ISSN : 1812-5379 (Print)
ISSN : 1812-5417 (Online)
http://ansijournals.com/ja

JOURNAL OF AGRONOMY

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Journal of Agronomy 8 (3): 93-99, 2009
ISSN 1812-5379

Effects of Flurenol on Soybean (*Glycine max* L. Merrill) Productivity and Electrophoretic Analysis of Seed and Root Nodule Proteins

[1,2]Y.M. Awad, [2]W.M. Hassan and [1]Y. Sik Ok
[1]Department of Biological Environment, College of Agriculture and Life Sciences,
Kangwon National University, Chuncheon 200-701, Korea
[2]Department of Agricultural Botany, Faculty of Agriculture, Suez Canal University, Ismailia, 41522, Egypt

Abstract: Recently, numerous studies have been conducted to evaluate the development of soybean crop productivity and the formation of root nodules with respect to the management of nitrogen fertilizers. Objective of this research was to evaluate the effects of flurenol foliar application on the productivity and root nodules formation of soybean (*Glycine max* L. Merrill) plants and the total protein patterns in seed and root nodules. Soybean (*Glycine max* L. Merrill) plants cv. Crawford were cultivated in newly reclaimed sandy soil during two growing seasons and treated by foliar application of flurenol at 50, 100 and 200 mg L^{-1} at 40 Days after Sowing (DAS). Furthermore, electrophoretic analysis of the seeds and root nodules was conducted to investigate the total protein bands and their similarity using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) method. Most flurenol treatments resulted in significantly decreased plant height and increased the number of branches. Furthermore, the number of root nodules and the dry weight of the plants significantly increased in response to treatment with 100 mg L^{-1} flurenol, with up to 47.67 and 35.67 root nodules and root nodule dry weights of 0.2287 and 0.1777 g being observed in 2006 and 2007, respectively. Evaluation of the root nodule protein patterns revealed a dissimilarity of 33.33% between all flurenol treatments and untreated plants. Based on these results, flurenol treatment at 100 mg L^{-1} with inoculating seeds with *Bradyrhizobium japonica* is recommended to enable the reduction of N fertilizers and increase the yield productivity of soybean cv. Crawford in Egypt.

Key words: Flurenol, soybean, nodulation, seed and nodule protein profiles, yield component

INTRODUCTION

Fabaceae is an important family of angiosperms that includes 650 genera and 18.000 species of higher plants (Young *et al*., 2003). One member of this family, soybean (*Glycine max* L. Merrill), has become one of the most important protein and oil crops. The development of an effective soybean cropping system is required to meet the current shortage in proteins and vegetable oils. Also, soybean seeds have 35-40% protein content on a dry weight basis (Derbyshire *et al*., 1976; Mujoo *et al*., 2003). Therefore, soybean (*Glycine max* L. Merrill) production in Egypt has increased to meet the demands of humans and live stock.

Increased attention has been raised regarding the role of auxin in apical dominance with respect to the degree of branching as a possible indicator of auxin activity (Shen *et al*., 1990; Estruch *et al*., 1991; Klee and Romano, 1994). Consequently, auxin controls shoot apical dominance either directly or indirectly via auxin apically driven into lateral buds, which leads to subsequent suppression of outgrowth in addition to other mechanisms (such as auxin-cytokinin ratio, secondary growth substances, nutrient diversion, etc.) (Tamas, 1987; Cline, 1994; Bangerth, 1994; Stafstrom, 1995; Ishikawa *et al*., 1997).

Polar auxin transport plays a role in the regulation of development by influencing cell growth, cell differentiation and organogenesis in plants, as well as the responses of cells, tissues and organs to internal and external stimuli (Morris, 2000). Accordingly, application of growth retardants can be used to maintain the balances of internal hormones and create efficient sink-source relationships that enhance crop productivity. Most growth retardants act by inhibiting GA biosynthesis (Kim *et al*., 2003).

The morphactin has recently generated interest due to its ability to enhance plant cell production of secondary metabolites with many health benefits including antioxidant and anti-cancer properties

Corresponding Author: Yong Sik Ok, Department of Biological Environment, Kangwon National University, Chuncheon 200-701, Korea Tel: 82-33-250-6443, 82-10-8951-6242 Fax: 82-33-241-6640

(Mathur and Ramawat, 2008: Roat and Ramawat, 2009). Morphactins are synthetic bioregulants that have a high morphogenetic potency and influence the stages of plant growth and development (El-Desoki *et al*., 1994; Mathur *et al*., 2007; Tanwar *et al*., 2007; Mathur and Ramawat, 2008). Also, flurenol is one of the morphactin compounds that have growth-regulating action (Ogura, 1975).

