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STUDIES ON PROPAGATION OF TOMATO IN VITRO: 1- EFFECT OF CYTOKININ TYPES AND CONCENTRATIONS AT DIFFERENT EXPLANT TYPES ON THE CALLUS INDUCTION IN TOMATO (*Lycopersicon esculentum* MILL.)

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ABSTRACT

The in vitro response of California Rock hybrid was tested using hypocotyls and leaf disc as an explants source for callus induction at different cytokinin types (KIN – BAP - TDZ) with different concentrations (0.0 -0.5 – 1.0 – 2.0 mg/l). Callus formation was noticed at all treatments, BAP was the most effective one followed by TDZ, the lowest values were obtained by KIN. Concentration at 1.0 mg/l recorded the greatest values. Hypocotyls was better than leaf disc for all recorded data. All treatments gave hard callus and didn't promote any shoots. No root was noticed with KIN or BAP at 2.0 mg/l with hypocotyls for each. TDZ at (0.5 – 1.0 – 2.0 mg/l) with any explants gave negative results to root formation. BAP at 1.0 mg with hypocotyls was the most effective one being recorded the highest values of callus *i.e*. (volume , fresh and dry weight).

Abbreviations: BAP = 6-benzylamino purine ; KIN = kinetin; TDZ, thidazuron; 2IP = 6-Δ^2 isopentenyl amino purine; IAA = indole-3-acetic acid; NAA = 1-Naphthalene acetic acid; MS, Murashige and Skoog (1962); ZEA = zeatin ; BA = 6- benzyladenine

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) belongs to the family Solanaceae. It is one of the most important and nutritional vegetable in the world with wide rang of adaptability of soil and climate. Tomato is cultivated almost all over the world. The demand of tomato is increasing day by day in the agro and food industries, also it has become cash crop (Faried *et al.*, 2004). Tomato is propagated by conventional methods, *i.e*, seeds which have many problems associated with a means of the transmission of plant pathogens such as viruses, fungi, bacteria, nematode parasitic weeds(Villareal, 1980). Over 200 diseases have been reported to affect tomato plant in the world. Also, seeds were very expensive especially of those of hybrids.

Application of tissue culture technique is recommended as a very effective method since very small pieces are used and a small space required to multiply large number of plants in a given short time under aseptic condition. Such technique can provide the growers with the required numbers of transplants and avoid the previous disadvantages.

The explants tissue of tomato from different organs are totipotent to regenerate into plant either through callogenesis or organogenesis however their morphogenesis responses are affected by different factors Such as explants type *i.e* meristems (Jatoi *et al*., 2001), Leaf (Jatol, 2002; Chaudary *et al*., 2004; EL-Meleigy *et al.,* 2004; Sarwarkhan *et al*., 2006;); stems

(Chaudary *et al.*, 2001; Faried *et al*., 2004; Sheeja *et al*., 2004), Cotyledons (Rzepka- Pleves *et a*l., 2006) and hypocotyls (Ohki *et al*., 1978; Chaudary *et al.*, 2001; Gubis *et al*., 2004; Chaudary *et al.,* 2004) and cytokinin types at different concentrations (Youhui *et al.*, 1999; Chaudary *et al.,* 2001;Faried *et al* .2004; Gubis *et al.,* 2004; Chaudary *et al.,* 2004; Gubis *et al*., 2005; Sarwarkhan *et al*., 2006; Rzepka- Pleves *et al*., 2006).

Nevertheless, morphogenesis in cultivated tomato has been achieved less frequently compared to other members of family *Solanaceae*, such as *Nicotiana* spp., *Capsicum* spp and *Solanum* spp (Gubis *et al*., 2004).

This study designed for explaining the role of certain factors on micro- propagation of tomato (*Lycopersicon esculentum* Mill) California rock hybrid plant through tissue culture technique as callus induction and regeneration through investigating effect of explant types and effect of cytokinin types at different concentrations.

MATERIALS AND METHODS

The present work was aimed to study the possibility of micro - propagation of tomato (*Lycopersicon esculentum* Mill) by tissue culture technique .This work was conducted at the tissue culture laboratory, Horticulture department, faculty of Agriculture, Mansoura University, Egypt.

Source of explants under study

Seeds of tomato (*Lycopersicon esculentum* Mill) California rock hybrid were surface sterilized with 70% (v/v) ethanol for 1 min and were rinsed two times in sterilized water. The seeds were soaked in 0.5 % NaOCl sodium hypochlorite (commercial Clorox bleach) at 0.1% (v/v) Tween 20 for 20 min with shaking by hand. The seeds were thoroughly rinsed three times in sterilized water to avoid the toxic effect of sodium hypochlorite. After sterilization, the seeds were germinated aseptically on free hormone Murashige & Skoog, (1962) basal medium supplemented with 30 g /l sucrose and 7 g /l agar).

Callus Induction

After 4 weeks, two different explant types : leaf disc of true leaf (7 x 7 mm) and hypocotyls (8 – 10 mm) were excised aseptically and placed individually directly into 200 ml sterilized jars, which contained 20 ml of the media, that consists of (30 g sucrose/l, MS 4.405 g/l, $NaH_2PO_4.H_2O$ 50 mg /l, L-tyrosine 100 mg/l, Adenine sulfate 40 mg/l, NAA 0.5 mg/l). The treatments were the combination of two explant types, three cytokinin types (KIN, BAP, TDZ) and four concentrations (0.0 – 0.5 – 1.0 – 2.0 mg/l) for each type. PH of various media was adjusted to 5.8. Also, purified agar was added at 0.7 % (w/v) .All the tested treatments were autoclaved at 121 c^0 and at pressure of 1.2 kg/cm^2 for 20 minutes.

All the various treatments (10 cultured jar per trial) were incubated at 25 ± 2 c^0 under 16 h/day and 8h/night and light intensity (1500 lux).

Observation of cultures

After four weeks, culture treatments were evaluated for each as characters of callus (volume – fresh weight – dry weight and texture),

characters of root (formation % - number – length - fresh and dry weight) and shoot induction.

Statistical analysis

The experiment was conducted as factorial in complete randomized design with 10 replications. Data from three replicates were used for analysis. Statistical analysis were done using a PC- SAS program (version 9, SAS Institute Inc, Cary, NC, USA). Treatment means showing significant differences were statistically separated using the test LSD.

RESULTS AND DISCUSSION

1-Effect of different cytokinin types :

This part of study was conduced to evaluate the influence of cytokinins (TDZ, BAP and KIN) on the development of tomato (*Lycopersicon esculentum* Mill) *i.e*, characters of callus (volume – fresh weight – dry weight – texture), characters of root (formation % - number – length - fresh weight – dry weight) and shoot induction.

