J. Agric. Sci. Mansoura Univ., 32 (8): 6249 - 6257, 2007

INFLUENCE OF CULTURE CONDITIONS ON BIOMASS FORMATION IN CALLUS CULTURE OF *Echinacea purpurea*

Rabie, Kawthar A. E.; M. Abderassoul and H.H. Manaf

Agric. Botany Dept., Faculty of Agric., Ain Shams University, Shoubra El-Keima, Cairo, Egypt

ABSTRACT

In order to study the effect of some growth regulators treatments on callus growth as affected with the interaction between three different temperatures (18, 23, 28^{o}C $\pm$ 2^{o}C), photoperiod, type of explants and incubation period. Three experiments were carried out during the two successive years 2001 and 2002. The results indicated that, the interaction between the different treatments led to significant difference in callus growth when incubation at different temperature degrees (18, 23 and 28^{o}C). The suitable temperature degree for growth of echinacea explants was 23^{o}C. Culturing the explant under the light condition encouraged the callus growth in light than dark.

The explants under investigation recorded significant difference in callus growth and the cotyledonary leaf explant was the best one. The first treatment activated the callus growth significantly than the second and third treatments. In addition, significant increment was achieved in callus growth with increasing incubation period, and the best incubation period for callus growth was 35 day.

The interaction between different treatments showed that the callus growth was increasing significantly with increasing the incubation period even with different growth regulators treatments, explant type, photoperiod, and different temperature degrees (18, 23, and 28^{o}C).

As for the interaction between factors controlling callus establishment, it is clear that the most convenient conditions on the optimum growth of echinacea callus tissues are application of cotyledonary leaf explant, MS basal medium supplemented with BA 1.5 mg/L + NAA 1 mg/L & BA 2 mg/L + NAA 1 mg/L and light condition for 16 hr./day photoperiod at intensity of 6000 Lux, at 23^{o}C for 35 day incubation period.

Keywords: *Echinacea purpurea*, callus culture, explant, growth regulators.

INTRODUCTION

Echinacea has gained considerable attention because of its increasing economic value and use as a medicinal plant. The genus *Echinacea* (purple coneflower) is represented by 11 taxa found in the United States and in south central Canada. *Echinacea purpurea* is the most widespread species (McGregor, 1968) and the most widely cultivated medicinal species of the genus (McKeown, 1999).

Echinacea is used for medicinal purposes by indigenous Americans, externally for snake or insect bites and burns (Busing, 1952 and Hill *et al.*, 1996) and internally for coughs, colds, sore throats, infections, and inflammations (Hobbs, 1989 and 1994). In addition, it possesses a broad spectrum of effects on the immune system because of its content of a diverse range of active components affecting different aspects of immune function (Hobbs, 1989 & Bauer and Wagner, 1991).

In spite of the millions of dollars that are generated each year from the sale of extracts and dried tissues of echinacea, numerous problems exist with preparations that have raised concerns about the quality of echinacea

products (Consumer Reports, 2000). The quality of echinacea and medicinal plant preparation in general can be seriously compromised by contamination of fungi, bacteria, and environmental pollutants (Laughlin and Munro, 1982), adulteration with the wrong plant species (Betz, 1998 and Slifman *et al.*, 1998).

Plant tissue culture techniques have become a powerful tool for studying basic and applied problems in plant biology. Furthermore, in the last years these techniques have found wide commercial application in the propagation of plants, mainly horticulture species. Success in the technology and application of *in vitro* methods is due largely to a better understanding of the nutritional requirements of cultured cells and tissues (Murashige and Skoog, 1962). Tissue culture protocols have been established for purple coneflower, depending on hormone regime which included the auxins indole acetic acid (IAA), naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) and the cytokinins benzyl adenine (BA) and kinetin (Camper *et al*., 1999). Callus and protoplast of *Rudbeckia purpurea* were cultured on MS medium with IAA 1mg/L and BA 2mg/L at 23$\pm$ 2^{o}C (Al-Atabee and Power, 1987 and 1990). The goal of this project was to establish the best protocol for the *in vitro* culture of *E. purpurea.*

MATERIAL AND METHODS

Achenes of *E. purpurea* L., were obtained from National Research Center, Pharmaceutical Science Department, Dokki, Egypt. Callus was initiated by using *in vitro* seedlings (10 to 12 mm in length). The disinfected seeds were washed with water and 2 to 3 drops of detergent (Tween 20) were added, rinsing with tap water, soaking in 70 percent ethyl alcohol (EtOH) for 1 min and 30% hydrogen peroxide (H_2O_2) with detergent (2-3 drops/ 100 ml) for 20 min then cultured on agar medium for 10 days. All further manipulations were done under sterile conditions. Seedlings were harvested and parted to hypocotyl + root (radical), tip (plumule bud) and cotyledonary leaf (divided to two parts). These explants were examined for giving callus growth in order to identify the more convenient parts for callus initiation.

