 0.33	abcd
T4	9.00 ± 0.58	efg
T5	8.66 ± 1.20	fg
T6	8.66 ± 0.33	fg
T7	11.0 ± 0.00	abcd
T8	10.0 ± 0.58	cdefg
T9	9.66 ± 1.20	defg
T10	10.66 ± 0.88	abcde
T11	9.66 ± 1.20	defg
T12	11.0 ± 0.00	abcd
T13	10.33 ± 0.33	bcdef
T14	11.66 ± 0.33	abc
T15	8.66 ± 0.67	fg
***LSD Value**	**1.95704**	

Number of Bolts (Flowers) per Plant

The data regarding mean values for number of bolts per plant showed that the plants are highly affected by the treatments (table II). There were significant variations among the treatments. Maximum value for number of bolts was observed for NAA at 10^{-3} M concentration. Early flowering was shown by the combined effect of NAA (10^{-3} M) and BAP (10^{-4} M). These results confirmed the findings of Mclaughin and Greene (1991) and Zeroni and Hall (1984) who reported that growth regulator NAA affect and induce early flowering.

Seed Yield

Seed yield per plant was determined at the end of the experiment. Non-significant variations observed among the treatments (shown in table III). Maximum seed yield was observed both for BAP and NAA at a concentration of (10^{-3} M) and (10^{-4} M). In early studies it has been investigated that the auxin (NAA) has greater role in increasing seed yield. In the present study combined affect of BAP and NAA was observed as affective. Similar findings have been reported by Upadhyay, (1994) and Kalpana and Krishnarajan, (2003).

Table-III ***Effect of foliar application of NAA and BAP on seed yield***

Treatments	**Seed Yield Per Plant (g) Mean ± SE**	**LSD Rank**
T0 (control)	1.74 ± 0.10	b
T1	1.89 ± 0.02	ab
T2	2.17 ± 0.17	a
T3	1.83 ± 0.02	ab
T4	1.77 ± 0.02	ab
T5	1.93 ± 0.04	ab
T6	1.90 ± 0.11	ab
T7	2.08 ± 0.09	ab
T8	2.01 ± 0.10	ab
T9	1.88 ± 0.03	ab
T10	2.18 ± 0.38	a
T11	1.72 ± 0.04	b
T12	2.09 ± 0.08	ab
T13	1.77 ± 0.44	ab
T14	1.76 ± 0.07	ab
T15	2.06 ± 0.09	ab
***LSD Value**	**0.423783**	

Seed Weight

It is evident from table (IV) that out of 16 treatments, 15 treatments increased the 1000-seed weight. Maximum seed weight was observed for NAA (9.20g) at (10^{-5} M) concentration. Both NAA and BAP have increased the seed weight as compared to control. The findings regarding seed weight are quite in conformation to Alagukannan and Vijay Kumar, (1999) and Kalpana and Krishnarajan, (2003).

Table-IV ***Effect of foliar application of NAA and BAP on 1000-seed wt of*** *Spinacia oleracea L.*

Treatments	**1000 –Seed Weight Mean ± SE**	**LSD Rank**
T0 (control)	**8.13 ± 0.15**	**gh**
T1	8.6 ± 0.06	cdef
T2	8.96 ± 0.12	ab
T3	9.2 ± 0.15	a
T4	8.66 ± 0.09	bcde
T5	8.6 ± 0.12	cdef
T6	8.9 ± 0.06	abc
T7	8.0 ± 0.06	h
T8	8.66 ± 0.15	bcde
T9	8.56 ± 0.19	cdef
T10	8.73 ± 0.15	bcd
T11	8.46 ± 0.15	defg
T12	8.33 ± 0.07	efgh
T13	9.1 ± 0.06	a
T14	8.3 ± 0.20	fgh
T15	8.4 ± 0.12	defg
***LSD Value**	**0.369751**	

Chlorophyll Content

Changes in chlorophyll contents occur due to nutrient deficiency, environmental stress, and exposure to herbicide. Non significant variations were observed for chlorophyll content (table V). The highest value for chlorophyll content was (46.85), in which BAP (10^{-5}M) was applied to the plants.