Data presented in Table (1) and Fig. (1a,b and 2a,b) indicate that callus characteristics (volume ,fresh and dry weight) were significantly affected by cytokinin types. The greatest values were achieved with BAP or TDZ treatments. Also, root characters (formation %, number , length ,- fresh and dry weight) were influenced significantly by type of cytokinin since KIN recorded the highest mean values followed by BAP then TDZ. The other tested characteristics *i.e*. shoot induction and callus texture were not affected significantly by cytokinin types.

These results are in agreement with Youmbi *et al.* (2006) who showed that TDZ increased multiplication rate. Sheeja *et al.* (2004) found that KIN was more effective than BAP at different IAA concentrations on tomato variety pant 5. Yu-wen *et al.* (1991) found that BA is more effective than ZEA and KIN for shoot regeneration frequency of pepper. Gubbuk and Pekmezccl (2004) reported that TDZ was more effective in producing more and better quality shoots in in-vitro banana propagation than BAP. Shoot proliferation was significantly greater on medium with TDZ than with BAP for each of the 3 banana types.

2- Effect of different concentrations of cytokinin :

This part of study was conduced to evaluate the influence of cytokinin typs (TDZ, BAP and KIN) at different concentrations (0.0, 0.50, 1.0 and 2.0 mg/l) on the development of tomato (*Lycopersicon esculentum* Mill) expressed callus characters(volume, texture , fresh and dry weight –), root (formation % - number – length - fresh weight – dry weight) and shoot induction.

Data recorded in Table (2) and illustrated in Fig. (1 a,b and 2 a,b) show that callus characters (volume, fresh and dry weight) were significantly affected by cytokinin concentrations, Where higher values were recorded at (0.5, 1.0 , and 2.0 mg/l with no significant difference between concentrations), while the lowest values were observed at control (0.0 mg/l). Also, the root characters (length , fresh and dry weight) were affected significantly by

concentrations and higher values were recorded at (0.0 - 0.5 – 1.0 mg/l). while the lowest value was observed at (2.0 mg/l). As for root number it was significantly affected by different concentrations, since the greatest values it were recorded at (0.5 and 1.0 mg/l) followed by 0.0 mg/l, then 2.0mg/l. However, the root formation % was affected significantly, since the highest value was achieved at 1.0mg/l. but 2.0 mg/l gave the lowest value. On the other hand, callus texture and shoot induction were not influenced significantly by cytokinin concentration..

These results were confirmed by results of Youmbi *et al.* (2006) who reported that the highest value was obtained at 0.4 µM TDZ with banana. Gyana (2004) found that concentration 2.5 mg/l of BA or 3.0 mg/l. of KIN is more effective. Aswath and Choudhary (2002) found that using BAP at 0.4 mg/l gave the highest value. On contrary, Kim (1987) found that callus, root and shoot of tomato were not affected by the concentrations of KIN at 1, 2 ,3, and 4 mg/l

3- Effect of explants type:

The influence of the two explant type , *i.e* leaf disc and hypocotyls on the development of tomato (*Lycopersicon esculentum* Mill) was studied. Callus characters (volume; texture , fresh and dry weight –), root characters (formation % ,- number , length , fresh and dry weight) and shoot induction were presented in Table (3) and Fig. (1 a,b and 2 a,b) . The results showed that callus (volume ,fresh and dry weight), root (formation % , number , length , fresh and dry weight) were significantly affected by explants type. Higher values of previous characteristics were obtained from hypocotyls explants as compared with leaf disc. On the other hand, callus texture and shoots induction were not influenced significantly by explants type**.**

The results are in harmony with Moghaleb *et al.* (1999) who reported that hypocotyls was better than cotyledons. Gubis *et al.* (2003) on tomato found that the hypocotyls explants was the most effective for percentage of regeneration for most tomato cultivars. Kim (1987) found that callus and root were formed by using hypocotyls as explants, otherwise no shoot was formed. Chaudary *et al*. (2004) reported that the hypocotyls were better than leaf discs because of recording the highest value of callus formation and growth. Chaudary *et al.* (2001) found that Hypocotyls have shown to be a better explants source for callus induction of tomato. EL-Meleigy *et al.* (2004) found that the use of hypocotyls segments of tomato cultivars (Castlrock or Oriet) produced better fresh and dry weights of callus than cotyledon or true leaf explants. Sheeja *et al.* (2004) reported that hypocotyls of tomato performed the best than cotyledon. Mok and Norzulaani (2007) reported that hypocotyls showed better response than cotyledons.

1+2+3

4 - Effect of interaction between cytokinin types and concentrations :

Data given in Table (4) and illustrated in Fig. (1 a,b and 2 a,b) show that callus characters (volume , fresh and dry weight), root characters(fresh and dry weight , number , length and formation %) were significantly affected by interaction between cytokinin types and concentrations. As for callus characters (volume and fresh weight), the BAP at (1.0 and 2.0 mg/l) and TDZ at 0.5 mg/l. recorded greatest values followed by the BAP at 0.5 mg/l and TDZ at 1.0 mg/l. While KIN recorded the lowest values. On other hand, callus dry weight tended to be different to the callus fresh weight with KIN at (0.5 – 1.0 and 2.0 mg/l.). The highest values were recorded at(0.5 - 1.0 and 2.0 mg/l) with BAP and at (0.5 and 2.0 mg/l.) with TDZ. The lowest value was observed at 0.0 mg/l. for each type. Regarding root (fresh weight – dry weight – number), the greatest values were noticed at 0.5 and 1.0 mg/l. of KIN. followed by 0.0 mg/l. for each type. But the lowest values of root cheaters resulted with TDZ at (0.5 – 1.0 and 2.0 mg/l.). As for the root length, the concentration 0.5 mg/l of KIN and 1.0 mg/l. of BAP recorded the highest value. While the lowest values of root cheaters with with TDZ at (0.5 – 1.0 and 2.0 mg/l.). In the case of root formation % the BAP at 1.0 mg/l. gave the best result (75 %), But the result was negative with TDZ at (0.5 – 1.0 and 2.0 mg/l.). On the other hand, callus texture and shoot induction were not influenced significantly either bu cytokinin type or concentration.