The basal medium of Murashige and Skoog, 1962 (MS inorganic salts and vitamins) and 30 g/L sucrose was used. The medium was modified by supplementation with growth regulators benzyl adenine (BA) and naphthalene acetic acid (NAA). The combination between growth regulators was as follows:-

- BA 1.5 mg/L + NAA 1.0 mg/L (T1).
- BA 2.0 mg/L + NAA 1.0 mg/L (T2).
- BA 2.0 mg/L + NAA 1.5 mg/L (T3).

Media were adjusted to a pH of 5.7, and solidified with 7 g/L agar, added prior to autoclaving at 121^{o}C and 1.1 kg/cm^2 for 20 min., media were dispensed into glass jars (100ml) containing 25 ml of the test media.

Cultures of all treatments were divided into two groups; the first group was maintained in light condition for 16 hr./day photoperiod at intensity of 6000 Lux from Gro-Lux and white fluorescent lamps. The second group was maintained in darkness (24hr.). All cultures were incubated at three different

temperatures (18, 23 and 28°C ± 2°C). The experiments were repeated twice in plant tissue culture laboratory, Agric. Botany Dept. Faculty of Agric., Ain Shams University. Growth measurement (fresh weight) was measured after 21, 28 and 35 days from culturing the explants in growth incubator (Heraeus).

In this part, three experiments were carried out consisting of 108 treatments, and each treatment was replicated three times.

Analysis of variance of the data was conducted and the comparisons among treatments (temperature, photoperiod, explant type, growth regulator, and incubation period) were carried out using Tukey's test (SAS 1996) for mean separation.

RESULTS AND DISCUSSION

1-Effect of growth regulators treatments on callus growth Incubation under 18°C

The results for the effect of growth regulators treatments on callus growth (mg) as affected with the interaction between temperature degree (18°C), photoperiod, type of explants and incubation period were illustrated in Table (1). Under the light condition, no significant difference in the callus growth was recorded between different growth regulators treatments with different type of explants at 21-day incubation period. The reverse was true for 35-day incubation period. Significant differences were recorded at 28-day incubation period between some treatments in case of plumule bud and cotyledonary leaf.

Generally, clear increment in callus growth was observed with increasing incubation period until 35 day. The explant response was differed to the growth regulators treatment for giving the best callus growth. The first treatment achieved the best growth for plumule bud explant, the same was happened for the second treatment with the cotyledonary leaf explant, while, the third treatment was the best for the radical explant. In this respect, **Al-**Atabee and Power (1990) incubated the *Rudbeckia purpurea* callus on MS medium with 1 mg/L IAA and 2 mg/L BA for 5 to 8 weeks.

As for darkness, callus growth was better in the light than the dark with different type of explants or growth regulators. In addition, the second treatment was the best one for increasing the callus growth from cotyledonal leaf explant even in light or dark. In this connection, Neumann and Raafat (1972) mentioned that the influence of light on cell division activity on carrot callus is mainly due to an interaction between IAA-inositol and light. Furthermore, the lower cell division activity in the kinetin treatment in the dark can be due to the absence of the latter interaction. Dagustu (1999) reported that cotyledon explants of seeds of sunflowers germinated in the dark or light were cultured to produce callus in the dark or light on a MS medium supplemented with 0.5, 1.0 or 1.5 mg/L NAA and BA.

Incubation under 23°C

The results for the effect of growth regulators treatments on callus growth (mg) as affected with the interaction between temperature degree (23°C), photoperiod, type of explants and incubation period were presented in Table (1).

T1

As for the light condition, significant difference was observed in callus growth between the different treatments after 28 & 35 day incubation period when using the radical and cotyledonary leaf explants. Whereas, the same observation was shown only after 35 day in case of plumule bud explant. The first treatment recorded the best response for radical explant growth, the cotyledonary leaf and plumule bud explants respectively.

