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BIOTECHNOLOGICAL STUDIES ON *Solanum viride* PLANT: A- *IN VITRO* PROPAGATION OF *Solanum viride* PLANT THROUGH TISSUE CULTURE TECHNIQUE.

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ABSTRACT

Leaves of Green nightshade (*Solanum viride* Solander ex Forst. f.) family: *Solanaceae* were used as source of explants to start micropropagation. Leaves were successfully sterilized using the treatment of sodium hypochlorite (NaOCl) at 1.0% that showed the highest percentage of survival without any contamination. The highest fresh weight of callus/explant was recorded when the leaf segments were cultured on MS media contained 0.5 mg/l BA and 1.5 mg/l NAA. Interestingly, some treatments recorded direct organogenesis (direct shoots and roots) from the leaf explants. The highest direct shoot formation was recorded with MS media supplemented with 2 mg/l BA alone. However, it was clear that all shoots obtained with the responded treatments were vitrified. Therefore, in a trial to obtain unvitrified shoots as indirect organogenesis through callus formation and differentiation, all indirect obtained shoots were vitrified except some shoots (4.7 % of them) obtained on MS media supplemented with 1.0 mg/l BA alone. These unvitrified shoots were successfully acclimatized in soil mixture of sand and peatmoss (1:2, v: v) that showed the best growth and percentage of survival (95%).

INTRODUCTION

Green nightshade (*Solanum viride* Solander ex Forst. f.) is belonging to family: *Solanaceae*. This plant is wide spread in warmer regions, it is found growing in different habitats, it occurs naturally or cultivated, especially on limestone soils at low elevations along coastlines, edges of forests, and open areas. The plant contains glycoalkaloids including solasodine. In Tahiti, the plant is used as a sedative, diuretic, to treat infection of the eye (conjunctivitis) and to treat pus-filled infections. In New Guinea, the swellings resulting from the parasitic disease, filariasis, is remedied with a tea made from the leaves. Crushed leaves are applied to boils, fungal infections and tumors of the breast. In Fiji, the leaves are used to treat wounds (Han, 1998). Solasodine has been reported as a valuable steroidal precursor for the supplementary source of commercial synthesis of several steroidal drugs (Jiradej and Aranya, 2005). Glycoalkaloids are reported to inactivate the *Herpes simplex*, *Herpes zoster* and *Herpes genitalis* viruses in humans, while Aglyones, including solasodine, may protect against skin cancer. Extracts of glycoalkaloids can be used to obtain a potential skin cancer preparation for clinical research (Nada, *et al.* 2005). So far there is no earlier report on *in vitro* plant regeneration of this species (*Solanum viride).* It is, therefore, necessary to develop efficient and reliable cultural techniques and formulate appropriate media composition for specific organ as a prerequisite for *in vitro*

propagation of the plant. The present investigation was conducted to establish a suitable regeneration protocol for *Solanum viride* plant using tissue culture technique.

MATERIALS AND METHODS

This work was carried out in the Lab. of Biotechnology, Genetic Engineering and Biotechnology Research Institute (GEBRI), Minufiya University, Sadat City, and the Lab. of Biotechnology, Horticulture Research Institute, Ministry of Agriculture, Egypt, on *Solanum viride* plant during the period from 2002 to 2007.

Plant Materials:

Seeds of *Solanum viride* were obtained from the Genetic Engineering and Biotechnology Research Institute. Mother plant was selected which was normal, healthy grown, free of any disease symptoms. Leaves were used as explants to start the tissue culture experiments. These explants were dissected from 2 months old plantlets produced by cultivation the seeds in the farm of GEBRI.

Culture medium:

Murashige and Skoog (1962) (MS) was used as a nutrient medium in all experiments of this study. The pH value of the used medium was adjusted at 5.7± 0.1 prior to addition of agar (7g/l). The medium was dispensed in jars of 120 ml capacity, each contained 25 ml medium. The jars were steam sterilized in an autoclave under pressure of 1.2 Kg/cm^2 and 121^o C over a period of 20 minutes. The medium was left for 4 days at room temperature and then used for culture.