Rhizobium-legume symbiosis is important to natural and agroecosystems under conditions of low soil nitrogen. Furthermore, nitrogen-fixing bacteria infect the roots of legumes and induce the formation of root nodules (Dalton *et al*., 2009). Auxin transport inhibitors induced the formation of nodule-like structures that resembled *Rhizobium*-induced nodules (Hirsch, 1992). For example, Mathesius *et al*. (1998) reported that auxin transport inhibition preceded root nodule formation in white clover (*Trifolium repens* L.) plants. Therefore, nodulating bacteria rapidly induced in expression of an auxin-responsive reporter gene at the site of nodule formation (Hirsch, 1992; Mathesius *et al*., 1998). So, the proposed research objective is based on the role of morphactin in the regulation of polar auxin transport to enhance soybean root nodule formation for the reduction of nitrogen chemical fertilizers.

Consequently, this study was conducted to evaluate the effects of flurenol foliar application on soybean productivity and root nodule formation of plants cultivated under high density in newly reclaimed sandy soil. Additionally, total protein fractions in seeds and root nodules were used as a useful tool to evaluate the studied morphactin treatments.

MATERIALS AND METHODS

Field experiments were conducted in newly reclaimed sandy soil using soybean plants (*Glycine max* L. Merrill) cv. Crawford at the Experimental Farm of Suez Canal University during 2006 and 2007 in Egypt. The seeds were obtained from the Legume Research Division, Field Crops Institute, Agricultural Research Centre, Giza, Egypt. Foliar application of the plant growth retardant, flurenol at 50, 100 and 200 mg L^{-1} was conducted at 40 Days after Sowing (DAS) and the effects of the treatments on seed and root nodule proteins patterns were evaluated using SDS-PAGE. In addition, the effects of the treatments on plant growth, yield and its components were evaluated (average number of pods $plant^{-1}$ and average number of seeds $plant^{-1}$). In addition, control plants that were sprayed with tap water were also evaluated.

Field experiment: The trial was conducted in a plot with an area of 7.2 m^2 (split plot design with three rows; 4 m in length and 60 cm in width). During seed-bed preparation, conventional applications (35.7 kg ha^{-1} of P_2O_5 as calcium superphosphate and 57.12 kg $ha^{-1}$of K_2O as potassium sulphate) were done. Soybean seeds were inoculated with *Bradyrhizobium japonica* and then they were sown in hills spaced 20 cm apart on two sides of the row (high plant population density). The stands were then thinned to two plants per hill. Prior to the second irrigation, 33.2 kg N ha^{-1} as ammonium nitrate was dressed beside the plants, after which other practices were done as recommended to region.

Vegetative and some yield components: Random plants were collected from each treatment group from the middle of the plot during the harvest stage (110 DAS) and used to record both vegetative characteristics (such as plant height, number of branches and dry weight of the plant shoot+root) and some yield components (such as number of pods $plant^{-1}$ and number of seeds $plant^{-1}$) of soybean cv. Crawford during 2006 and 2007. In addition, the number and dry weight of the nodules were recorded at 60 DAS.

Extraction of total proteins: Fifty milligrams of soybean seed cotyledon flour collected at 80 DAS (0.5 g of the powder was defatted twice with 25 mL 70% ethanol for 10 min each) and root nodules taken at 60 DAS were extracted in 400 µL of 0.05 M Tris [Tris(hydroxymethyl) aminomethane] buffer (pH 8.0) containing 0.01 M EDTA [ethylenediaminetetraacetic acid] and 5 mM cysteine. The samples were then centrifuged at room temperature for 20 min at 6000 rpm, after which 10 µL of the extracted protein was mixed with 10 µL of SDS-sample buffer (0.15 M Tris-HCl, pH 6.8, 4% SDS (Sodium dodecyl sulfate), 5% β-mercaptoethanol) and heated at 96°C for 3 min as described by Mujoo *et al*. (2003). Finally, 100 µL of 50% glycerol in addition to a trace amount of bromophenol blue were added to the samples.

SDS polyacrylamide gel electrophoresis of proteins: Ten microliters of extracted protein was subjected to SDS-PAGE in a 10% acrylamide slab gel at constant voltage (150 V) for 1 h, followed by 90V for 2 h until the tracking dye migrated to the bottom edge of the gel using the method described by Laemmli (1970) and Walker (2002).