These results agree with Knopp and Mix (1986), who cultured hypocotyl, cotyledon, internode and leaf explants and apical meristems of sunflower on a MS medium supplemented with sucrose and BA. Callus formation was observed in cultures of cotyledon and hypocotyl fragments. Lupi *et al.* (1987) also suggested that hypocotyl and cotyledon explants from *Helianthus annuus* formed callus on medium containing 2.0 mg/L NAA and 0.5 mg/L BA. Moreover, Coker and Camper (2000) mentioned that 4-5 mm long hypocotyl sections of *Echinacea purpurea* were cultured on a MS basal media plus NAA (1 to 3 mg/L) + kinetin (1 to 2 mg/L) and 2,4-D (0.5 to 1.0 mg/L) + kinetin (1.5 to 2.0 mg/L). They found that explants treated with 2,4-D : kinetin combinations produced more callus than explants treated with NAA : kinetin combinations. In addition, Trigiano and Gray (2000) reported that auxins play a role in many development processes, including cell elongation and swelling of tissue, apical dominance, adventitious root formation and somatic embryogenesis. In addition, cytokinins promote cell division; stimulate initiation and growth of shoots *in vitro*.

Under the dark condition, the effect of growth regulators treatments appeared on the callus growth that produced from different explants after 28 & 35-day incubation period, but at 21-day incubation period, usually, no significant difference appeared between treatments because of simple callus growth. Difference in the response of different explants to all growth regulators treatments was observed in light than dark to obtain the best callus growth.

Incubation under 28ºC

The effect of growth regulators treatments on callus growth (mg) as affected with the interaction between temperature degree (28ºC), photoperiod, type of explants and incubation period was illustrated in Table (1). Under light condition, growth regulator treatments did not create significant increment in callus growth from different types of explant until 21 day from incubation, except for the first treatment with radical explant. On the other hand, the first treatment was the best for callus growth from the plumule bud explant with the different incubation periods. The same was happened by the second treatment with the other explants at 35 day. Moreover, callus fresh weight from cotyledonary leaf explant was equal with the second and third treatments after 35 day, which were the best treatments that achieved the biggest increment in callus growth under these conditions.

These results agree with Greco *et al.* (1984), who tested leaf and cotyledon pieces, shoot apices and hypocotyl segments of the sunflower on MS medium supplemented with 2 to 5 mg/L 2,4-D and/or 0.1 to 5.0 mg/L BA. Responses differed according to explant and hormonal treatment. In addition, Lupi *et al.* (1987) found that sunflower callus induction and growth in tissue cultures using a MS medium supplemented with different concentrations of

auxins (IAA, NAA and 2,4-D) and cytokinins (BA and kinetin) depended on the genotype of the lines cultured. Moreover, Mohmand (1991) cultured sunflower cotyledon and hypocotyl explants on Linsmaier-Skoog (LS) culture medium supplemented with varying concentrations of 2,4-D, BA and NAA. Abundant growth of compact green callus was induced on LS + 2 mg/L NAA + 0.5 to 1.0 mg/L BA. Riao *et al.* (1993) also used two varieties of sunflower for tissue culture studies. They cultured different explants i.e. cotyledons, leaves, transition zone and hypocotyls, on modified MS media supplemented with different concentrations of auxins and cytokinins. Both the varieties and the various explants showed differential response to the different media compositions.

As for darkness, the plumule bud or the cotyledonary leaf explants response did not differ with growth regulators treatment for inducing optimum callus growth in the dark than the light. Whereas the radical explant response differed, this explant gave the highest callus growth with the first treatment.

2-Effect of the interaction between different treatments on callus growth

The interaction between different treatments (temperature, photoperiod, type of explant, growth regulators and incubation periods) were illustrated in Table (2). The interaction between the different treatments led to significant differences in callus growth when incubation at different temperature degrees (18, 23 and 28^{o}C). The suitable temperature degree for growth of echinacea explants was 23^{o}C. Culturing the explant under light condition encouraged the callus growth than darkness.

Significant difference was observed in callus growth, which produced from different explants under investigation; the cotyledonary leaf explant was the best one. The first and second treatments activated the callus growth significantly than the third treatment. In addition, significant increment was found in callus growth with increasing incubation period, and the best incubation period for callus growth was 35 day.

