J. Agric. Sci. Mansoura Univ., 25 (6): 3303 - 3313, 2000.

RESPONSE OF EGYPTIAN CLOVER (*Trifolium alexandrinum L.*) TO NITROGEN FERTILIZATION AND/OR IAA AND KINETIN

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ABSTRACT

Response of Egyptian clover plants to N-fertilization and/or application of IAA or kinetin was studied. Application of either N, IAA, or kinetin and the combination between N and each of IAA and kinetin enhanced growth of plants with a maximum green yield obtained from N-fertilized plants treated with kinetin at 30 mg/l. In addition, N fertilization and IAA as well as kinetin application stimulated greening of the treated plants through enhancing chlorophyll content.

N-fertilization increased the nutritional value of the shoots through increasing N and P contents in both leaves and stems. The effects of IAA and kinetin on N and P contents were variable and prominent in the stem rather than the leaf, and the most favorable effect was recorded in case of kinetin at 30 mg/l. The highest N content in the stem was obtained in N-fertilized plants treated with the above mentioned level of kinetin.

Plants showed the best growth enhancement were subjected to certain anatomical measurements. Anatomical study revealed that growth stimulation due to IAA at 100 mg/l and kinetin at 30 mg/l was, anatomically, based on increasing no. of vascular bundles, dimensions of the bundle, and no. of xylem vessels/bundle, as well as, increasing cortex thickness

INTRODUCTION

Egyptian clover is the main winter forage crop in Egypt, not only to its nutritional value for livestock but also to its effect on improving physical characteristics and fertility of cultivated soils. As a forage crop, enhancing the vegetative growth as well as greening of the plants will be beneficial for increasing the yield and its nutritional value.

N-application, generally, stimulates vegetative plant growth through enhancing most aspects of plant metabolism (Helaly *et al.*, 1985). So, it has been used to enhance vegetative growth of crops cultivated for their vegetative yield such as spinach (El-Gizawy *et al*., 1992 and Abdel Razzik, 1996). It is also employed to enhance growth, hence yield of some fabaceous plants (El-Hamdi *et al*.; 1992, Abdel-Gawad and El-Batal, 1995).

There are certain previous attempts aimed to enhance vegetative yield of Egyptian clover through the use of some growth regulating chemicals (El-Kady and Nassar, 1978). IAA, as a growth stimulating auxin has been used to enhance growth and yield of some crops (Hamail *et al*., 1991; Shams El-Din and Salwau, 1994, and Salama *et al*., 1995). Kinetin also was used to enhance growth, yield and chlorophyll content of some plants (El-Shafey *et al*., 1981; Arafa and Harb, 1989; Patel and Saxena, 1994, and Younis *et al*., 1994). So, the present study aimed at increasing vegetative growth, hence, yield and its quality of Egyptian clover plants through N- fertilization, and IAA or kinetin application either alone or in combination.

MATERIALS AND METHODS

A field experiment was conducted in a farm at Meet Khamis, near El-Mansoura City, Dakahlya Governorate during the growing season 1997/98. Certain physical and chemical characteristics of the used soil are illustrated in Table (1). Two levels of nitrogen (N) were tested 0 (N^-), 10 kg N (as urea 46% N)/fad. of each harvest (N^+). In addition, Indole-3-acetic acid (IAA) was applied at 50 and 100 ppm, and kinetin (6-furfurylaminopurine) at 15 and 30 ppm, either on unfertilized or N-fertilized plants. A split-plot design (R.C.B.) with 4 replicates was employed, the main plots were assigned to N-levels whereas the subplots were assigned to growth substances levels. The experimental unit was 2×2 m^2, occupying an area of 1/1050 fad.

Rhizobium-inoculated seeds of Egyptian clover (*Trifolium alexandrinum L.*), cv. Miskawi, were broadcated at the rate of 20 kg/fad. on 15th November. For the first harvest, N was applied during the first irrigation whereas growth substances were foliarly sprayed twice, 3 and 4 weeks after sowing. In the second harvest, N was applied one-week after the first cut whereas growth substances were sprayed twice 2 and 3 week after the first cut. Calcium superphosphate and potassium sulphate were added during soil preparation for cultivation at 100 and 50 kg/fad. respectively.