Gel staining solution: Staining solution (2 g Coomassie Brilliant Blue R 250, 0.5 g Coomassie Brilliant Blue G250, 425 mL anhydrous ethanol (99.9%), 50 mL methanol (≥99%), 100 mL glacial acetic acid (≥99%) and 425 mL sterilized deionized water) was used to stain the slab gels after electrophoretic separation of both the seed and root nodule total protein banding patterns. The gel was then

immersed in fast destaining solution (45% ethanol, 10% acetic acid and 75% sterilized deionized water) for 2 h (the destaining solution was changed 3-5 times), after which the gel was rinsed three times using sterilized deionized water and then preserved in 7% glacial acetic acid in sterilized deionized water for 24 h (Biometra, 1995).

Statistical analysis: Relative molecular weight (M_r) of polypeptide bands was estimated by SDS-PAGE using Pharmacia (Germany) calibration kit of low protein molecular weight (LMW) that contains phosphorylase b (94 KDt), albumin (67 KDt), ovalbumin (43 KDt), carbonic anhydrase (30KDt), trypsin inhibitor (20.1 KDt) and α-lactalbumin (14.4 KDa). Differences in the seed and root nodule proteins were evaluated based on the individual band frequency for each accession. Therefore, a similarity matrix based on Jaccard's coefficient was determined using the UPGMA (Unweighted Pair Group Method with Arithmetic mean) based on each protein marker (Rohlf, 1993). One-way ANOVA was used for data analysis and the means were compared using Tukey's multiple range test using SAS 9.1 (SAS, 2003).

RESULTS

The effects of various concentrations of flurenol on growth parameters, the number and dry weights of root nodules and the protein patterns of seeds and root nodules of soybean (*Glycine max* L. Merrill) cv. Crawford were evaluated throughout two sets of field experiments conducted in 2006 and 2007. The results obtained are as follows:

Vegetative and some yield components: As shown in Table 1, treatment with all concentrations of flurenol led to a significant decrease in the length of the main stem when compared with untreated plants (being 50.4 and 38.7 cm in 2006 and 2007, respectively). The shortest length observed was 38.7 cm in response to treatment with 50 mg L^{-1} flurenol during the first cultivation season (2006). A similar trend was found during the second season (2007), with the shortest length observed being 34.4 cm in response to 200 mg L^{-1} flurenol. Moreover, foliar application of flurenol led to a significant increase in the number of lateral branches when compared with untreated plants. Specifically, plants treated with 200 and 100 mg L^{-1} flurenol in 2006 and 2007 had 3.6 and 3.13 branches $plant^{-1}$, respectively. Furthermore, ANOVA revealed that there were significant differences in the shoot dry weight of plants treated with 200 mg L^{-1} flurenol treatment by 16.53 g when compared with the control plants in 2006, but no other significant differences in shoot dry weights were observed in either season. Evaluation of the root dry weight revealed a similar trend in response to flurenol treatment as observed in the shoot dry weight.

Table 1: The effects of flurenol treatments effects on soybean (*Glycine max* L. Merrill) cv. Crawford growth parameters during 2006 and 2007

			Morphactin concentrations (mg L^{-1})		
Parameter	Season	Control	50	100	200
Average plant height (cm)	2006	50.400a	38.700b	43.700ab	46.500a
	2007	38.700a	35.100b	34.800b	34.400b
Average number of branches $plant^{-1}$	2006	2.800d	3.200c	3.400b	3.600a
	2007	2.130bc	3.000ab	3.130a	1.750c
Average shoot dry weight (g)	2006	13.470b	11.000b	12.270b	16.530a
	2007	6.730a	8.400a	9.470a	7.330a
Average root dry weight (g)	2006	3.200a	3.200a	3.700a	4.100a
	2007	3.000ab	3.700a	2.900ab	2.600b
Average number of pods $plant^{-1}$	2006	15.100a	27.900a	29.800a	34.800a
	2007	15.100a	19.800a	16.300a	18.000a
Average number of seeds $plant^{-1}$	2006	54.900a	57.500a	59.300a	71.600a
	2007	23.300a	38.300a	39.900a	33.600a
Average number of nodules $plant^{-1}$	2006	12.670b	17.670b	47.670a	20.330b
	2007	8.330b	15.330b	35.670a	17.330b
Average nodules DW $plant^{-1}$	2006	0.132b	0.136b	0.229b	0.073b
	2007	0.039b	0.108ab	0.178a	0.053b

Means followed by the same letter within a row are not significantly different at the 0.05 probability level

As shown in Table 1, the average number of pods $plant^{-1}$ and seeds $plant^{-1}$ increased for all treatments, but these increases were not significant in either season.