The interaction between different treatments showed that the callus growth was increasing significantly with increasing the incubation period even with different growth regulators treatments, explant type, photoperiod, and different temperature degrees (18, 23, and 28^{o}C). For the interaction between factors controlling callus establishment and maintenance of echinacea plant. It is clear that the more convenient conditions on the optimum growth of echinacea callus tissues are application of cotyledonary leaf explant, MS basal medium supplemented with (BA 1.5 mg/L + NAA 1.0 mg/L & BA 2.0 mg/L + NAA 1.0 mg/L), and light condition for 16 hr./day photoperiod at intensity of 6000 Lux, at 23^{o}C for 35 day incubation period.

In this regard, Al-Atabee and Power (1987 and 1990) incubated the *Rudbeckia purpurea* callus on MS medium with 1 mg/L IAA and 2 mg/L BA for 5 to 8 weeks at 23$\pm$ 2^{o}C. They found that the best incubation period was 5 weeks. Moreover, Punia and Bohorova (1990) mentioned that bud, stem, leaf and cotyledon explants of sunflower formed calluses on MS medium containing 0.05 mg/L BA and 0.5 mg/L NAA. In addition, Ceriani et al. (1992) cultured cotyledon explants of *Helianthus annuus* on modified MS medium (MS-Ha) containing 1 mg/L BA. The optimum conditions for callus proliferation involved the use of 0.5, 0.75, or 1.0 mg/L NAA alone or in

combination with low concentrations of BA. Camper *et al*. (1999) also showed that *in vitro* cultures of purple coneflower were initiated using seedlings. They incubated the hypocotyl in MS basal media with NAA (1 to 3 mg/L) + kinetin (1 to 2 mg/L) and 2,4-D (0.5 to 1.0 mg/L) + kinetin (1 to 2 mg/L) at 25°C under a 16-hr photoperiod.

Table (4).Effect of temperature degree, photoperiod, type of explants, growth regulators treatments, incubation period and their interactions on callus growth (mg).

Temperature	**18°C** 82 C	**23°C** 158 A	**28°C** 130 B
Photoperiod	**Light** 145 A	**Dark** 101 B	
Explants type	**Radical** 119 B	**Cotyl. leaf** 147 A	**P. bud** 105 C
Growth regulators treat.	**T1** 131 A	**T2** 130 A	**T3** 109 B
Incubation period (day)	**21** 27 C	**28** 80 B	**35** 264 A

-Temperature values are means of 162 replicates.
-Photoperiod values are means of 243 replicates.
-Type of explants values are means of 162 replicates.
-Growth regulators treatments values are means of 162 replicates.
-Incubation period values are means of 162 replicates.
-Values having the same letter within a row are not statistically different at 5% level.

REFERENCES

Al - Atabee, J.S. and J.B. Power (1987). Plant regeneration from protoplasts of Dimorphotheca and Rudbeckia. Plant Cell Rep. 6:414-416.

Al - Atabee, J.S. and J.B. Power (1990). Protoplast isolation and plant regeneration in ornamental compositae. Acta Hort. 280:255-258.

Bauer, R. and H. Wagner (1991). Echinacea species as potential immunostimulatory drugs. In: H. Wagner and H. N. Farnsworth, eds., Economic and Medicinal Plant Research, vol. 5. Acad. Press, London; pp 253-321.

Betz, W. (1998). Epidemic of renal failure due to herbals. Sci. Rev. Alt. Med. 2; 12-13.

Busing, K. (1952). Hyaluronidasehemmung durch echinacin. Arzneim Forsh. 2:467-469.

Camper, N.D.; P.S. Coker; K.C. Hall; J.A. McCoy and A.U. Turker (1999). Tissue culture of medicinal plants. SNA Research conference. 44:347-349.

Ceriani, M.F.; H.E. Hopp; G. Hahne and A.S. Escandon (1992). Cotyledons: an explant for routine regeneration of sunflower plants. Plant and Cell Physiology. 33: 2, 157-164 (CAB abstr., 941603900).

Consumer Reports (2000). The mainstreaming of alternative medicine. May. (pp 17-25). Yonkers, New York.