The following experiments were conducted:

Experiment 1: Effect of different materials of surface sterilization on survival and contamination percentages of *Solanum viride* explants:

Leaves were washed thoroughly under a running tap water for about 30 minutes, and these leaves were immersed in 3 levels of sodium hypochlorite (NaOCl) 0.5, 1.0 and 1.5% with 2-3 drops of tween 20 (as wetting agent) for 20 minutes. Then, leaves were washed three times with sterile distilled water. Half of explants (leaves) in each treatment were immersed in 95 % ethanol alcohol for 10 second. Then, leaves were washed three times with sterile distilled water. Data were recorded after 2 weeks as survival and contamination percentage of *Solanum viride* cultures.

Experiment 2: **Callus initiation from *Solanum viride* explants as affected by plant growth regulators (P.G.R.):**

Segments of sterilized leaf (1 x 0.5 cm) were cultured on MS medium supplemented with 30 g/l sucrose, 7 g/l agar and 2,4-Dichlorophenoxy acetic acid (2,4-D) or naphthalene acetic acid (NAA) as auxin each at 0.0, 0.5, 1.0, 1.5, 2.0 mg/l and combined with benzyl adenine (BA) as cytokinin at 0.0, 0.5, 2.0, 3.5, 5.0 mg/l. Each treatment was represented by 6 replicates (jars), each jar contained three explants. Then, the cultured jars were incubated in the growth room. Data were recorded after six weeks including percentage of callus formation and callus fresh weight/explant (g).

Some treatments of this experiment showed direct shoot or root formation or both (shoots and roots), so that data included the shoot number/explant and root number/explant.

Experiment 3: Effect of plant growth regulators on callus differentiation:

This experiment was designed to study the effect of different plant growth regulators on callus differentiation. Sterilized leaves were cultured on the best concentration (1.5 mg/l NAA with 0.5 mg/l BA) for obtaining callus as previously obtained during the above experiment. 5 g of the produced fresh callus were cultured on 40 ml MS medium supplemented with 30g sucrose, 7g/l Agar and combinations between NAA as auxin at 0.0, 0.5, 1.0, 1.5 mg/l and BA as cytokinin at 0.0, 0.5, 1.0, 1.5 mg/l. The pH value was adjusted to 5.7 before adding the agar. Media were autoclaved at 121C^{o} and 1.2 Kg/cm^{2} for 20 min. Each treatment was represented by 6 replicates (jars). Data were recorded after six weeks as shoot number/cluster, root number/cluster and vitrification percentage.

Incubation conditions:

The cultures of all *in vitro* experiments were incubated at 25^{0}c $\pm$ 2 day and night. Light was provided by white fluorescent tubes giving light intensity of 2000 lux at the level of cultures for 16 hours per day.

Experiment 4: Acclimatization of plantlets:

Unvetrified plantlets obtained from experiment 3 (3 to 5cm in length) were cultured on MS basal medium supplemented with 20 g sucrose + 7 g agar + 0.5 mg/l BA (the same medium of experiment 3 with less sucrose), and were incubated in growth room. After 4 weeks, the plantlets were transferred to the greenhouse for acclimatization. Unvetrified plantlets of *Solanum viride* were transferred individually and planted in pots (6cm diameter) filled with different mixtures of sand and peatmoss (1:1, 1:2 and 2:1, v: v)**.** Each pot contained one plantlet and was covered with transparent polyethylene bags (for 2 week) to maintain a high humidity around the plantlets. Data were recorded after 30 days of transplanting as survival percentage, plantlet height (cm) and leaf number/plantlet.

All experiments were repeated twice and the represented data were averages. Results of these experiments were analyzed by analysis of variance (ANOVA) according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Experiment (1): Effect of different materials of surface sterilization on survival and contamination percentages of *Solanum viride* explants after 2 weeks *in vitro*:

The effect of different concentrations of NaOCl and ethanol (95%) and their combinations on survival percentages is presented in Table (1). Data on the main effect of NaOCl clearly indicate that, a negative response was significantly recorded with increasing the concentration of NaOCl. Moreover, the highest survival percentage (73.33) was significantly observed with the lowest level of NaOCl (0.5%).

Data on the main effect of ethanol (95%) show that, significant decrease in survival percentage was obtained with using ethanol at 95% for surface sterilization of *Solanum viride* explants (leaves).