Soybean nodulation: Table 1 shows the number of root nodules in plants treated with flurenol during 2006 and 2007. Statistical analysis illustrated that the number of root nodules increased significantly in response to treatment with 100 mg L^{-1} flurenol. The greatest numbers of root nodules number were 47.67 and 35.67, which were observed in plants treated with 100 mg L^{-1} flurenol in 2006 and 2007, respectively. In addition, similar trends were observed in the dry weight of the root nodules, with values of 0.229 and 0.178 g being observed in plants treated with 100 mg L^{-1} flurenol in 2006 and 2007, respectively.

Seed and root nodule protein patterns

Total (seed) protein: The SDS-PAGE data describing the total seed protein fractions from plants treated by flurenol foliar application are shown in Fig. 1 and 2. The total seed protein banding pattern appeared to be similar among all treatments, including the controls.

The results of UPGMA clustering analysis of the total protein fractions are shown in Fig. 2. Based on the similarity of polymorphisms, there were two main clusters in which all treatments and their control were located at

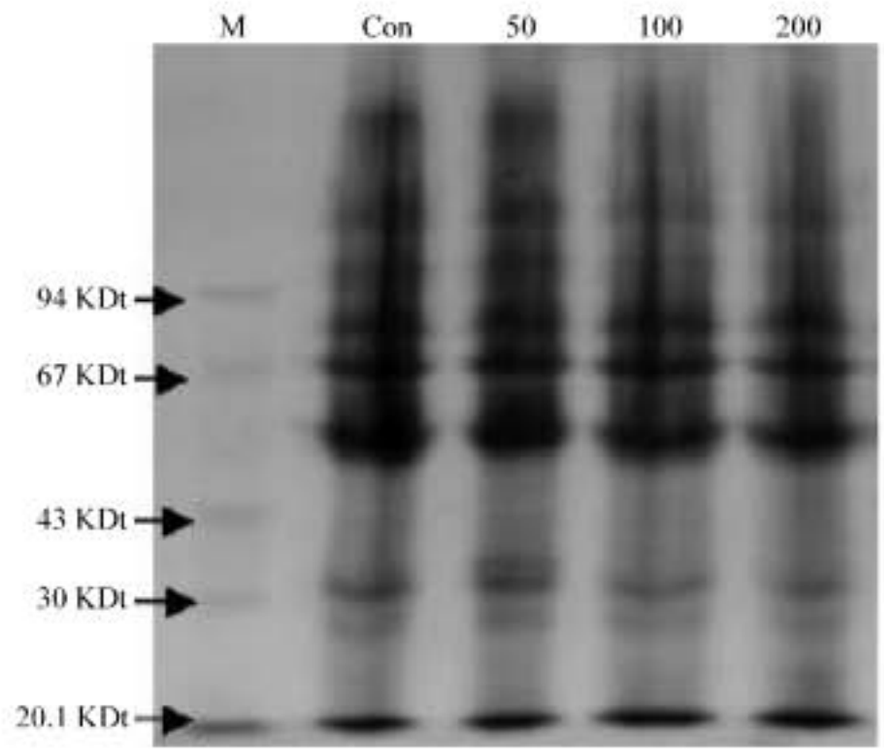

Fig. 1: Electrophoregram of the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of soybean cv. Crawford seed protein patterns in plants subjected to different flurenol treatments: M: Protein marker, Con: Control, 50: Flurenol at 50 mg L^{-1}, 100: Flurenol at 100 mg L^{-1}, 200: Flurenol at 200 mg L^{-1}

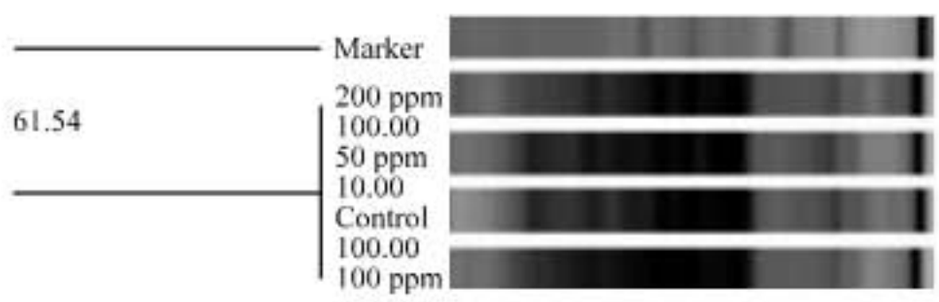

Fig. 2: Dendrogram produced by UPGMA cluster analysis based on the similarity polymorphism of seed protein patterns of soybean cv. Crawford subjected to different flurenol treatments obtained by SDS-PAGE. UPGMA clustering using simple band match (Tolerence: 3.20%) Jan 16 2009

one cluster with a similarity 100% and a dissimilarity 38.46% with protein marker which was located in the second cluster. These findings demonstrate that there were no significant differences in the total protein content of seeds from plants that were subjected to different treatments.