Coker, P.S. and N.D. Camper (2000). *In vitro* culture of *Echinacea purpurea*. Journal of Herbs, Spices & Medicinal plants, vol. 7: 4,1-7.

Greco, B.; O.A. Tanzarella; G. Carrozzo and A. Blanco (1984). Callus induction and shoot regeneration in sunflower (*Helianthus annuus* L.). Plant Science Letters. 36: 1, 73-77 (CAB abstr., 841634050).

Dagustu, N. (1999). The effects of light and hormone concentrations on embryo induction and plant regeneration in sunflower (*Helianthus annuus* L.). Turkish Journal of Field Crops. 1999, 4: 1, 29-34 (CAB abstr., 19990707207).

Hill, N.; C. Stam; R.A. and R.A. van Haselen (1996). The efficacy of prikweg R. gel in the treatment of insect bites: A double–blind, placebo–controlled clinical trial. Pharmacy World Sci. 18:35-41.

Hobbs, C. (1989). The Echinacea handbook. Eclectic Medical Publications, Portland, Oregon. Pp. 1-118.

Hobbs, C. (1994). Echinacea-A literature review. Herbalgram. 30:33-49.

Knopp, E. and G. Mix (1986). In vitro shoot and root regeneration from tissue fragments of sunflower (*Helianthus annuus* L.). Landbauforschung-Volkenrode. 36: 2,127-129 (CAB abstr., 861654663).

Laughlin, J.C. and D. Munro (1982). The effect of fungal colonization on the morphine production of poppy *Papaver somniferum L*. capsules. Journal of Agriculture Science. 98: 679-686.

Lupi, M.C.; A. Bennici; F. Locci and D. Gennai (1987). Plantlet formation from callus and shoot-tip culture of *Helianthus annuus* (L.). Plant Cell, Tissue and Organ Culture. 11: 1, 47-55.

McGregor, R.L. (1968). The taxonomy of the genus Echinacea (Compositae). Univ. Kansas Sci. Bull., 48:113-142.

McKeown, K.A. (1999). A review of taxonomy of the genus Echinacea. In: Janick J. (ed) perspectives on New Crops and New Uses (pp 482-490). Purdue University, USA.

Mohmand, A.S. (1991). Tissue culture of sunflower (*Helianthus annuus* L.). In-Vitro; 27, 3, Pt.2, 117A.

Murashige, T. and F.A. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Punia, M.S. and N.E. Bohorova (1990). Callus induction and plant regeneration from explants of wild diploid species of sunflower (*Helianthus* L.). Annals of Biology Ludhiana. 6: 1, 1-5 (CAB abstr., 911619173).

Riao A.M.M; S.K. Reddy and S.A. Farook. (1993). Induction of callus from different explants in sunflower. In Vitro, 29A, 3, Pt.2, 78.

Slifman, N.R.; W.R. Obermeyer; S.M. Musser; W.A. Corrrell; S.M. Cichqwicz; J.M. Betz and L.A. Love (1998). Contamination of botanical dietary supplements with *Digitalis lanata*. New Eng. J. Med. 399: 806-811.

Trigiano, R.N. and D.J. Gray (2000). Plant tissue culture concepts and laboratory exercises 2nd ed. CRC press, New York. P.28.

تأثير ظروف الزراعة على تكوين الكتلة الخلوية فى مزارع الكالس للإشيناسيا بربوريا

كوثر على أمام ربيع، محمد عبد الرسول، حسام حسن أحمد مناف
قسم النبات الزراعي - كلية الزراعة - جامعة عين شمس - شبرا الخيمة

صممث ثلاث تجارب لزراعة الأنسجة خلال سنتين متتالتين ٢٠٠٢،٢٠٠١ لدراسة تأثير معاملات بعض منظمات النمو على نمو الكالس متأثرا بالتداخل مع ثلاث درجات حرارة مختلفة (١٨، ٢٣، ٢٨ °م ± ٢°م) و الفترة الضوئية و نوع المنفصل النباتى و فترة التحضين. و أشارت النتائج الى أن التداخل بين المعاملات المحتلفة أدى الى وجود فرق معنوى فى نمو الكالس عند التحضين على درجات حرارة مختلفة (١٨، ٢٣، ٢٨ °م) و كانت أنسب درجة حرارة لنمو منفصلات نبات الأشيناسيا ٢٣°م. و قد شجعت زراعة المنفصل النباتى تحت ظروف الإضاءة من نمو الكالس عن الظلام.