As for the interaction, data reveal that, using lower levels of NaOCl (0.5 or 1.0%) alone was more effective in increasing survival percentage, in the contrary, using the highest level of NaOCl (1.5 %) alone or combined with ethanol in surface sterilization of *Solanum viride* explants decreased the survival percentage of explants. However, the highest percentage of survival (80%) was significantly recorded with the treatment of NaOCl alone at the lowest level (0.5%) compared to the other treatments.

Concerning the effect of different concentration of NaOCl and ethanol (95%) and their combinations on contamination percentages, data in Table (1) indicate that, all treatments of surface sterilization showed explants free of any contamination except the treatment of NaOCl alone at the lowest level (0.5%) that recorded 20% contaminated explants and these explants were dead.

Table (1): Effect of different sterilization materials and their combinations on the survival, and contamination percentages of *Solanum viride* after 2 weeks *in vitro*.

Ethanol (95%) NaOCl	Survival %			Contamination %		
	Without Ethanol	With Ethanol (95%)	Mean	Without Ethanol	With Ethanol (95%)	Mean
0.5 %	80.00 a	66.66 c	73.33 a	20.00 a	00.00 b	10.50 a
1.0 %	73.33 b	60.00 d	66.66 b	00.00 b	00.00 b	00.00 b
1.5 %	57.00 e	53.33 f	55.16 c	00.00 b	00.00 b	00.00 b
Mean	70.11 a	60.00 b		7.00 a	00.00 b	

Experiment (2): Callus initiation from *Solanum viride* explants as affected by plant growth regulators after 6 weeks *in vitro*:

(a): The effect of different concentrations of BA and NAA or 2,4-D and their combinations on callus formation from Solanum viride explants was studied.

In Table (2), when BA was used with NAA, data on the main effect of BA on the percentage of callus formation show that, the percentage of callus formation significantly increased by increasing BA concentration compared with the medium devoid of BA. The highest response in callus formation (80.40 %) was obtained with the highest concentration of BA (5.0 mg/l).

Data on the main effect of NAA on the percentage of callus formation reveal that, callus formation increased gradually with the gradual increase in concentration of NAA up to 1.5 mg/l, then callus formation was decreased by using the highest level of NAA (2.0 mg/l). Moreover, the lowest record of callus percentage was observed with the control.

Data of the interaction indicate that, all combination of BA and NAA significantly showed sporadic high percentages of callus formation compared to the control and the treatments contained 0.5 or 2.0 mg/l BA alone that showed no callus formation. The combination of 0.5 mg/l BA and 1.5 mg/l NAA was effective and resulted in the highest response in that concern (100 % callusing), (Fig., 1).

In the same Table (2), when BA was used with 2,4-D, data on the main effect of BA show the same trend as previously mentioned.

While data on the main effect of 2,4-D observed a gradual increase in callus formation with the gradual increase of 2,4-D up to 1.0 mg/l. The higher levels of 2,4-D (1.5 or 2.0 mg/l) negatively affected the callus formation compared to the treatment of 1.0 mg/l. However, the lowest response (16.13%) was significantly recorded with the control compared to all other treatments.

Concerning the interaction, data show that, the percentage of callus formation was highest (100%) with the combination contained both BA at highest level (5.0 mg/l) and 2,4-D at 0.5, 1.0 or 1.5 mg/l. However, significant similar result (100%) was observed with the treatment of 3.5 mg/l BA and 1.0 mg/l 2,4-D. Interestingly, no callus was formed with the control and the treatments of the low levels of BA alone (0.5 and 2.0 mg/l).

Results in this study were in harmony with Jawahar, *et al.* (2003) who indicated that, the callus was induced from leaf explants of *Solanum nigrum* on MS medium supplemented with different concentrations of IAA and NAA (0.5-3.0 mg/l) with or without BA (1.0 mg/l) and GA3 (0.01 mg/l). Moreover, Salih and Al-Mallah, (2000) reported that, the combination of 1 mg/l NAA and 2 mg/l BA were suitable for callus induction from *S. nigrum* explants. Similarly Anwar, *et al.* (1999) found that, the MS basal medium augmented with 2,4-D (2 mg/l) alone and in combination with BA (0.5 mg/l) proved superior for the induction of compact nodular green calluses from *S. nigrum* explants.