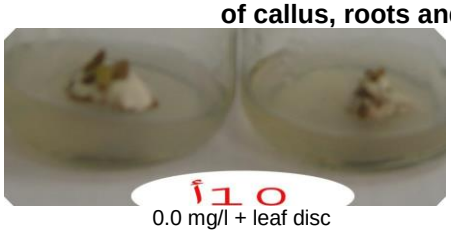
In this respect Rzepka- Pleves *et al.* (2006) reported that the highest explants forming callus % of tomato cultivar was obtained when media enriched with IAA at 2.0 mg/ll. and BAP at 1.0 mg/l. Gyana (2004) reported that shoot elongation was faster on media with BA alone (1.5 or 2.5 mg/l). Farid *et al.* (2004) reported that callus fresh weight and shoots number of tomato was increased by increasing BAP from 3.0 to 4.0 mg/l or IAA from 0.2 to 0.5 or 1.0 mg/l. Mok and Norzulaani (2007) reported that percentage of bud formation of pepper was increased by increasing BA from 2.0 mg/l up to 4.0 mg/l. then decreased after that. Sheeja *et al.* (2004) found that in general KIN was more effective than BAP with different IAA concentrations for regeneration of tomato. Woo and Sendon (2007)found that friable callus growth was observed in BAP (1 or 2mg/l), and BAP 2 mg/l + NAA 1 mg/l. Moderately compact callus was occurred in BAP 1 mg/l + NAA 0.5mg/l. Rhizogenesis was occurred in BAP (1 mg/l) + NAA (0.5mg/l) and BAP (2 mg/l) + NAA (1 mg/l). Pirinc *et al.* (2003) observed the highest value when the medium was supplemented with BA (0.5 and 1.0 mg/l) compared to all kinetin tested concentrations. Sultana and Bari (2003) observed that the combinations of BA with NAA were found to be superior to BA only and the combination of 1.0 mg/l BA + 0.2 mg/l NAA was superior to all other combinations of BA with NAA in watermelon micro- propagation. Sanatombi and Sharma (2007) clarified that the maximum number of buds was produced on an MS medium containing 10 mg/l Zea followed by 5 mg/l BAP in combination with 1 mg/l IAA.

5 - Effect of interaction between cytokinin and explants types :

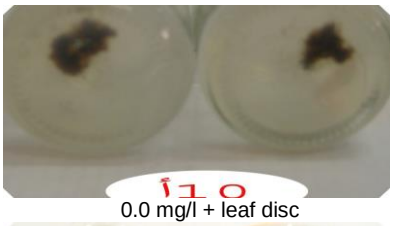
Data presented in Table (5) and illustrated in Fig. (1 a,b and 2 a,b) indicate that, callus characters(volume and fresh weight) were significantly affected by interaction between cytokinin and explant types.

4+5

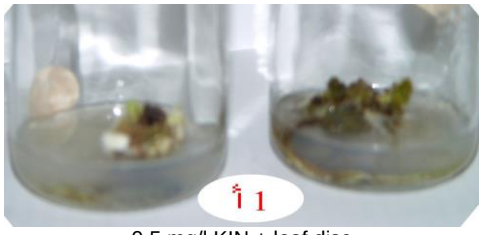
Fig. (1a,b) : Effect of interaction between cytokinin types and concentrations with leaf disc explants on characters of callus, roots and shoots induction.



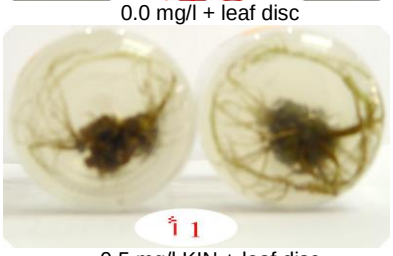
0.0 mg/l + leaf disc



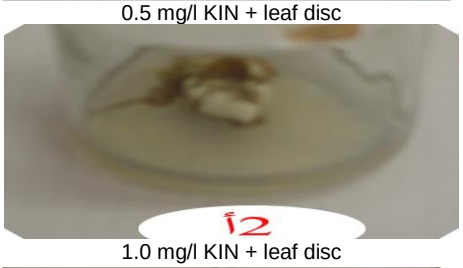
0.0 mg/l + leaf disc



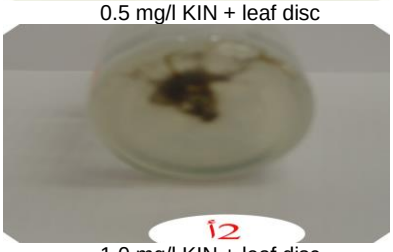
0.5 mg/l KIN + leaf disc



0.5 mg/l KIN + leaf disc

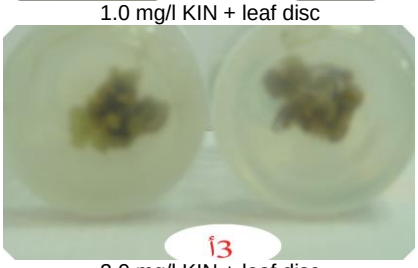


1.0 mg/l KIN + leaf disc

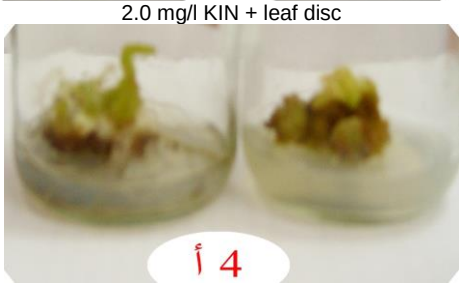


1.0 mg/l KIN + leaf disc

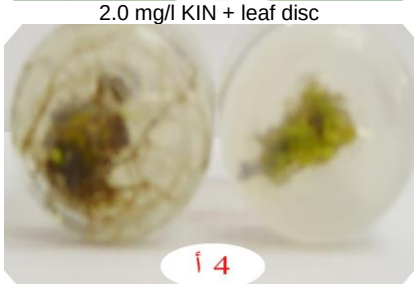
2.0 mg/l KIN + leaf disc



2.0 mg/l KIN + leaf disc

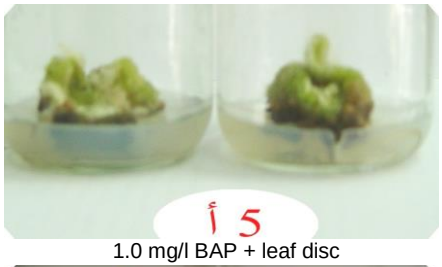


0.5 mg/l BAP + leaf disc

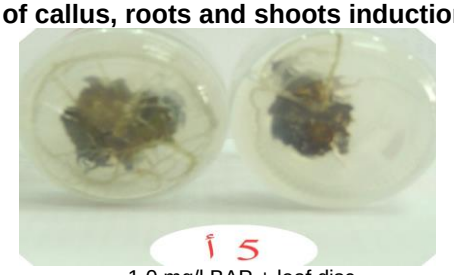


0.5 mg/l BAP + leaf disc

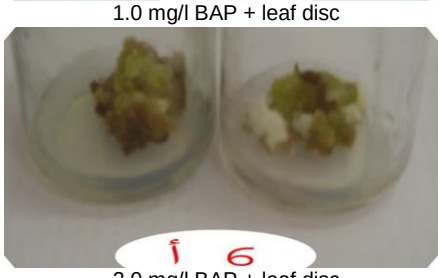
Fig. (1a,b) continuous: Effect of interaction between cytokinin types and concentrations with leaf disc explants on characters of callus, roots and shoots induction.