Root nodule total protein: The SDS-PAGE results of the total protein fractions in the root nodules of plants treated by flurenol foliar application are shown in Table 2 and Fig. 3 and 4. Variations in the total protein fractions were observed among groups, with treated plants producing 6 bands and the controls producing 3 bands.

Two polymorphic banding patterns of the total proteins in soybean root nodules of plants treated with

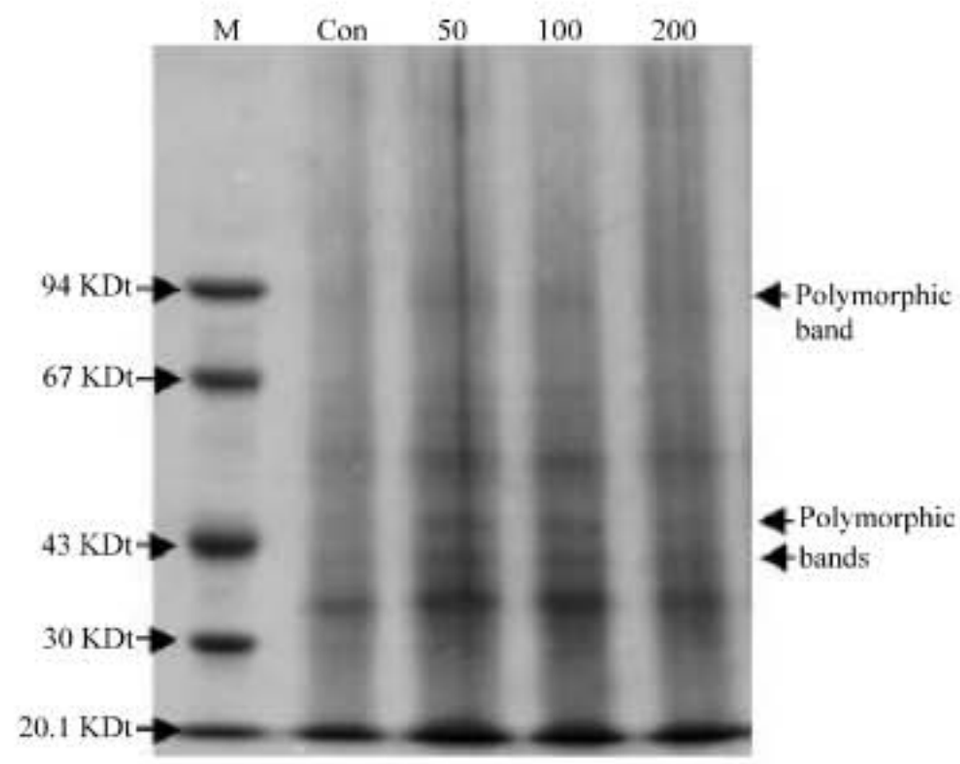

Fig. 3: Electrophoregram of the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) showing root nodule protein patterns of soybean plants subjected to different flurenol treatments: M: Protein marker, Con: Control, 50: Flurenol at 50 mg L^{-1}, 100: Flurenol at 100 mg L^{-1}, 200: Flurenol at 200 mg L^{-1}

Table 2: Protein banding patterns and distribution of soybean (*Glycine max* L. Merrill) cv. Crawford root nodules as determined by SDS-PAGE

				50	100	200
Band No.	M_r	Marker	Control	----------	(mg L^{-1})	----------
1	94	33.85(+)	0.00(-)	0.00(-)	0.00(-)	0.00(-)
2	90	0.00(-)	0.00(-)	0.00(-)	15.14(+)	0.00(-)
3	89	0.00(-)	0.00(-)	16.30(+)	0.00(-)	15.16(+)
4	67	22.70(+)	0.00(-)	0.00(-)	0.00(-)	0.00(-)
5	56	0.00(-)	34.33(+)	26.00(+)	0.00(-)	0.00(-)
6	55	0.00(-)	0.00(-)	0.00(-)	26.31(+)	24.61(+)
7	47	0.00(-)	0.00(-)	4.15(+)	0.00(-)	3.73(+)
8	46	0.00(-)	0.00(-)	0.00(-)	4.50(+)	0.00(-)
9	43	19.74(+)	0.00(-)	0.00(-)	0.00(-)	0.00(-)
10	42	0.00(-)	0.00(-)	6.56(+)	2.58(+)	2.27(+)
11	36	0.00(-)	0.00(-)	42.16(+)	44.12(+)	9.20(+)
12	35	0.00(-)	58.22(+)	0.00(-)	0.00(-)	0.00(-)
13	30	12.44(+)	0.00(-)	0.00(-)	0.00(-)	0.00(-)
14	20	11.27(+)	7.45(+)	0.00(-)	0.00	45.03(+)
15	19	0.00(-)	0.00	4.83(+)	7.35(+)	0.00(-)
Total banding		5	3	6	6	6