(b): The effect of different concentrations of BA and NAA or 2,4-D and their combinations on fresh weight (g) of the obtained callus from *Solanum viride* explants was studied.

Referring to the effect of different concentrations of BA and NAA or 2,4-D on callus weight in Table (3), data on the main effect of BA clearly indicate that, the addition of BA to the medium was effective in increasing the callus mass production compared with control. Callus weight was increased gradually by increasing BA concentrations up to 3.5 mg/l, then, the rate of callus weight was decreased with the highest concentration of BA (5.0 mg/l).

Data on the main effect of NAA on callus weight show that, the addition of NAA to the medium positively affected the callus fresh weight compared to the medium devoid of NAA (control). However, the highest value of fresh weight (2.39 g) was recorded with 1.5 mg/l NAA.

Regarding the interaction between BA and NAA, it was clear that, the addition of BA with NAA to the medium appeared to be more effective in enhancing the callus fresh weight compared to the control and the treatments contained BA alone at low concentrations (0.5 and 2 mg/l). However, the heaviest callus weight (3.61 g) was significantly obtained with the treatment contained 0.5 mg/l BA and 1.5 mg/l NAA compared to control (hormone-free medium) and all other treatments, (Fig., 1).

When the medium contained BA and 2,4-D, data on the main effect of BA reveal that, the addition of BA to the medium enhanced callus fresh weight and the gradual increase of BA level was combined with a gradual increase in the fresh weight.

2-3

Data on the main of 2,4-D on callus fresh weight show that, the gradual increase of 2,4-D level up to 1.0 mg/l significantly had a positive gradual effect on callus fresh weight. The higher level of 2,4-D (1.5 and 2 mg/l) showed a negative effect in that concern. However, the lowest value (0.40 g) was recorded with the control.

Concerning the interaction, although all treatments contained both growth regulators observed a significant positive effect compared to control and the treatments contained low BA alone (0.5 and 2.0 mg/l) which showed no callus formation. However, the heaviest callus fresh weight (2.42 g) was significantly recorded with the treatment contained 5.0 mg/l BA and 1.0 mg/l 2,4-D compared to the control and all other treatments.

According to results of this experiment, it was recommended to use 0.5 mg/l BA with 1.5 mg/l NAA in the culture medium to enhance the callus formation.

The above mentioned results are in harmony with, Mahmood, *et al*. (1995) who showed that, Callus cultures from stem, leaf and root segments of *S. mammosum* were successfully established on Abou-Mandour's (AM) and MS media. Growth of undifferentiated callus was achieved on both media, having varying concentrations of 2,4-D and BA, respectively. Salih and Al-Mallah, (2000) initiated callus from different explants (stems, leaves, roots) of *S. nigrum* on MS medium supplemented with different concentrations of NAA and BA. The combination of 1 mg NAA and 2 mg BA/l were suitable for callus induction and maintenance. Moreover, Jawahar, *et al*. (2003) found that, the callus was induced from leaf explants of *Solanum nigrum* on MS medium supplemented with different concentrations of IAA and NAA (0.5-3.0 mg/l) with or without BA (1.0 mg/l) and GA3 (0.01 mg/l). The highest frequency of green compact callus was obtained on the MS medium containing 2.0 mg IAA+1.0 mg BAP+0.01 mg GA3/litre.

During this experiment (2), some explants in some treatments showed direct organogenesis (shoots and roots formation) from the leaf explants. Accordingly, in Table (4) data on the main effect of BA on shoot formation reveal that, the highest shoot number significantly was observed with the medium contained 2.0 mg/l BA compare to the control and the other levels of BA. No shoots were observed with the control, (Fig., 1).

Data on the main effect of NAA on shoot number reveal that, the addition of NAA to the media suppressed the shoot formation as the gradual decreasing of shoot number combined with the gradual increase of NAA levels. Interestingly, the highest response for shoot formation (8.03) was significantly obtained with the medium devoid of NAA (control).

Concerning the interaction, it was clear that, the highest direct shoot number (20.67) was significantly obtained with the medium contained BA alone at 2.0 mg/l (Fig., 1) followed by the medium contained BA alone at 0.5 mg/l (12.33) when both treatments compared with control and the other treatments. Moreover the media contained the high concentrations of both BA and NAA showed no shoot formation, and the same negative effect on shoot formation was observed with the media devoid of BA even with the presence of NAA at any of the used concentrations.