Table (1): Some physical and chemical characteristics of the experimental soil.

Soil properties	Content
Clay %	42.00
Silt %	25.2
Fine sand %	26.7
Coarse sand %	1.20
$CaCo_3$ %	2.90
Organic matter %	2.00
Total nitrogen %	0.14
Available phosphorus (ppm)	8.00
Total soluble salts %	0.20
PH	7.80

At the time of 1st and 2nd harvests, the following parameters were recorded on 20 randomly selected plants for each treatment: plant height, no. of developing lateral buds (1st cut) or no. of lateral shoots (2nd cut), leaf area/plant, fresh and dry weights of the shoots/plant as well as green & dry yields (kg/m^2). In addition, photosynthetic pigments in the 3rd (1st harvest) and the 5th of the upper 2nd order shoot (2nd harvest) upper leaf were determined (Wettstein, 1957). Two weeks before the 1st and 2nd harvests plant, dry weight and leaf area/plant were determined in order to analyze growth represented by relative growth rate (RGR) and net assimilation rate (NAR), which were calculated according to Radford (1967).

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The dry matter of the leaves and stems were ground and wet digested as described by Peterburgski (1968) to determine nitrogen (N) and phosphorus (P). N was determined by the microkjeldahl method (Jackson, 1967). P was determined colorimetrically using the chlorostannous reduced molybdo-phosphoric blue colour method as described by Jackson (1967). Data were statistically analyzed according to Snedecor and Cochran (1980).

For the anatomical investigation, specimens were taken from the 5th internode of the upper 2nd order shoot during the second harvest. Only treatments, which showed the most growth responses were anatomically tested. Specimens were killed and fixed in Formalin-Aceto-Alcohol (FAA), dehydrated in Ethyl alcohol series, embedded in paraffin wax, transversely sectioned (15 μ thick), stained in safranin-light green combination and mounted in canada balsam (O'Brien and Mc Cully, 1981). Four specimens were examined from each, 4 randomly selected sections were measured. Measurements were taken using a calibrated eyepiece micrometer.

RESULTS AND DISCUSSION

One) Growth parameters and yield

Data in Table (2) show that application of IAA and kinetin at the used rates increased shoot fresh and dry weights, leaf area/plant, net assimilation and relative growth rates as well as green and dry yields at the two harvests. The higher levels of IAA (100mg/l) and kinetin (30 mg/l) proved to be more effective compared with the lower levels (50 and 15 mg/l, respectively). On the other hand, the two used levels of IAA increased plant height whereas kinetin decreased this parameter at its higher level. The differential effect of the two used growth substances was apparent also with respect to no. of developing lateral buds/lateral shoots, where IAA decreased and kinetin increased this parameter, though the effect was significant only at the used higher rate.

It has been reported that growth, hence vegetative yield, of Egyptian clover could be enhanced through the application of some growth substances (Al-Kady and Nassar, 1978). In addition, IAA was employed to stimulate vegetative growth of cabbage plants (Hamail *et al*., 1991), where an increase in fresh weight/plant, no. of leaves and leaf area/plant was obtained. Moreover, it has been reported that IAA not only increased plant height of faba bean plants, but also increased their seed yield (Shams El-Din and Salwau, 1994). The enhanced growth of IAA-treated plants could be attributed to increased wall extensibility and cell turgor (Okamoto *et al*., 1995), which may be the result of reduced lignification (Zin Huang *et al*., 1996).

As a growth stimulator, kinetin was widely known to enhance growth of fabaceous plants, so an increase in biological yield was obtained (El-Shafey *et al*., 1981; Arafa and Harb, 1989; Dashora and Jain, 1994; Patel and Saxena, 1994, and Younis *et al*., 1994). Enhanced growth was reflected in increased leaf area (El-Shafey *et al.,* 1981; Dashora and Jain, 1994), and dry weights of vegetative organs (El-Shafey *et al*., 1981; Arafa and Harb, 1989; and Patel and Saxena, 1994) in kinetin-treated plants.