+: Present, -: Absent

100 mg L^{-1} flurenol were observed, while only one band existed in the control (Table 2). Moreover, the results shown in Table 2 demonstrated that the highest total amount of protein present in the bands were 42.16 and 44.12% in band No. 11 (molecular weight 36 KDt) for plants treated with 50 and 200 mg L^{-1} flurenol, respectively, while the highest value of plants treated with 200 mg L^{-1} flurenol was 45.03% protein in band No. 14 (MW 20 KDt) and the highest value observed in the control was 58.22% protein exist in band No. 12 (molecular weight 35 KDt).

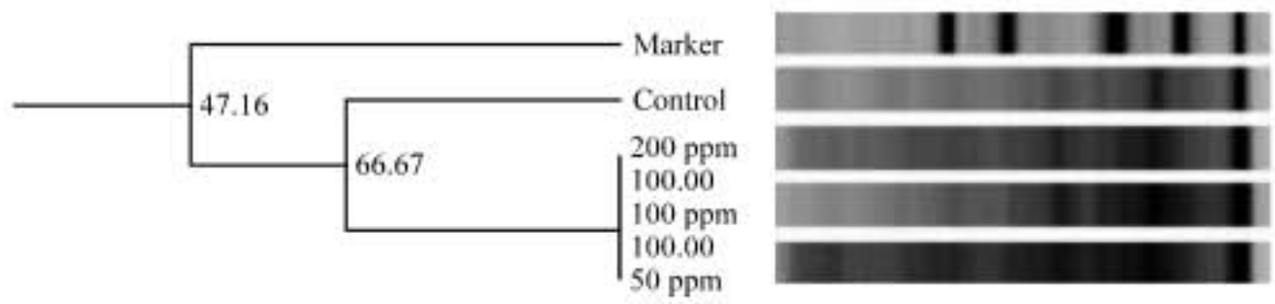

Fig. 4: Dendrogram produced by UPGMA cluster analysis based on the similarity polymorphism of protein banding patterns produced by soybean cv Crawford root nodules subjected to different flurenol treatments as determined by SDS-PAGE. UPGMA clustering using simple band match (Tolerence: 3.20%) Jan 16 2009

The results of UPGMA clustering analysis of the total protein fractions based on the similarity of the polymorphisms revealed the presence of three clusters (Fig. 4). The first cluster included all of the plants subjected to flurenol treatments with 100% similarity and 33.33% dissimilarity with their control in the second cluster, while the last group contained the protein marker. These findings demonstrated that there were significant differences in the total proteins of root nodules in plants treated with flurenol. These findings indicate that SDS-PAGE was a more useful tool for evaluation of the effects of treatments on the total proteins in the root nodules. Flurenol foliar application led to an increase in the total protein fractions in root nodules when compared with untreated plants, which indicated that plant growth retardants provided valuable effects on the formation of soybean root nodules that grown under high plants density in newly reclaimed sandy soil. In addition, all of the studied treatments enhanced the presence of polymorphic bands with molecular weights of 90, 89, 47 and 42 KDt when compared to the control.

DISCUSSION

It is well known that auxins, gibberellins and cytokinins are endogenous and exogenous plant growth regulators and growth inhibitors that play an important role in many physiological processes in plants (Baz *et al*., 1984). Moreover, these processes are greatly influenced by environmental conditions. Consequently, the application of growth retardants can be used to maintain the balance of internal hormones and efficient sink-source relationships, thereby enhancing crop productivity. Most growth retardants act by inhibiting GA biosynthesis (Leopold, 1964; Baz *et al*., 1984; Kim *et al*., 2003).

Data concerning the average length of the main stem in this study are in agreement with the results of studies conducted by Nooden and Nooden (1985), Castro *et al*. (1990), Ali *et al*. (1994) and El-Desoki *et al*. (1994), who stated that the length of the main stem of different leguminous plants was decreased in response to the morphactin treatments. The average plant dry weights observed in the present study are in accordance with those of studies conducted by Zayed *et al*. (1985) and El-Desoki *et al*. (1994), who treated soybean (*Glycine max* L. Merrill). var. Anoka and *Vicia faba* L. var. Giza 3, respectively, with the morphactin. Consequently, flurenol treatment increased the levels of auxins in the roots, which enhanced root nodule formation as reported by Baz *et al*. (1984), who stated that the effect of foliar application of growth retardants increased root nodule formation, numbers and dry weight in soybean plants via a reduction in GA-like substances.