In the same Table (4), data show that, almost the same trend of results mentioned above (BA with NAA) was obtained when BA was used with 2,4-D.

Results of this work were in harmony with several studies, Kannan*, et al.* (2006) mentioned that, the highest shoot multiplication rates were observed when explants of *S. nigrum* grown in the medium supplemented with 2.0 mg/l of BA. Shoots were elongated on MS-B5 medium fortified with 0.5 mg/l BA. Jabeen, *et al.* (2005) cleared that, direct organogenesis was obtained in *S. nigrum*. The highest frequency and number of multiple shoots were obtained from leaf and nodal explants cultured on MS medium supplemented with 2.0 mg/l BA and 1.0 mg/l IAA. Okrslar, *et al.* (2002) found that, direct Shoot induction on leaf explants of *S. laciniatum* was most successful on MS medium supplemented with 10 micro M BA and 1 micro M NAA. BA (13 micro M) was optimum for further shoot multiplication. Similarly, Hassanein and Soltan, (2000) recorded that, direct regeneration from *Solanum nigrum* was possible using basal media B5, B5C (B5 supplemented with 5% coconut endosperm milk), Schenk and Hildebrandt (SH), and MS, leaf, stem, shoot tips as explants, cytokinins BA or Kin at concentrations from 0.25 to 2 mg dm-3, and different light treatments (dark, dim and normal light). The best culture conditions for shoot formation was the culture of stem internodes segments on B5 medium supplemented with 0.5 mg dm-3 BA at 16-h photoperiod (irradiance of 100 micro mol m-2 s-1).

The effect of NAA with BA on root formation is presented in Table (4), data on the main effect of NAA observe that, the highest root number (4.11) was significantly recorded with 1 mg/l NAA followed by the control treatment (3.52). Moreover, the lowest root number (2.17) was recorded with the highest level of NAA (2.0 mg/l).

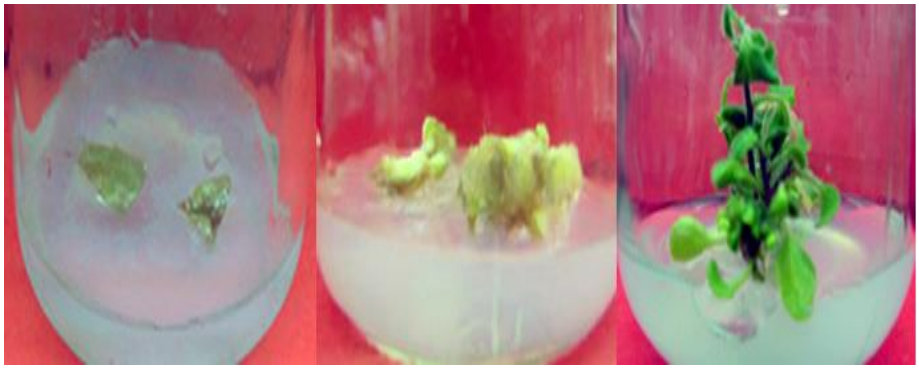

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Fig. (1): Effect of growth regulators on callus initiation and direct organogenesis from leaf explants of *Solanum viride* after 6 weeks *in vitro*.

1-Hormon-free medium (control) (no shoot or callus formation).

2-MS medium contained 0.5 mg/l BA + 1.5 mg/l NAA, and showed the highest callus formation.

3-MS medium contained 2 mg/l BA alone, and showed the highest direct shoot number/explant (all shoots were vitrified).

Table (5): Effect of different concentrations of NAA and BA and their combinations on differentiation of *Solanum viride* after 6 weeks *in vitro*.