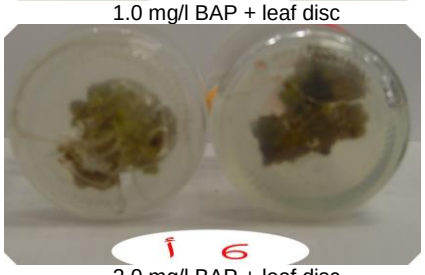
1.0 mg/l BAP + leaf disc



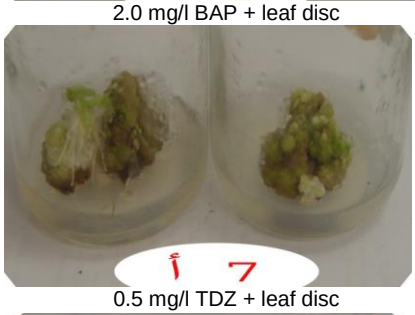
1.0 mg/l BAP + leaf disc



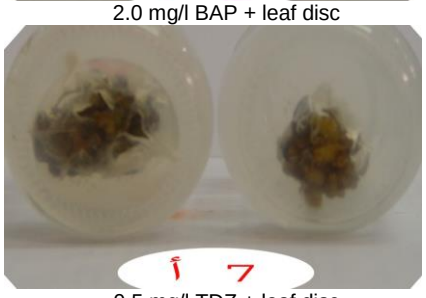
2.0 mg/l BAP + leaf disc



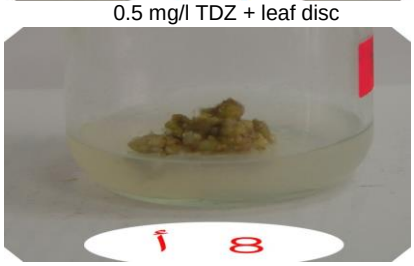
2.0 mg/l BAP + leaf disc



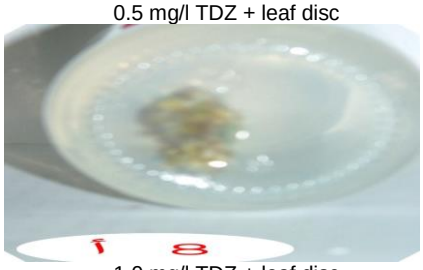
0.5 mg/l TDZ + leaf disc



0.5 mg/l TDZ + leaf disc



1.0 mg/l TDZ + leaf disc



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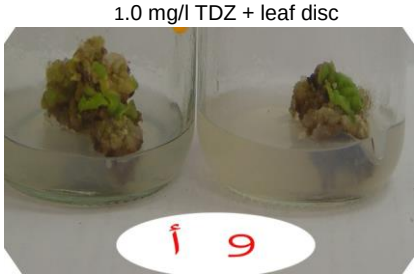
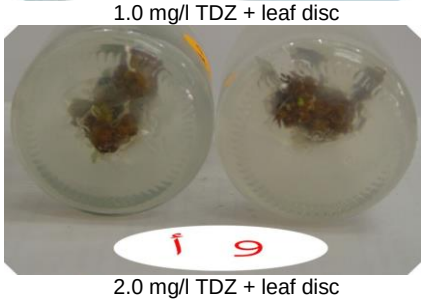
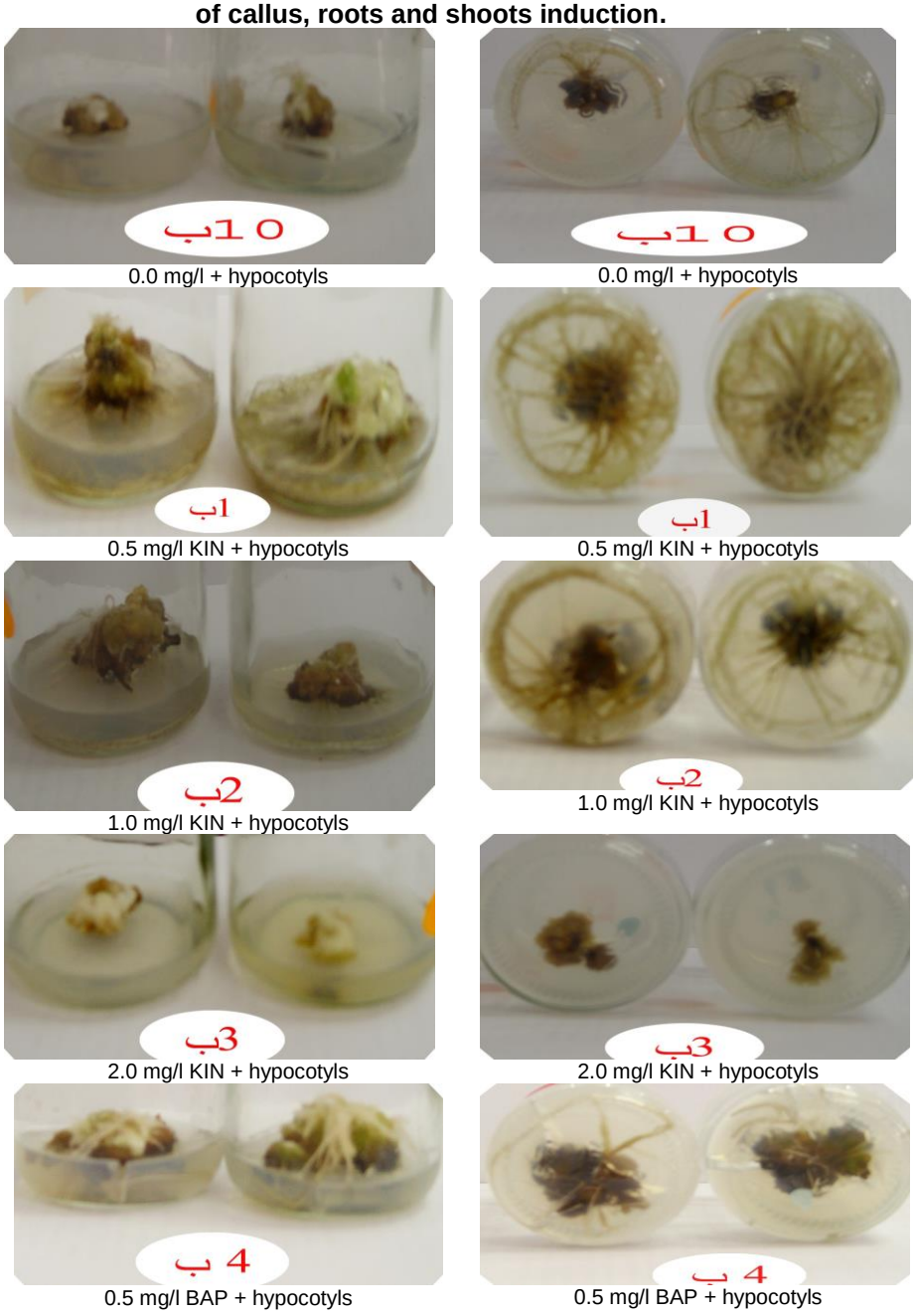


2.0 mg/l TDZ + leaf disc



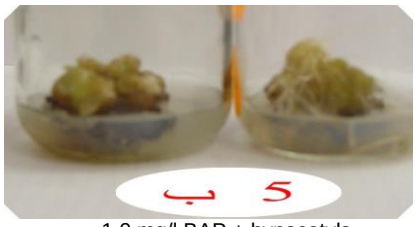
2.0 mg/l TDZ + leaf disc

Fig. (2a,b): Effect of interaction between cytokinin types and concentrations with hypocotyls explants on characters of callus, roots and shoots induction.