Table V ***Effect of Foliar Application of NAA and BAP on Chlorophyll Content of*** *Spinacia oleracea L.*

Treatments	**Chlorophyll Content Mean ± SE**	**LSD Rank**
T0 (control)	38.74 ± 0.36	ab
T1	40.98 ± 1.21	a
T2	42.21 ± 1.12	a
T3	42.84 ± 0.10	b
T4	45.49 ± 0.39	a
T5	46.6 ± 0.69	a
T6	46.85 ± 0.24	a
T7	43.76 ± 0.31	a
T8	45.84 ± 0.67	a
T9	43.9 ± 0.69	a
T10	39.78 ± 0.51	a
T11	39.95 ± 0.29	a
T12	45.05 ± 0.38	a
T13	44.09 ± 0.05	a
T14	42.94 ± 0.61	a
T15	40.65 ± 0.85	a
***LSD Value**	**12.16163**	

Protein Content

During protein analysis 16 bands were recorded (Fig. I) and out of these six bands (B6, B7, B9, B11, B15 and B16) were monomorphic, while remaining banding profile showed polymorphism (Table VI), which indicating the presence (1) and absence (0) of protein bands for each cluster. Cluster analysis (Table VI) sorted the different treatments of spinach into two lineages at linkage distance 4.5. At 50% linkage distance the two lineages were further divided into 4 clusters. Among these, Lineage 1 was divided into two clusters, the C1 (cluster 1) and C2 (cluster 2), cluster 1 comprises of T10, T5 and T15, while cluster 2 encircle T13, T9, T14, T8, T11, T6 and T4. Lineage 2 has also been divided into two clusters (C3 and C4). Cluster 3 grouped T12 and T7, while cluster 4 has T1, T3, T2 and T0.

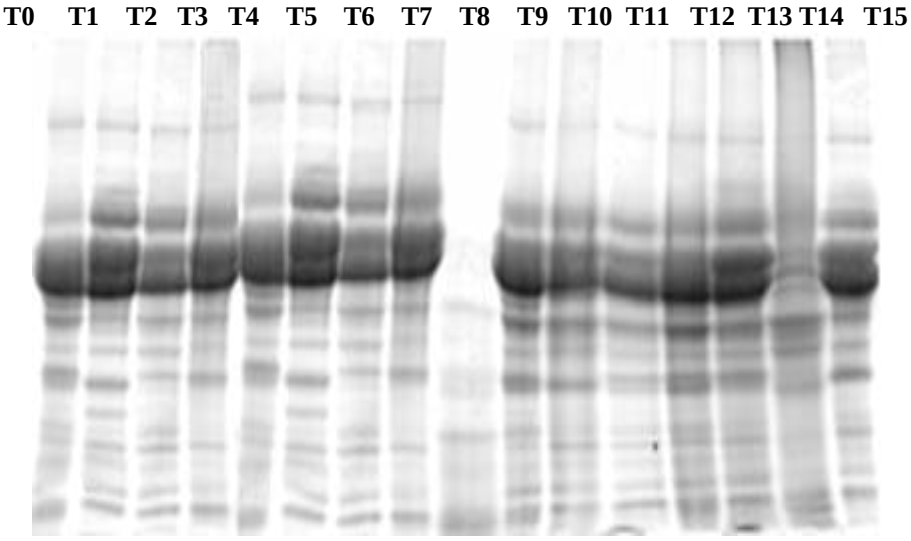

Fig I: Electrophorogram showing seed protein banding profile in Spinacia oleracea L.