BA (mg/l)	NAA (mg/l)														
	Shoot number					Root number					Vitrification %				
	0.0	0.5	1.0	1.5	Mean	0.0	0.5	1.0	1.5	Mean	0.0	0.5	1.0	1.5	Mean
0.0	0.0i	0.0i	0.0i	0.0i	0.0 d	0.0f	6.8b	7.8a	8.3a	5.8 a	100a	100a	100a	100a	100a
0.5	12.3c	3.3g	1.8h	0.0i	4.4 c	5.5c	6.7b	7.8a	0.0f	5.0 b	100a	100a	100a	100a	100a
1.0	15.3b	5.3e	3.9f	2.2h	6.7 b	4.2de	4.5d	5.2c	5.7c	4.9 b	95.3b	100a	100a	100a	98.8b
1.5	16.3a	6.8d	5.2e	3.2g	7.9 a	3.3e	3.7e	4.5d	5.2c	4.2 c	100a	100a	100a	100a	100a
Mean	11.0a	3.9b	2.7c	1.3d		3.3d	5.4b	6.3a	4.8c		98.8b	100a	100a	100a	

Concerning the main effect of BA on root formation, data indicate that, the addition of BA up to 2.0 mg/l to the medium significantly increased the root number. Increasing the level of BA to 3.5 and 5.0 mg/l significantly decreased the root number comparing to the lower levels of BA and control.

As for the interaction, high responses were significantly recorded with the different combinations between NAA and BA compared with the control and the treatment of 0.5 mg/l BA + 1.5 mg/l NAA (both had no root formation). Moreover, the highest root number (6.77) was significantly obtained with the medium contained 0.5 mg/l BA alone, and the lowest root number (1.00) was significantly recorded with the medium contained the highest level of both growth regulators together (5.0 mg/l BA + 2.0 mg/l NAA).

Concerning the effect of 2,4-D with BA on root number, data on the main effect of 2,4-D show that, sporadic negative effects were significantly recorded with the addition of 2,4-D to the media compared to control treatment. However, the highest root number (3.52) was obtained with the control.

Data on the main effect of BA on root number indicate that, the addition of BA to the medium positively affected the root number compared to the control that showed no roots formation. However, the gradual increase of BA level in the medium observed a significant gradual decrease in root number. Accordingly, the highest root number (3.27) was significantly obtained with the lowest level of BA (0.5 mg/l).

Concerning the interaction between 2,4-D and BA on root number, sporadic responses were recorded with the different combinations between 2,4-D and BA. The highest root number (6.77) was significantly formed with the medium contained BA alone at the lowest level (0.5 mg/l). However, the treatments of 2,4-D alone at all used levels or 2,4-D at the lowest rate (0.5mg/l) combined with BA at all used concentrations showed no root formation.

However, many other studies referred to the importance of auxins in the medium for root formation, Kannan*, et al.* (2006) who mentioned that, the well developed shoots of *S. nigrum* were easily rooted in medium containing 1.0 mg/l of IBA. Jabeen, *et al.* (2005) cleared that direct organogenesis and in vitro flowering was obtained in *S. nigrum*. Regenerated plants rooted and

flowered when transferred to rooting medium supplemented with 0.5-2.0 mg/l IBA or IAA. Jawahar, *et al.* (2004) cultured the stem explants of *S. nigrum* on MS medium supplemented with various concentration of IAA and NAA (0.5-3.0 mg/l) in combination with 1.0 mg/l BA and 0.01 mg/l GA3. The highest number of multiple shoots (8.6 shoots/stem explant) was observed in medium supplemented with 2.0 mg /l IAA + 1.0 mg/l BA + 0.01 mg/l GA3. All the regenerated shoots rooted in the same medium. Also, Manjula and Nair, (2002) investigated the *in vitro* plant regeneration via organogenesis from leaf explants of *Solanum aculeatissimum*, and found that, microshoots were rooted on MS medium contained IBA (1 mg/l).

It's very interesting to mention that, all shoots obtained directly from the explants (leaf segments) during this experiment as direct organogenesis were clearly vitrified. For this reason, another following experiment was designed as a trial to produce unvitrified plantlets through callus tissue (as indirect organogenesis).

Experiment (3): Effect of plant growth regulators on callus differentiation after 6 weeks *in vitro*:

In Table (5), data on the main effect of BA on indirect shoot formation (from callus) indicate that, shoot number increased gradually with the gradual increase in the level of BA. The highest shoot number (7.9) was significantly recorded with the highest concentration of BA (1.5 mg/l) compared to the control and the other treatments. No shoots were observed with control.