Finally, protein analysis of root nodules using SDS-PAGE revealed that they increased in response to the combination of morphactin treatments and seed inoculation with *Bradyrhizobium japonicum*. The proposed hypothesis that may be explained the effects of morphactin treatment on the enhancement of root nodule formation is via providing the necessary conditions for nitrogen fixation such as low oxygen and high level of auxin in root as supported by researches of Eckardt (2006) and Dalton *et al*. (2009). In this concern, it may be enhanced plant cell production of antioxidant by which strong antioxidant defenses gave strong reducing conditions for the oxidation of enzymes such as nitrogenase (Dalton *et al*., 2009; Roat and Ramawat, 2009). On the other hand, auxin has a central and stimulatory role in adventitious root formation (Jarvis and Yasmin, 1987). Further research is required to understand it's mechanism in inducing the formation of root nodules.

CONCLUSION

The results of the this study indicate that most flurenol treatments had a remarkable effect on the number of soybean root nodules plant^{-1} and the root nodule protein patterns, while no differences were found regarding seed protein patterns. In addition, flurenol treatment was capable of inducing plant growth and root nodule formation even though the plants were grown in a newly reclaimed sandy soil with poor quality using low

amounts of NPK fertilizers. However, its treatment at 100 mg L^{-1} should be recommended to induce a pronounced increase in the soybean (*Glycine max* L. Merrill) cv. Crawford yield and this concentration will likely give better results in clay soils treated with conventional fertilizers. Finally, these findings indicate that flurenol can be used to reduce the amount of N fertilizers applied in soybean production systems. So, it could be recommended to all soybean growers to the region since its application is very easy.

ACKNOWLEDGMENT

This research was supported by a grant (Code No. 2-2-3) from Sustainable Water Resources Center of 21st Century Frontier Research Program.

REFERENCES

Ali, Z.A., E.R. El-Desoki, R.R. El-Masry and S.I. Omara, 1994. Effect of morphactin CF125 on the anatomical structure of *Vicia faba* L. Ann. Agric. Sci. Moshl., 32: 875-888.

Bangerth, F., 1994. Response of cytokinin concentration in the xylem exudates of bean plants (*Phaseolus vulagris* L.) to decapitation and auxin treatment and relationship of apical dominance. Planta, 194: 439-442.

Baz, A.G.I.O., M.S.A. Safwat and A.R. Abdallah, 1984. Some physiological studies on soybean plants 1: Effect of some growth regulators on growth, yield, nodulation and chemical composition. Ann. Agric. Sci. Moshtohor Univ., 21: 479-494.

Castro, P.R., B. Appezzato, C.W. Lara, A. Pelissari and M. Pereira *et al*., 1990. Action of plant regulators on growth, nutritional aspects, anatomy and the productivity of dry beans (*Phaseolus vulgaris* cv. Carioca). Anais da Escola Superior de Agricultura Luiz de Queiroz, 47: 11-28.

Cline, M.G., 1994. The role of hormones and apical dominance. New approaches to an old problem in plant development. Physiol. Plant., 90: 230-237.

Dalton, D.A., C. Boniface, Z. Turner, A. Lindahl and H.J. Kim *et al*., 2009. Physiological roles of glutathione s-transferases in soybean root nodules. Plant Physiol., 150: 521-530.

Derbyshire, E., D.J. Wright and D. Boulter, 1976. Legumin and vicilin storage protein of legume seeds. Phytochemistry, 15: 3-24.

Eckardt, N.A., 2006. The Role of flavonoids in root nodule development and auxin transport in *Medicago truncatula*. Plant Cell, 18: 1539-1540.

El-Desoki, E.R., S.I. Omara, Z.A. Ali and R.R. El-Masry, 1994. Effect of morphactin Cf125 on the growth and development of *Vicia faba* L. and *Orobanche crenata* Forsk. J. Agric. Sci. Mansoura Univ., 19: 1691-1699.

Estruch, J.J., D. Chriqui, K. Grossmann and A. Spena, 1991. The plant oncogene rolC is responsible for the release of cytokinins from glucoside conjugates. EMBO J., 10: 2889-2895.

Hirsch, A.M., 1992. Developmental biology of legume nodulation. New Phytol., 122: 211-237.