Cluster 1, consisting of three treatments BAP and NAA at 10^{-3}M and 10^{-4}M, BAP at 10^{-4}M and BAP and NAA) at 10^{-5}M (each) showed the lowest degree of Euclidian distance and highest degree of genetic variation in banding profile, cluster 2 and cluster 3 also have seven and two treatments respectively. Cluster 2 contained NAA (10^{-4} M) and BAP (10^{-5}M), NAA (10^{-3}M) and BAP (10^{-5}M), BAP (10^{-4}M) and NAA (10^{-5}M), NAA (10^{-4}M) and BAP (10^{-3-4}M), BAP (10^{-3}M) and NAA (10^{-5}M), BAP at 10^{-5}M and 10^{-3}M concentrations. Cluster 3 has NAA and BAP at 10^{-4}M (each) and NAA and BAP at 10^{-3}M (each). Cluster 2 and cluster 3 have treatments of different foliar application of growth regulators and having moderate level of variability. While cluster 4 has highest degree of Euclidian distance, but showed lowest degree of variability (Fig. II). Cluster 4 having treatments of NAA at 10^{-3}M, NAA at 10^{-5}M, NAA at 10^{-4}M and control (having zero concentration of hormones). From the present work it was concluded that foliar application of growth regulators individually have no such clear effect upon the genetic makeup of the plant, while in combination their effect was impressive and showing effect on plant genetic make up. The treatments in which NAA and BAP were applied in combination showed better results.

Table-VI ***Banding Profile in 16 treatments of Spinach***

Bands	To	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15
B1	0	0	0	0	1	1	1	1	1	0	1	1	1	1	1	1
B2	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0
B3	0	1	0	0	0	1	0	1	0	0	0	0	0	1	0	1
B4	0	1	0	0	1	1	1	0	1	1	1	1	1	1	1	1
B5	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1
B6	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
B7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B8	1	0	0	0	1	0	0	1	0	1	0	0	1	0	0	0
B9	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1
B10	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0
B11	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1
B12	0	1	0	0	0	1	0	1	0	0	1	0	1	0	0	0
B13	1	0	1	0	1	1	1	1	0	1	1	1	0	1	0	1
B14	1	1	1	1	1	1	1	0	1	0	0	1	1	1	1	1
B15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table-VII ***Dendrogram based on SDS- PAGE analysis showing average linkage in Spinacia oleracea*** *L.*

Lineage 1				**Lineage 2**			
Cluster 1		**Cluster 2**		**Cluster 3**		**Cluster 4**	
Treat.	**Hormones & Conc.**	**Treat.**	**Hormones & Conc.**	**Treat.**	**Hormones & Conc.**	**Treat.**	**Hormones & Conc.**
T10	(BAP+NAA) 10^{-3-4}M	**T13**	(NAA+BAP) 10^{-4-5}M	**T12**	(NAA+BAP) 10^{-4-4}M	**T1**	(NAA) 10^{-3}M
T5	(BAP) 10^{-4}M	**T9**	(NAA+BAP) 10^{-3-5}M	**T7**	(NAA+BAP) 10^{-3-3}M	**T3**	(NAA) 10^{-5}M
T15	(NAA+BAP) 10^{-5-5}M	**T14**	(BAP+NAA) 10^{-4-5}	*	*	**T2**	(NAA) 10^{-4}M
*	*	**T8**	(NAA+BAP) 10^{-3-4}M	*	*	**T0**	(Control)
*	*	**T11**	(BAP+NAA) 10^{-3-5}M	*	*	*	*
*	*	**T6**	(BAP) 10^{-5}M	*	*	*	*
*	*	**T4**	(BAP) 10^{-3}M	*	*	*	*