Data on the main effect of NAA show that, the addition of NAA to the medium negatively affected the shoot number with significant difference when compared with control. The gradual increase of NAA level showed a gradual decrease in shoot number. Interestingly, the highest record of shoot number (11) was obtained with control.

Data of the interaction reveal that, all combinations of BA and NAA significantly showed sporadic high shoot numbers compared to either the treatments devoid of BA or the treatment contained 0.5 mg/l BA and 1.5 mg/l NAA that showed no shoot formation. Moreover, the addition of BA alone to the medium showed a significant positive effect on shoot formation compared to all treatments of the interaction between NAA and BA. The highest shoot number (16.3) was significantly obtained with the highest level of BA alone (1.5 mg/l).

In this regard, Jawahar, *et al.* (2004) found that, the highest number of indirect shoots of *S. nigrum* (8.6 shoots/stem explants) were observed in medium supplemented with 2.0 mg IAA/l + 1.0 mg BA/l + 0.01 mg GA3/l. Moreover, Manjula and Nair, (2002) investigated the *in vitro* plant regeneration via organogenesis from leaf explants and production of solasodine from *Solanum aculeatissimum*. The best shoot regeneration was achieved from IAA-derived leaf callus when transferred to MS medium containing IAA (0.5 mg/l) and kinetin (4 mg/l), which gave the maximum number of shoots (38+or-0.6) per gram callus.

As for the effect of BA with NAA on root number in Table (5), data on the main effect of NAA show that, the medium contained NAA was more effective for root formation compared to the control and the gradual addition of NAA up to 1.0 mg/l showed a significant gradual increase on root number.

Data on the main effect of BA reveal that, the presence of BA was negatively effective on root formation compared to the control. Moreover, a significant gradual decrease on root number was recorded with the gradual increase in used levels of BA. However, the highest root number (5.8) was observed with the control.

Concerning the interaction, data indicate that, all combinations contained both growth regulators observed a significant positive effect compared to the control. The highest root number was significantly obtained with the treatments of 1.0 mg/l or 1.5 mg/l NAA each alone and the treatment of 1.0 mg/l NAA + 0.5 mg/l BA (without significant differences between the three treatments) compared to all other treatments.

In this regard, Kannan*, et al.* 2006 found that, the well developed shoots of *S. nigrum* were easily rooted in medium containing 1.0 mg/l of IBA. Also, Jawahar, *et al.* (2004) described a protocol for indirect shoot regeneration from stem explants of *S*. *nigrum*. The highest numbers of multiple shoots were observed in medium supplemented with 2.0 mg IAA/l + 1.0 mg BA/l + 0.01 mg GA3/l. All the regenerated shoots rooted in the same medium. Moreover, Manjula and Nair, (2002) investigated the *in vitro* plant regeneration via organogenesis from leaf explants of *Solanum aculeatissimum*. Microshoots were rooted on MS medium contained IBA at 1 mg/l.

As for the effect of growth regulators on shoot vitrification in the same Table (5), interestingly, data reveal that, all used treatments that had either NAA or BA each alone or in combinations resulted in vitrified shoots except the medium contained 1.0 mg/l BA alone (that observed 4.7 % unvitrified shoots and 95.3% vitrified shoots). So, this latest treatment was a source for obtaining unvitrified plantlets that were transferred to the greenhouse, Fig. (2).

1 **2**

Fig (2): Shoots obtained from callus of *Solanum viride* (vitrified and unvitrified)

1- Normal (unvitrified) shoots obtained on MS medium contained 1 mg/l BA.

2- Vitrified shoots obtained from MS medium contained 1.5 mg/l BA.