Addition of nitrogen was beneficial for enhancing growth of Egyptian clover plants where a significant increase in almost all studied parameters was recorded. The enhancement of plant growth due to N-application was previously reported (El-Hamdi *et al*., 1992; Abdel-Gawad and El-Batal, 1995 and Abdel-Razik, 1996). N-addition led to highly significant increases in plant fresh and dry weights, no. of leaves/plant and plant height of common bean plants (El-Hamdi *et al*., 1992). Moreover, Abdel-Gawad and El-Batal (1995), found that N-fertilization increased plant height and biological weight/plant. The enhanced growth of N-treated plants may be attributed to a stimulated effect on the metabolic processes in plant tissues due to its involvement in all enzymes, co-enzymes, all proteins and hence, of protoplasm (Helaly *et al*., 1985).

Data also show that there was a synergistic effect between N and the used growth substances on some studied parameters where their interaction was significant with respect to no. of lateral buds/shoots, shoot fresh and dry weights/plant as well as green yield (kg/m^2). The maximum values of shoot fresh and dry weights as well as green yield were obtained in N-fertilized plots treated with kinetin at 30 mg/l.

Two) Photosynthetic pigments

Data in Table (3) show the effect of different treatments on photosynthetic pigment content in the leaves of egyptian clover plants. Data show that, the two used growth substances increased chlorophyll (a) [chl (a)], chl (b), total chls as well as carotenoid content in the leaves. Generally, the higher concentration of kinetin was more effective whereas the lower level of IAA was less effective in this respect. In addition, N-application increased all studied aspects of leaf photosynthetic pigments. The interaction between growth substances and nitrogen was significant only with respect to the total chl content during the second harvest.

Greening of the vegetative organs of a fodder crop would be advantageous which, reasonably, the result of enhanced chl. content. Treatments adopted in the present investigation enhanced total chl content of Egyptian clover plants, in accordance with previous investigations dealing with the effect of N-fertilization (Abdel-Razik, 1996), IAA (Hamail *et al*., 1991; Salama *et al*., 1995), and kinetin (El-Shafey *et al*., 1981; Arafa and Harb, 1989; Aldesuquy and Gaber, 1993; Upadhay *et al*., 1993) on chlorophyll content of treated plants. Kinetin not only enhances chl content but also carotenoid content in the leaves of treated plants (El-Shafey *et al*., 1981; Arafa and Harb, 1989). The favourable effect of kinetin on chl content may be attributed to its inhibiting effect on chlorophyllase, hence retarding chl. breakdown (Wittenbach, 1977) or to its stimulating effects on carotenoids which may protect chl against degradation by photoxidation (El-Shafey *et al*., 1981).

Three) N and P contents

Data in Table (4) show that the effect of the applied growth substances on N and P contents in plant shoots is prominent in the stems comparing with the leaves. In the leaves, only kinetin at 30 mg/l increased N content during the second harvest. In the stems, IAA decreased whereas kinetin increased N content. Regarding P content, the effect of growth substances was significant only during the second harvest, where IAA and kinetin at 30 mg/l increased P content.

N-fertilization increased N and P contents in leaves and stems of the plants during the two harvests, though the increase was insignificant with respect to leaf N content during the first harvest. There was a significant interaction between growth substances application and N-fertilization with respect to stem N content throughout the experimental period and stem P content only during the second harvest. The highest N-content in the stems was found in N-fertilized plants treated with kinetin at 30 mg/l. Similar results were reported by El-Hamdi (1987).

Table (3): Photosynthetic pigments (mg/g f. wt.) in the leaves of Egyptian clover plants as affected by N-fertilization and/or IAA and kinetin.