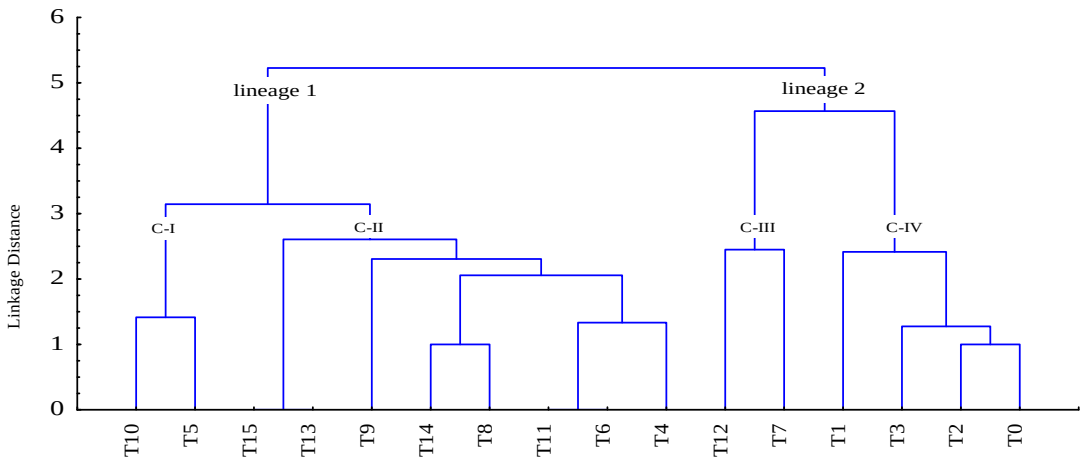

Fig. II ***Dendrogram based on SDS-PAGE analysis using Ward's Method in*** *Spinacia oleracea L.*

COCLUSIONS AND RECOMENDATIONS

From the present research work it was clearly concluded that some of the growth parameters were strongly affected by the foliar application of hormones. The parameters which showed significant results were number of bolts per plant and seed weight. While non significant results were achieved in case of seed yield and chlorophyll content. Moreover, maximum variability in seed protein electrophoratic banding profile was noted which wants to be exploited in the improvement of protein content in spinach.

REFERENCES

Alagukannan, G. and M. Vijaykumar. 1999. Effect of plant growth substances in yield attributing parameters, yield and quality in fenugreek. South Indian Horti. 47(1-6): 130-133.

Deremiens, J. 1995. Foliar feeding strawberries. Northland Berry News. June. pp: 8–9.

Greene, L., M. George and R. Crassweller. 1995. Correct calcium disorders. Fruit Grower. Oct. pp. 19–20.

Holden, J.M., A.L. Eldridge, G.R. Beecher, I.M. Buzzard, S.A. Bhagwat, C.S. Davis., L.W. Douglass, S.E. Gebhardt, D.B. Haytowitz and S. Schakel. 1999. Carotenoid content of U.S. foods: an update of the database. J. Food Comp Anal, 12: 169–96.

Kalpana, R. and J. Krishnarajan, 2003. Effects of combined application of nutrients and hormones on soybean yield. Deptt. of Agro. Tamil Nadu Agric. Univ. Coimbatore, India. Legume Res. 26(2): 151-152.

Mclaughin, J.M. and D.W. Greene, 1991. Fruit and hormone influence flowering of Apple. J. Amer. Soc. Horti. Sci. 116(3): 446-449.

Nisar, M., A., Ghafoor, M. R. Khan, and A.S. Qureshi. 2006. Screening of *Pisum sativum* germplasm against *Erysiphe pisi*. Syd. Acta biologica. Cracoviensia series botanica. 48/2 pp 33-37

Nisar, M., A., Ghafoor, M. R. Khan, H. Ahmad., A.S. Qureshi and H. Ali. 2007. Genetic diversity and geographic relationship among local and exotic chickpea germplasm. Pak J. Bot. 9(5): 1575-1581.

Sneath, P.H.A. and R.R. Sokal. 1973. Numerial Taxanomy: The principal and practical of numerical classification. Freeman, San Francisco. pp 572-573

Steel, R.G.D. and J.H.Torrie. 1984. Principles and procedures of statistics. McGraw Hill Book Co., Inc., Singapore. pp: 173-177.

Upadhyay, R.G. 1994. Effect of bioregulators on growth, development, flowering behavior and yield of chickpea (*Cicer arietinum* L.). Legume Res. 17(1): 60-62.

U.S. Deptt. of Agric. Agric Res. Service. 2003. Bethseda, Md.: USDA Natl. Nutrient Database for Standard Reference. Release 16. Nutrient Data Laboratory Home Page. Available from: http://www.nal.usda.gov/fnic/foodcomp. Accessed, 12 Aug. 2003.

Williams, G. and Williams, P. (1986). Zinc foliar sprays need not be fancy, at least not for grapes. HortIdeas?. March. pp: 32.

Zeroni, M. and Hall, M.A. (1984). Molecular effects of hormonal treatments on tissue. In: Mac. Millon J. (Edit.). Hormonal regulation of development. Encycl. of Plant Physiol, N.S. (9): 511-586.