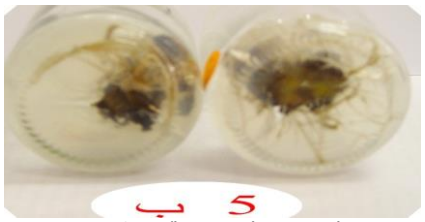


0.0 mg/l + hypocotyls
0.0 mg/l + hypocotyls
0.5 mg/l KIN + hypocotyls
0.5 mg/l KIN + hypocotyls
1.0 mg/l KIN + hypocotyls
1.0 mg/l KIN + hypocotyls
2.0 mg/l KIN + hypocotyls
2.0 mg/l KIN + hypocotyls
0.5 mg/l BAP + hypocotyls
0.5 mg/l BAP + hypocotyls

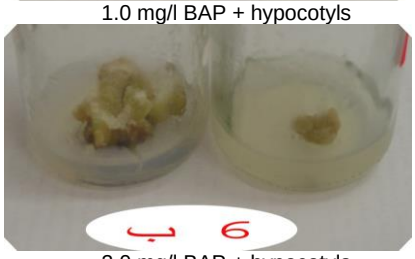
Fig. (2a,b) continuous: Effect of interaction between cytokinin types and concentrations with hypocotyls explants on characters of callus, roots and shoots induction.



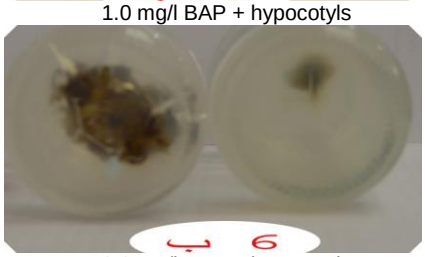
1.0 mg/l BAP + hypocotyls



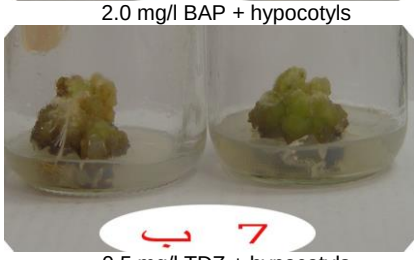
1.0 mg/l BAP + hypocotyls



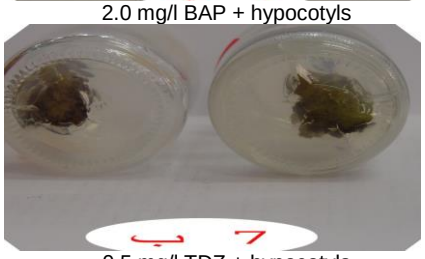
2.0 mg/l BAP + hypocotyls



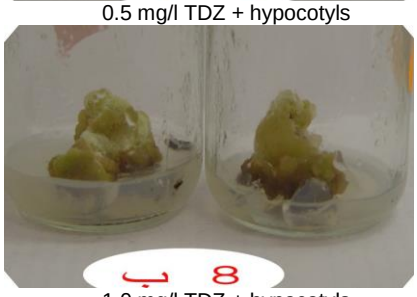
2.0 mg/l BAP + hypocotyls



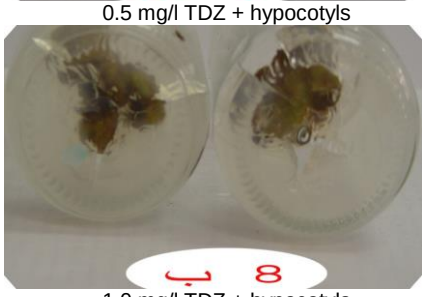
0.5 mg/l TDZ + hypocotyls



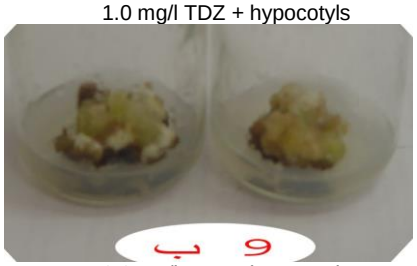
0.5 mg/l TDZ + hypocotyls



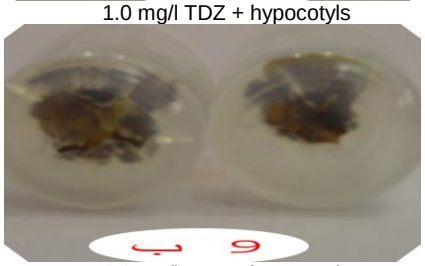
1.0 mg/l TDZ + hypocotyls



1.0 mg/l TDZ + hypocotyls



2.0 mg/l TDZ + hypocotyls



2.0 mg/l TDZ + hypocotyls

The greatest results were observed by BAP with leaf disc or hypocotyls or TDZ with hypocotyl . The lowest values were recorded at KIN with leaf disc or hypocotyls. Also, callus dry weight was significantly affected by interaction between cytokinin and explants type. The highest values were recorded with TDZ and hypocotyls or by BAP with both explants. On contrast, TDZ with leaf disc gave the lowest value. As for the root, fresh and dry weight were significantly affected. The interaction between KIN and hypocotyls was the most effective one being recorded the highest mean values (0.71 and 0.0280 gram, respectively).The least effective treatments in this respect were BAP and TDZ with both explant types. Regarding root characters (number , length and formation %), the means were affected significantly with interaction between cytokinin and explant types. The greatest value of root number was recorded in case of KIN with hypocotyls . on the other hand, TDZ with leaf disc gave the lowest value. Regarding root length, KIN with hypocotyls or BAP with hypocotyl recorded the higher values, while TDZ with leaf disc gave the lowest. As for root formation %, the KIN with hypocotyls or BAP with hypocotyl recorded the highest values (47.5 and 50 %, respectively). On the other hand, the least treatment in this respect was TDZ with leaf disc (4.5 %). The rest interactions treatments gave values between those two extremes. On the contrast, callus texture and shoot induction were not influenced significantly.

These results are not in agreement with those of Gubis *et al*. (2005) who reported that the best regeneration frequency was noticed with hypocotyls and 0.5 mg/l ZEA and 0.05 mg/l IAA. Sarwarkhan *et al.* (2006) found that, in tomato Based on media composition, more callus formation and less regeneration were observed at 1mg/l ZEA and 0.1mg/lIAA from either leaves or cotyledons. In wild tomato (*Lycopersicon cheesmanii*) regeneration of 5.8 and 6.0 shoots using leaf and cotyledon explants, respectively was reported when zeatin was added to the culture medium (Arriliage *et al.* (2001). Jatoi *et al*. (2002) mentioned that formation of callogenesis and shoot regeneration from leaf explants depend on cytokinin and genotype.