Treat.	Chl (a)		Chl (b)		Total chls		Carotenoids	
	h1	h2	h1	h2	h1	h2	h1	h2
N^-	1.74	1.79	0.535	0.561	2.273	2.351	0.350	0.315
N^+	1.92	1.87	0.575	0.590	2.493	2.460	0.410	0.365
$I_{50}N^-$	1.85	1.80	0.543	0.550	2.393	2.345	0.361	0.330
$I_{50}N^+$	1.99	1.91	0.602	0.614	2.597	2.524	0.415	0.396
$I_{100}N^-$	1.90	1.81	0.551	0.562	2.448	2.372	0.405	0.357
$I_{100}N^+$	2.00	1.99	0.635	0.646	2.636	2.641	0.463	0.433
$K_{15}N^-$	1.82	1.78	0.541	0.565	2.356	2.345	0.431	0.400
$K_{15}N^+$	2.01	1.98	0.615	0.626	2.620	2.606	0.460	0.450
$K_{30}N^-$	1.84	1.87	0.553	0.570	2.388	2.440	0.440	0.415
$K_{30}N^+$	2.08	2.01	0.632	0.657	2.707	2.676	0.490	0.460
LSD (5%)								
GS	0.054	0.046	0.020	0.022	0.058	0.054	NS	NS
N	0.034	0.029	0.013	0.014	0.037	0.034	0.013	0.018
GS×N	NS	NS	NS	NS	NS	NS	NS	NS

Table (4): N & P contents in stems and leaves of Egyptian clover plants as affected by N fertilization and/or IAA and kinetin.

Treat.	Leaves				Stems			
	N %		P %		N %		P %	
	h1	h2	h1	h2	h1	h2	h1	h2
N^-	3.250	3.300	0.24	0.230	1.180	1.060	0.150	0.140
N^+	4.200	3.890	0.25	0.247	1.440	1.337	0.180	0.160
$I_{50}N^-$	3.520	3.300	0.20	0.200	1.200	1.200	0.160	0.148
$I_{50}N^+$	3.818	3.700	0.23	0.220	1.020	1.000	0.180	0.170
$I_{100}N^-$	3.543	3.200	0.19	0.200	1.007	1.000	0.150	0.140
$I_{100}N^+$	3.925	3.800	0.23	0.220	1.350	1.300	0.170	0.180
$K_{15}N^-$	3.800	3.350	0.19	0.180	1.200	1.180	0.140	0.120
$K_{15}N^+$	4.313	4.020	0.22	0.210	1.630	1.590	0.190	0.180
$K_{30}N^-$	3.940	3.900	0.20	0.200	1.180	1.090	0.158	0.170
$K_{30}N^+$	3.787	4.550	0.22	0.230	2.200	1.830	0.190	0.170
LSD (5%)								
GS	NS	0.164	NS	NS	0.123	0.089	NS	0.009
N	NS	0.104	0.023	0.022	0.078	0.056	0.007	0.006
GS×N	NS	NS	NS	NS	0.174	0.125	NS	0.013

d) Anatomical parameters

Data in Table (5) show the effect of selected treatments on some anatomical features of egyptian clover stems. Data show that, generally, N application proved to be effective in enhancing the studied anatomical features of the stem. However, no. of vascular bundles was almost unaffected (Table 5 and Fig. 1). IAA or kinetin added to non-fertilized plants stimulated the anatomical structure of the stem with superiority to IAA concerning both no. of vascular bundles and no. of xylem vessels/bundle. When IAA or kinetin was added to N-fertilized plants, a cumulative effect was resulted, so that the enhancing effect of the combined treatments surpassed that of either N or the growth substances added alone. It is apparent from Table (5) and Fig. (1) that the effect of either N, or the growth substances and their combinations on increasing the cross sectional diameter of the stem was primarily a result of increasing cortex thickness and vascular bundle dimensions rather than increasing no. of vascular bundles which was less responsive. Because stem cortex contains actively synthesizing chloroenchymatous cells, increasing cortex thickness will positively affect the growth attributes of the treated plants.