سجلت المنفصلات النباتية تحت الدراسة فرق معنوى فى نمو الكالس و كان أفضلها المنفصل النباتى للورقة الفلقية. نشطت كل من المعاملة الأولى (١,٥ ملجم/لتر بنزيل أدنين + ١ملجم/لتر نفثالين حمض الخليك) و الثانية (٢ ملجم/لتر بنزيل أدنين + ١ملجم/لتر نفثالين حمض الخليك) من نمو الكالس عن المعاملة الثالثة (٢ ملجم/لتر بنزيل أدنين + ١,٥ملجم/لتر نفثالين حمض الخليك). هذا بالأضافة الى تحقيق زيادة معنوية فى نمو الكالس بزيادة فى فترة التحضين و كانت أفضل فترة تحضين لنمو الكالس ٣٥ يوم.

أظهر التفاعل بين المعاملات المختلفة لنمو الكالس زيادة نمو الكالس معنويا بزيادة فترة التحضين سواء مع المعاملات المختلفلة لمنظمات النمو أو نوع المنفصل النباتى أو الفترة الضوئية أو درجات الحرارة المختلفة.

بالنسبة للتفاعل بين العوامل المتحكمة فى تكوين الكالس و المحافظة عليه. فأنه من الواضح أن أفضل الظروف الملائمة للنمو الأمثل لكالس نبات الإشيناسيا هى استعمال منفصلات الأوراق الفلقية و زراعتها على بيئة MS مضاف اليها ١,٥ ملجم/لتر بنزيل أدنين + ١ملجم/لتر نفثالين حمض الخليك (المعاملة الاولى) و ٢ ملجم/لتر بنزيل أدنين + ١ملجم/لتر نفثالين حمض الخليك (المعاملة الثانية) تحت ظروف إضاءة (١٦ ساعة/اليوم) و شدة الأضاءة ٦٠٠٠ لكس و على ٢٣ درجة مئوى لمدة ٣٥ يوم (فترة تحضين).

Table (1).Effect of some growth regulators treatments on callus growth (mg) as affected with the interaction between temperature degree, photoperiod, type of explants and incubation period.

Light											Dark								
Explants		**Radical**			**Cotyledonal leaf**			**Plumule bud**			**Radical**			**Cotyledonal leaf**			**Plumule bud**		
Temperature	**I.P. (day)**	**21**	**28**	**35**	**21**	**28**	**35**	**21**	**28**	**35**	**21**	**28**	**35**	**21**	**28**	**35**	**21**	**28**	**35**
18°C	**T1**	10A	48A	265B	14A	65A	236B	21A	59A	239A	7A	22A	121A	12A	46B	139B	15B	33B	155B
	T2	13A	53	159C	23A	47AB	255A	15A	63A	152B	20A	31A	106AB	18A	66A	229A	16B	59A	163B
	T3	16A	44A	354A	26A	42B	111C	17A	36B	114C	13A	27A	94B	24A	56AB	111C	35A	44AB	289A
23°C	**T1**	77A	369A	534A	46B	89C	327C	36A	74A	271B	13A	44B	167B	34A	152A	262A	67A	142A	386A
	T2	31B	112B	385B	135A	248A	664A	28A	72A	334A	23A	184A	443A	36A	122B	245B	14B	51C	196C
	T3	29B	71C	315C	45B	117B	440B	24A	32B	238C	5A	19C	77C	31A	103C	220C	32B	100B	244B
28°C	**T1**	58A	99A	318B	41A	113A	278B	22A	99A	258A	7A	27B	378A	31A	91A	341A	19A	58A	265A
	T2	15B	74B	342A	43A	61B	637A	21A	95A	175C	14A	68A	180B	27A	62B	135B	24A	69A	169B
	T3	17B	99A	225C	32A	108A	637A	7A	58A	238B	16A	42B	169B	27A	71B	350A	20A	61A	135C

-Values are means of 3 replicates.
-Values having the same letter within a column are not statistically different at 5% level.
-T1: BA 1.5 mg/L + NAA 1.0 mg/L.
-T2: BA 2.0 mg/L + NAA 1.0 mg/L.
-T3: BA 2.0 mg/L + NAA 1.5 mg/L.
-I.P. = Incubation period.