6 - Effect of interaction between concentration and explants type :

Results presented in Table (6) and illustrated in Fig. (1 a,b and 2 a,b) show that callus characters (volume , fresh and dry weight), and root (number , length , formation %, fresh and dry weight) were significantly affected by interaction between concentration and explant types. Regarding callus (volume , fresh and dry weight), data showed that the highest value was noticed with (0.5 mg/l and hypocotyls) , (1.0 mg/l and hypocotyls) or (2.0 mg/l with leaf disc). On contrast, the 0.0 mg/l with leaf disc or hypocotyls gave the lowest value for each character. As for root fresh and dry weight, the 0.0 mg/l with hypocotyls or 0.5 mg/l with hypocotyls gave the greatest value. On the other hand , all used concentrations with leaf disc gave the lowest values. As for root (fresh weight – dry weight - number – length) the response to the interaction has the same trend. While the 0.0, 0.5 and 1.0 mg/l with hypocotyls were the most effective one being recorded the highest mean values. On contrast, the rest interactions registered the lowest value. As for root formation %, the 0.0 mg/l with hypocotyls gave the higher

percentage followed by 1.0 mg/l with leaf disc or hypocotyls. But the rest interactions recorded the lowest root formation. On the contrast, callus texture and shoot induction were not influenced significantly either by concentration of cytokinins or explant type.

This result corresponds with that obtained by Rabbia *et al*. (2007) who found that 3.0 mg/l of BA is the most effective. Sanatombi and Sharmain (2006) observed that the shoot-tip explants of pepper with 22.2 µM BAP or 44.4 µM BAP were the most effective.

7- Effect of interaction among cytokinin type, concentration and explant type :

This part of study was conduced to evaluate the influence of cytokinin types (KIN, BAP and TDZ) at different concentrations (0.0, 0.50, 1.0 and 2.0 mg/l) on callus characters (volume, texture, fresh and dry weight), root characters (formation %, number, length, fresh and dry weight) and shoot induction.

Data presented in Table (7) and illustrated in Fig. (1 a,b and 2 a,b) indicate that, all studied characters were influenced significantly by interaction between cytokinin type, concentration and explant types except callus texture and shoot induction were not significantly affected by any treatment. Regarding callus volume and fresh weight, data clarified that BAP at 1.0 mg/l with leaf disc or hypocotyls and BAP at (0.5 , 2.0 mg/l) with hypocotyl recorded the greatest value callus volume and fresh weight. The concentration 0.0 mg/l with leaf disc of each type gave the lowest values. Regarding callus fresh weight the highest value observed with (BAP 0.5 + hypocotyls ,1.0 and 2.0 mg/l with each explant) or TDZ at 2.0 mg/l with hypocotyls. As for callus dry weight the greatest value was obtained from (KIN at 0.5 + each explant , 1.0 mg/l with hypocotyls , 2.0 mg/l + leaf disc) or (BAP at 0.5 ,1.0 with each explant and 2.0 mg/l with lef disc) or (TDZ at 0.5 , 1.0 and 2.0 with hypocotyls). On contrast, the concentration 0.0 mg/l with leaf disc of each type produced the lowest values. As for root fresh weight, data reveal that, KIN at 0.5 or 1.0 mg/l with hypocotyls was the most effective and gave the highest value. On the contrary, TDZ at (0.5 or 1.0 or 2.0 mg/l) with leaf disc or hypocotyls gave negative result (0.0 value). Regarding root dry weight, using KIN at 0.5 mg/l with hypocotyls recorded the greatest value, followed by 0.0 mg/l with hypocotyls for each type of cytokinin or KIN at 1.0 mg/l with hypocotyls. On the contrast, TDZ at 0.5 or 1.0 or 2.0 mg/l with leaf disc or hypocotyls gave negative result (0.0 value). As for root number, Kin at 0.5 or 1.0 mg/l with hypocotyls gave the best results followed by BAP at 1.0 mg/l with hypocotyls. On the other hand, TDZ at (0.5 or 1.0 or 2.0 mg/l) with leaf disc or hypocotyls gave negative result (0.0 value). As root length, using KIN at 1.0 mg/l with hypocotyls gave rise to the greatest value followed by KIN at 0.5 mg/l with hypocotyls or BAP at 1.0 mg/l for both of explants. On contrast, TDZ at (0.5 or 1.0 or 2.0 mg/l) with leaf disc or hypocotyls gave negative result (0.0 value). As for root formation %, BAP at 1.0 mg/l with hypocotyls recorded the highest value, followed by 0.0 mg/l with hypocotyls. On the other hand, TDZ at (0.5 or 1.0 or 2.0 mg/l) with leaf disc or hypocotyls recorded negative result (0.0 value)

7

Results reported herein are similar to those obtained by Duran and Semanick (1981) who found that the optimum concentration of KIN for stimulating bud and shoot regeneration was 3 mg/l for leaf disc and 1 mg/l. for stem segments, KIN at 30 mg/l were toxic to leaf disc. Sarwarkhan *et al.* (2006) mentioned that more callus formation and less regeneration was observed at 1mg/l Zea and 0.1mg/lIAA either taken from leaves or cotyledons. Otherwise, Sanatombi and Sharma (2007) clarified that the maximum number of buds was produced on MS medium containing 10 mg/l Zea followed by 5 mg/l BAP in combination with 1 mg/l IAA. MS medium containing Kin alone was found to be the least effective among the three cytokinins (BAP, Zea, and Kin). Woo and Sendon (2007) showed the responses of the different kinds of explants of pepper to auxin-cytokinin combinations. Formation of yellowish, friable callus was observed in all the treatments. Root formation was found to occur predominantly in cotyledon explants without petiole in the medium containing BAP (1mg/l) + NAA (0.5mg/l). Shoot bud development was not observed in any of the treatments. Rubluo and Barroso (1992) found similar results since callus production in pepper hypocotyls was the most conspicuous expression and appeared with all the combinations test. While a poor morphogenetic response was apparent in this explants, also shoot induction ranged from 0.0 to 13.0 with apical meristem or from 0.0 – 1.0 with hypocotyls. Gubis *et al*. (2004) mentioned that the frequency of adventitious shoot regeneration differed depending on the type of explants and both the type and concentration of growth regulators.

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دراسات على إكثار الطماطم باستخدام تكنيك زراعة الأنسجة.
١- تأثير نوع وتركيز السيتوكينن مع اجزاء نباتية مختلفة على تحفيز تكوين الكالس للطماطم.