Increasing stem cross sectional diameter due to kinetin application, found in the present investigation, was previously reported by El-Banna *et al*. (1988), working on tomato and Arafa and Harb (1989) on pea. In their investigations, this effect was accompanied by increasing cortex thickness, no. of vessels/bundle and vascular bundle dimensions. Increasing the vascular system proportion is a logic result of enhanced cambial activity, an effect of kinetin, which was proved in the studies of Sorokin *et al*. (1962) and El-Banna (1985).

It is logically accepted that enhancing growth reflects a well-developed conductive system. So, it could be assumed that enhancing growth of Egyptian clover plants is based upon an enhanced vascular system, which is a result of induced cambial activity. In many investigations, auxins were found to induce cambial activity e.g. Sorokin *et al*. (1962), Digby and Wareing (1966), Esau (1977), El-Banna (1985), Arafa *et al.* (1987) and Mohamed *et al*. (1993). Sorokin *et al*. (1962) found that IAA stimulated cell division in the vascular cambium and initiated some intervascular cambium in segments from etiolated pea internodes. Digby and Wareing (1966) found that cambium divisions were stimulated by application of IAA and the cambial derivatives differentiated to produce xylem. Moreover, Esau (1977) stated that, for secondary growth in pea roots, a relatively high concentration of auxin must be present in the medium.

Fig. (1): Part of a cross section of Egyptian clover stem as affected by N-fertilization and/or IAA (100mg/l) and kinetin (30 mg/l). V. B., vascular bundle; C, cortex; X40.

Table (5)*: Counts and measurements of some anatomical characters of Egyptian clover stem as affected by N-fertilization and/or IAA and kinetin.

Items		Treatments					
		N^-	N^+	$I_{100}N^-$	$I_{100}N^+$	$K_{30}N^-$	$K_{30}N^+$
C. S. diameter (μ)		3393.0	3977.3	3901.9	4769.0	4052.7	4938.7
**		0	+17.2	+14.9	+40.5	+19.4	+45.5
No. of vasc. Bund.		23.7	24.0	27.0	26.9	25.0	26.3
**		0	+1.2	+13.9	+13.5	+5.4	+10.9
Cortex thick. (μ)		450.7	576.8	527.8	678.6	562.8	716.3
**		0	+27.9	+17.1	+50.5	+24.8	+58.9
Dimensions of the vascular Bundle	L	293.1	386.5	378.9	457.1	360.0	429.6
	**	0	+31.8	+29.2	+55.9	+22.8	+46.5
	W	271.7	358.9	307.5	357.6	314.6	397.5
	**	0	+32.0	+13.1	+31.6	+15.7	+46.3
No. of xyl. Vess.		20.6	27.0	25.8	31.7	23.4	28.9
**		0	+31.0	+25.2	+53.8	+13.5	+40.2

*** Each entry is the mean of 16 measurements. ** ± % of control (N^-).**

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استجابة نباتات البرسيم المصرى للتسميد النيتروجيني و/أو المعاملة بإندول حامض الخليك والكينتين.

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أجريت دراسة بهدف معرفة مدى تأثر إنتاجية نبات البرسيم المصري بالتسميد النيتروجيني مع أو بدون المعاملة بإندول حامض الخليك والكينتين. أدى التسميد النيتروجيني والمعاملة بإندول حامض الخليك والكينتين وكذا الإضافة المشتركة للنيتروجين مع كل مادة نمو مستخدمة على حدة إلي زيادة نمو النباتات وتم التحصل علي أعلي محصول أخضر من النباتات التي سمدت بالنيتروجين وعوملت بالكينتين عند تركيز 30 مجم/لتر. ولقد أدت المعاملات إلي زيادة اخضرار النباتات نتيجة زيادة محتواها من الكلورفيلات.