Ishikawa, K., H. Kamada and H. Harada, 1997. Another evidence for inhibitory effect of auxin in adventitious bud formation of decapitated Flax (*Linum usitatissimurn* L.) seedlings. J. Plant Res., 110: 387-392.

Jarvis, B.C. and S. Yasmin, 1987. Plant growth regulators and adventitious root development in relation to auxin. Biol. Pl., 29: 189-198.

Kim, S.K., S.C. Lee, K.M. Kim, B.H. Lee and I.J. Lee, 2003. Possible residual effects of gibberellic acid and gibberellin biosynthesis inhibitors on sprouting, early bulbil formation and tuber yield in Chinese yam. J. Agron. Crop Sci., 189: 428-432.

Klee, H.J. and C.P. Romano, 1994. The roles of phytohormones in development as studied in transgenic plants. Crit. Rev. Plant Sci., 13: 311-324.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of head of bacteriophage T_4. Nature, 227: 680-685.

Leopold, A.C., 1964. Plant Growth and Development. Mc Graw-Hill, New York, pp: 144-325.

Mathesius, U., H.R.M. Schlaman, H.P. Spaink, C. Sautter, B.G. Rolfe and M.A. Djordjevic, 1998. Auxin transport inhibition precedes root nodule formation in white clover roots and is regulated by flavonoids and derivatives of chitin oligosaccharides. Plant J., 14: 23-34.

Mathur, M., A.K. Jain, S. Dass and K.G. Ramawat, 2007. Optimization of guggulsterone production in callus cultures of *Commiphora wightii* (Arnott.) Bhandari. Indian J. Biotechnol., 6: 525-531.

Mathur, M. and K.G. Ramawat, 2008. Improved guggulsterone production from sugars, precursors and morphactin in cell cultures of *Commiphora wightii* grown in shake flasks and a bioreactor. Plant Biotechnol. Rep., 2: 133-136.

Morris, D.A., 2000. Transmembrane auxin carrier systems-dynamic regulators of polar auxin transport. Plant Growth Regul., 32: 161-172.

Mujoo, R., D.T. Trinh and P.K.W. Ng, 2003. Characterization of storage proteins in different soybean varieties and their relationship to tofu yield and texture. Food Chem., 82: 265-273.

Nooden, L.D. and S.M. Nooden, 1985. Effect of morphactin and other auxin transport inhibitors on soybean senescence and pod development. Plant Physiol., 78: 263-266.

Ogura, H., 1975. The effects of a morphactin, chlorflurenol, on organ redifferentiation from *Tobacco Calluses* cultured in vitro. Bot. Mag. Tokyo, 88: 1-8.

Roat, C. and K.G. Ramawat, 2009. Morphactin and 2iP markedly enhance accumulation of stilbenes in cell cultures of *Cayratia trifolia* (L.) Domin. Acta Physiol. Plant., 31: 411-414.

Rohlf, F.J., 1993. NTSYS-pc, Numerical Taxonomy and Multivariate Analysis System. Applied Biostatistics Inc., New York.

SAS, 2003. SAS/STAT User's Guide, Release 9.1. SAS Institute Inc., Cary, NC, USA.

Shen, W.H., E. Davioud, C. David, H. Barbier-Brygoo, J. Tempe and J. Guern, 1990. High sensitivity to auxin is a common feature of hairy root. Plant Physiol., 94: 554-560.

Stafstrom, J., 1995. Developmental Potential of Shoot Buds. In: Plant Stems: Physiology and Functional Morphology, Gartner, B.L., (Ed). Academic Press, San Diego, pp: 257-279.

Tamas, I.A., 1987. Hormonal Regulation of Apical Dominance. In: Plant Hormones and their Role in Plant Growth and Development, Davies, P.J. (Ed.). Martinus Nijhoff Publishers, Dordrecht, pp: 393-410.

Tanwar, Y.S., M. Mathur and K.G. Ramawat, 2007. Morphactin influences guggulsterone production in callus cultures of *Commiphora wightii*. Plant Growth Regul., 51: 93-98.

Walker, J.M., 2002. The Protein Protocols Handbook. 2nd Edn., Humana Press Inc., USA., pp: 61-67.

Young, N.D., J. Mudge and T.H.N. Ellis, 2003. Legume Genomes: More than Peas in a Pod. Curr. Opin. Plant Biol., 6: 199-204.

Zayed, E.A., A.I. El-Zawily, E.S. Nofal and M. Hassan, 1985. Studies on growth, productivity and physiological aspects of hot pepper (*Capsicum anmam* L. variety red cherry) 1: Effect of morphactin, gibberellic acid and their combination. J. Agric. Sci., Mans Univ., 10: 183-190.