محمود محمد زغلول ، سمير طه العفيفى و السعيد السيد متولى
قسم الخضر والزينة – كلية الزراعة – جامعة المنصورة

اجريت هذة الدراسة فى معمل زراعة الانسجة بقسم الخضر والزينة كلية الزراعة – جامعة المنصورة بهدف دراسة تأثير ثلاثة أنواع من السيتوكينن هى (الكينيتين – بنزيل امينو بيورين ثيدازرون) عند تركيزات مختلفة (صفر - ٠,٥ – ١,٠ ٢,٠ مجم/ لتر) مع جزئين نباتيين هما جزء من كلا من السويقة الجنننية السفلى و الورقة. ولقد لوحظ تكوين الكالس مع كل المعاملات وكان البنزيل امينو بيورين افضل نوع يليه الثيدازرون ثم الكينتين. سجل التركيز٠,٥ و ١,٠ مجم /لتر اعلى القيم مقارنة بباقى التركيزات. وتفوقت السويقة الجنينية السفلى على الورقة فى تسجيل اعلى القيم. الكالس المتكون ذو قوام هارد (صلب) ولم يتكون اى نموات خضرية على الكالس. كذلك لم يتكون جذور مع الكينتين والبنزيل امينو بيورين عند تركيز ٢,٠ مجم/ لتر مع السويقة الجنينية السفلى وأيضا الثيدازرون مع التركيزات (٠,٥ -١,٠ – ٢,٠ مجم/ لتر) مع اى جزء نباتى. .وللتفاعل الثلاثى فلقد تحققت اعلى القيم لانتاج الكالس (الحجم والوزن الطازج والجاف) مع البنزيل امينوبيورين بتركيز ١,٠ مجم/لتر مع السويقة الجنينية السفلى.

Table (1): Effect of cytokinin types on characters of callus , root and shoot induction.

cytokinin types	Callus volum (cm^3)	Callus fresh weight (g)	Callus dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root number	Root length (cm)	Callus texture	Root formation %	Shoot induction
KIN	2.66 b	2.326 b	0.187 b	0.440 a	0.018 a	5.416 a	2.38 a	hard	40.5 a	0.0
BAP	5.36 a	4.632 a	0.229 a	0.173 b	0.009 ab	3.125 b	2.73 a	hard	46.25 a	0.0
TDZ	4.69 a	4.188 a	0.207 ab	0.077 b	0.003 b	0.625 c	0.504 b	hard	12.5 b	0.0

(0.0) : none values with the same letters are not significantly differed due to LSD at 5%.

Table (2): Effect of cytokinin concentrations on characters of callus , root and shoot induction.

Concent-ration mg/l	Callus volum (cm^3)	Callus fresh weight (g)	Callus dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root number	Root length (cm)	Callus texture	Root formation %	Shoot induc-tion
0.0	1.966 b	1.442 b	0.100 b	0.308 a	0.015 a	2.50 b	2.01 a	Hard	50 c	0.0
0.5	5.116 a	4.732 a	0.249 a	0.339 a	0.016 a	4.61 a	2.37 a	Hard	56.66 b	0.0
1.0	4.738 a	4.193 a	0.234 a	0.262 a	0.009 ab	4.61 a	2.73 a	Hard	86.66 a	0.0
2.0	5.144 a	4.494 a	0.248 a	0.010 b	0.001 b	0.50 c	0.36 b	hard	33.33 d	0.0

(0.0) : none values with the same letters are not significantly differed due to LSD at 5%.

Table (3): Effect of explant types on characters ofcallus , root and shoot induction.

Explant types	Callus volume (cm^3)	Callus fresh weight (g)	Callus dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root number	Root length (cm)	Callus texture	Root formation %	Shoot induction
Leaf disc	3.813 b	3.26 b	0.1839 b	0.994 b	0.0056 b	1.52 b	1.95 b	hard	32.0 a	0.0
hypocotyls	4.669 a	4.17 a	0.2324 a	0.361 a	0.0159 a	4.58 a	2.55 a	hard	31.0 a	0.0

(0.0) : none values with the same letters are not significantly differed due to LSD at 5%.

Table (4): Effect of interaction between cytokinin types (cyto. Types)and concentration (conc.)on characters of callus , root and shoot induction .

Treatment		Callus volume (cm^3)	Callus fresh weight (g)	Callus dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root number	Root length (cm)	Callus texture	Root formation %	Shoot induction
Cyto. types	Con. mg/l										
KIN	**0.0**	1.96 d	1.44 d	0.1005 c	0.308 bc	0.015 bc	2.50 cd	2.01 bcd	hard	50 c	0.0
	0.5	2.68 cd	3.09 c	0.2271 ab	0.829 a	0.036 a	10.50 a	4.15 a	Hard	50 c	0.0
	1.0	2.80 cd	2.49 cd	0.2183 ab	0.610 ab	0.0190 ab	8.00 ab	3.23 ab	Hard	55 b	0.0
	2.0	3.21 cd	2.27 cd	0.204 ab	0.014 c	0.0033 bc	0.66 d	0.12 d	Hard	25 e	0.0
BAP	**0.0**	1.96 d	1.44 d	0.1005 c	0.308 bc	0.015 bc	2.50 cd	2.01 bcd	Hard	50 c	0.0
	0.5	6.08 ab	5.26 ab	0.264 ab	0.190 c	0.0125 bc	3.33 cd	2.96 abc	Hard	35 d	0.0
	1.0	7.08 a	6.24 a	0.2966 a	0.175 c	0.0088 bc	5.83 bc	4.98 a	Hard	75 a	0.0
	2.0	6.33 a	5.57 a	0.257 ab	0.018 c	0.0021 bc	0.83 d	0.96 cd	Hard	25 e	0.0
TDZ	**0.0**	1.96 d	1.44 d	0.1005 c	0.308 bc	0.015 bc	2.50 cd	2.01 bcd	Hard	50 c	0.0
	0.5	6.58 a	5.84 a	0.255 ab	0.00 c	0.00 c	0.00 d	0.00 d	Hard	0.0 f	0.0
	1.0	4.33 bc	3.83 bc	0.1883 bc	0.00 c	0.00 c	0.00 d	0.00 d	Hard	0.0 f	0.0
	2.0	5.88 ab	5.63 a	0.2838 ab	0.00 c	0.00 c	0.00 d	0.00 d	hard	0.0 f	0.0

(0.0) : none values with the same letters are not significantly differed due to LSD at 5%.

Table (5): Effect of interaction between cytokinin types (cyto. Types)and explants types on characters of callus , root and shoot induction.

Cyto. types	Explant type	Callus volume (cm^3)	Callus fresh weight (g)	Callus dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root number	Root length (cm)	Callus texture	Root form-ation %	Shoot induc-tion
KIN	**Leaf disc**	2.458 c	2.10 b	0.175 bc	0.170 b	0.0093 b	2.00 bc	1.22 bc	hrad	42.5 ab	0.0
	Hypocotyl	2.875 c	2.55 b	0.200 bc	0.71 0a	0.0280 a	8.83 a	3.54 a	Hrad	47.5 a	0.0
BAP	**Leaf disc**	5.133 ab	4.487 a	0.227 ab	0.111 b	0.0071 b	2.50 bc	2.35 ab	Hrad	42.5 ab.	0.0
	Hypocotyl	5.60 a	4.77 a	0.232 ab	0.235 b	0.0124 b	3.75 b	3.11 a	Hrad	50 a	0.0
TDZ	**Leaf disc**	3.85 bc	3.19 b	0.149 c	0.016 b	0.0005 b	0.08c	0.016 c	Hrad	4.5 b	0.0
	Hypocotyl	5.533 a	5.18 a	0.264 a	0.1377 b	0.0072 b	1.66 bc	0.99 bc	hrad	17.5 ab	0.0

(0.0) : none values with the same letters are not significantly differed due to LSD at 5%.