كان التسميد النيتروجيني أكثر تأثيراً في زيادة القيمة الغذائية للمحصول الأخضر حيث أدى إلي زيادة محتوى الأوراق والسيقان من النيتروجين والفوسفور. ومن الناحية الأخرى كان تأثير المعاملة بمواد النمو علي المحتوى من النيتروجين والفوسفور غير ثابت وإن كان أكثر وضوحاً في السيقان عنه في الأوراق، وكانت أفضل المعاملات في هذا الشأن هي المعاملة بالكينتين عند 30 مجم/لتر للنباتات المسمدة بالنيتروجين حيث احتوت سيقان النباتات المعاملة علي أعلي مستوى من النيتروجين.

تم إجراء دراسة تشريحية للنباتات التي عوملت معاملات معينة وأظهرت زيادة واضحة في نموها وأظهرت هذه الدراسة أن زيادة النمو في النباتات التي سمدت بالنيتروجين وعوملت في نفس الوقت بمواد النمو كان راجعاً إلي زيادة عدد الحزم الوعائية وأبعادها وعدد الأوعية بالحزمة الواحدة وكذا سمك القشرة في النباتات المعاملة.

Table (2): Growth attributes and yield of Egyptian clover plants as affected by N-fertilization and/or IAA and Kinet.

Treat.	Plant height (cm)		No. of axillary buds/lateral shoots		Shoots F. wt./plant (gm)		Shoots D. wt./plant (gm)		Leaf area/plant (cm^2)		NAR mg/cm^2 .day		RGR mg/g.day		Green yield kg/m^2		Dry yield kg/m^2	
	h1	h2	h1	h2	h1	h2	h1	h2	h1	h2	h1	H2	h1	h2	h1	h2	h1	h2
N^-	52.4	79.6	2.0	2.3	3.88	8.78	0.38	0.85	35.1	80.5	1.21	1.25	18.1	18.8	3.6	8.3	0.41	0.98
N^+	58.9	86.4	3.2	3.3	4.35	13.9	0.40	1.05	43.7	121	1.35	1.38	18.8	19.5	4.4	9.5	0.48	1.10
$I_{50}N^-$	55.4	83.1	2.0	2.3	4.09	9.15	0.40	0.86	38.5	84.1	1.25	1.29	18.4	19.0	4.0	8.7	0.44	1.00
$I_{50}N^+$	60.1	87.0	2.5	2.9	4.67	13.6	0.45	1.20	49.8	111	1.35	1.40	18.6	19.3	4.8	9.7	0.51	1.20
$I_{100}N^-$	56.0	86.5	2.1	2.4	4.30	10.5	0.47	1.06	40.0	86.1	1.30	1.32	18.9	19.2	4.5	9.4	0.50	1.20
$I_{100}N^+$	62.3	89.7	2.4	2.6	5.51	14.2	0.50	1.35	53.8	127	1.37	1.41	19.5	20.0	5.6	12.2	0.59	1.40
$K_{15}N^-$	53.2	80.0	2.5	2.6	3.92	8.90	0.42	0.98	36.8	83.0	1.36	1.38	18.5	18.9	4.5	9.4	0.48	1.10
$K_{15}N^+$	59.0	86.0	3.2	3.4	4.46	14.0	0.43	0.99	50.2	125	1.37	1.40	19.0	19.5	4.8	9.9	0.55	1.20
$K_{30}N^-$	43.3	71.7	2.9	3.0	4.15	10.4	0.47	0.95	47.7	101	1.36	1.37	18.9	19.3	4.4	9.3	0.59	1.20
$K_{30}N^+$	53.8	81.5	3.5	3.7	5.35	16.9	0.57	1.42	57.3	135	1.42	1.48	19.9	20.6	5.2	13.0	0.65	1.50
LSD 5% GS	2.17	5.54	0.21	0.29	0.27	0.29	0.03	0.06	3.5	6.7	0.08	0.06	0.61	0.55	0.20	0.50	0.03	0.14
N	1.37	3.50	0.13	0.18	0.17	0.18	0.02	0.04	2.2	4.2	0.05	0.04	0.38	0.58	0.13	0.30	0.02	0.09
GS×N	NS	NS	0.29	0.41	0.38	0.41	0.04	0.09	NS	NS	NS	NS	NS	NS	0.28	0.70	NS	NS