Table (6): Effect of interaction between cytokinin concentration (conc mg/l) and explant types on characters of callus, root and shoot induction.

Cyto. Conc. mg /l	Explants types	Callus volume (cm^3)	Callus fresh weight (g)	Callus dry weight (g)	Root fresh weigh (g)	Root dry weight (g)	Root number	Root length (cm)	Callus texture	Root formation %	Shoot induction
0.0	Leaf disc	1.70 e	1.23 c	0.077 d	0.066 cd	0.0023 c	0.3 c	0.06 d	hard	30 bc	0.0
	Hypo-cotyl	2.23 de	1.65 c	0.124 cd	0.551 a	0.0290 a	4.6 ab	3.96 a	Hard	70 a	0.0
0.5	Leaf disc	4.56 bc	4.10 ab	0.2332 b	0.186 bcd	0.0121 bc	2.5 bc	1.62 bc	Hard	20 bc	0.0
	Hypo-cotyl	5.66 ab	5.35 a	0.265 ab	0.493 ab	0.020 ab	6.6 a	2.82 ab	Hard	36.67 b	0.0
1.0	Leaf disc	3.31 cd	3.10 b	0.1606 c	0.123 cd	0.0046 c	2.2 bc	2.06 bc	Hard	40.0 ab	0.0
	Hypo-cotyl	6.16 a	5.28 a	0.3082 a	0.401 abc	0.0138 abc	7.0 a	3.41 ab	Hard	46.67 ab	0.0
2.0	Leaf disc	5.67 ab	4.59 a	0.264 ab	0.021 d	0.003 c	1.0 c	6.72 cd	Hard	33.33 b	0.0
	Hypo-cotyl	4.61 bc	4.39 ab	0.232 b	0.000 d	0.000 c	0.0 c	0.00 d	hard	0.0 c	0.0

(0.0) : none values with the same letters are not significantly differed due to LSD at 5%.

Table (7): Effect of interaction among cytokinin types, concentrations (mg/l)and explants types on characters callus , root and shoot induction.

Cyto.type	Cyto. Conc.	C.	Callus volume (cm^3)	Callus fresh weight (g)	Callus dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root number	Root length (cm)	Callus texture	Root formation %	Shoot induction
KIN	0.0	C1	1.70 g	1.23 i	0.077 d	0.066 cd	0.0023 c	0.33 f	0.06 e	hard	30 f	0.0
		C2	2.23 fg	1.65 hi	0.1240 cd	0.551 bc	0.0290 b	4.66 bcde	3.96 abc	Hard	70 b	0.0
	0.5	C1	2.53 fg	2.72 fghi	0.222 abc	0.306 cd	0.0193 bc	5.00 bcd	3.40 abcd	Hard	40 e	0.0
		C2	2.83 feg	3.46 efg	0.2316 abc	1.351 a	0.0543 a	16.00 a	4.90 ab	Hard	60 c	0.0
	1.0	C1	1.26 g	1.43 hi	0.1286 cd	0.280 cd	0.0090 bc	1.33 ef	1.16 de	Hard	50 d	0.0
		C2	4.33 def	3.56 efg	0.308 ab	0.941 ab	0.0290 b	14.66 a	5.30 a	Hard	60 c	0.0
	2.0	C1	4.33 def	3.01 fgh	0.271 ab	0.028 cd	0.006 bc	1.33 ef	0.25 e	Hard	50 d	0.0
		C2	2.11 fg	1.52 hi	0.1373 cd	0.00 d	0.00 c	0.00 f	0.00 e	Hard	0.00 h	0.0
PAB	0.0	C1	1.70 g	1.23 i	0.077 d	0.066 cd	0.0023 c	0.33 f	0.06 e	Hard	30 f	0.0
		C2	2.23 fg	1.65 hi	0.1240 cd	0.551 bc	0.0290 b	4.66 bcde	3.96 abc	Hard	70 b	0.0
	0.5	C1	5.00 cde	4.37 def	0.268 ab	0.252 cd	0.017 bc	2.66 cdef	2.36 bcde	Hard	20 g	0.0
		C2	7.16 abc	6.15 abc	0.261 ab	0.127 cd	0.008 bc	4.00 bcde	3.56 abcd	hard	50 d	0.0
	1.0	C1	6.50 abcd	6.00 abcd	0.278 ab	0.088 cd	0.005 bc	5.33 bc	5.03 ab	hard	70 b	0.0
		C2	7.66 a	6.49 ab	0.314 ab	0.262 cd	0.012 bc	6.33 b	4.93 ab	Hard	80 a	0.0
	2.0	C1	7.33 ab	6.34 ab	0.285 ab	0.036 cd	0.004 bc	1.66 def	1.93 cde	Hard	50 d	0.0
		C2	5.33 bcd	4.81 bcde	0.229 abc	0.00 d	0.00 c	0.00 f	0.00 e	Hard	0.0 h	0.0
TDZ	0.0	C1	1.70 g	1.23 i	0.077 d	.066 cd	0.0023 c	0.33 f	0.06 e	Hard	30 f	0.0
		C2	2.23 fg	1.65 hi	0.1240 cd	0.551 bc	0.0290 b	4.66 bcde	3.96 abc	Hard	70 b	0.0
	0.5	C1	6.16 abcd	5.22 abcde	0.208 bc	0.00 d	0.00 c	0.00 f	0.00 e	Hard	0.0 h	0.0
		C2	7.00 abc	6.46 ab	0.303 ab	0.00 d	0.00 c	0.00 f	0.00 e	Hard	0.0 h	0.0
	1.0	C1	2.16 fg	1.87 ghi	0.074 d	0.00 d	0.00 c	0.00 f	0.00 e	Hard	0.0 h	0.0
		C2	6.50 abcd	5.80 abcd	0.302 ab	0.00 d	0.00 c	0.00 f	0.00 e	hard	0.0 h	0.0
	2.0	C1	5.36 abcd	4.44 cdef	0.237 abc	0.00 d	0.00 c	0.00 f	0.00 e	Hard	0.0 h	0.0
		C2	6.40 abcd	6.82 a	0.330 a	0.00 d	0.00 c	0.00 f	0.00 e	hard	0.0h	0.0

C- explants types c1- leaf disc c2- hypocotyls
(0.0) : none values with the same letters are not significantly differed due to LSD at 5%.