In this regard, Ghasemi *et al.* (2007) reported that, vitrification of regenerates was promoted by increasing the auxin NAA or cytokinin BAP, and ABA in the nutrient medium. Also, Lee *et al.* (2004) recorded that, the highest regeneration rate was obtained in most shoots from young leaves of *Pyrus pyrifolia* on a medium based on MS media supplemented with 0.1-1.0 mg/l TDZ and 0.1-1.0 mg/l IBA and/or NAA in each cultivar. Physiological differences with BA and TDZ treatments were compared. In the regeneration medium with BA treatments, green foci appeared on the callus surface after 8 days. Then, some adventitious buds were induced on those green foci, resulting in normal shoots. On the other hand, in the medium with TDZ, callus surface turned compact and greenish, and many adventitious buds were formed over the whole area of the callus surface. In some shoots, cultured on the medium with TDZ, there were morphologically abnormal shoots, including vitrified shoots. However, Vincent, *et al*. (2001) found that, tomato (*Lycopersicon esculentum* Mill.) plants grown on the medium supplemented with 3.5 µM zeatin (Z) were rather different. A basal callus developed, root formation never occurred and numerous shoots (up to 18) with short internodes were produced, conferring a bushy habit to the explants. Almost all leaves were vitrified and the total number of leaves on the longest shoot was also reduced compared with the plants growing on the other used cytokinins {Kin, BA and isopentenyladenine (2iP)}.

Experiment (4): Acclimatization of plantlets:

Data in Table (6) clearly indicate that, the indirect regenerated plantlets were successfully established in the soil contained different mixtures of sand and peatmoss. However, the mixture of sand and peatmoss (1:2, v: v) was the best treatment for survival percentage, plant height and leaf number, Fig. (3).

Table (6): Effect of different mixtures of sand and peatmoss on acclimatization of *Solanum viride* plantlets after one month in greenhouse.

sand : peat moss	Survival %	Plant height (cm)	Leave number
1:1	90.67 b	16.00 b	17.50 b
1:2	95.00 a	20.67 a	21.00 a
2:1	82.33 c	14.33 c	10.66 c

In that concern, Kannan*, et al.* (2006) recorded that, the rooted plantlets of *S. nigrum* were successfully established in the soil. Also, Jawahar, *et al.* (2004) found that, *S. nigrum* plantlets were successfully hardened and transferred to the field,

1 **2** **3**

Fig. (3): Effect of different mixtures of sand and peatmoss on acclimatization of *Solanum viride* plantlets after one month in greenhouse.

1- Plantlets grown in soil mixture contained sand and peatmoss (1:1, v: v).

2- Plantlets grown in soil mixture contained sand and peatmoss (1:2, v: v).

3- Plantlets grown in soil mixture contained sand and peatmoss (2:1, v: v).

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دراسات بيوتكنولوجية على نبات السولانم فيرايد:
أ- إكثار نبات السولانم فيرايد باستخدام تقنيات زراعة الأنسجة

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استخدمت أوراق نبات السولانم فيرايد كمصدر للأجزاء النباتية (explants) المستخدمة لبدء إكثار النبات داخل المعمل0 و قد تم تعقيم تلك الأوراق باستخدام هيبوكلوريت صوديوم بتركيز 1% حيث اظهر هذا التركيز اعلى نسبة حيوية للأجزاء النباتية المستخدمة بعد زراعتها بدون ظهور أى تلوث. وقد تم الحصول على نسيج كالس من تلك الأجزاء النباتية في بعض المعاملات، وكان أعلى وزن طازج للكالس الناتج واضحا مع الأجزاء النباتية المنزرعة على بيئة موراشيج وسكوج المحتوية على 0.5 مجم/لتر بنزيل أدنين مع 1.5 مجم/لتر نفثالين حامض الخليك. وقد لوحظ أن بعض المعاملات قد شجعت تكوين أفرع أو جذور على الأجزاء النباتية مباشرة (بدون تكوين كالس كمرحلة وسطية)، وكانت أكثر المعاملات تشجيعا لتكوين أفرع مباشرة على الجزء النباتي هي بيئة موراشيج وسكوج المحتوية على 2 مجم/لتر بنزيل أدنين. وقد كان جديرا بالملاحظة أن جميع الأفرع التي تكونت مباشرة على الأجزاء النباتية كانت غير طبيعية النمو (متزججة vitrified). ولذلك أجريت تجربة كمحاولة لإنتاج أفرع طبيعية غير متزججة وذلك عن طرق تكوين كالس على أحسن بيئة لتكوين الكالس في التجربة السابقة (0.5 مجم/لتر بنزيل أدنين مع 1.5 مجم/لتر نفثالين حامض الخليك) ثم زراعة هذا الكالس على بيئات مختلفة لتكشفه. وقد تم الحصول على أفرع طبيعية النمو (غير متزججة) مع معاملة واحدة فقط وهى بيئة موراشيج وسكوج المحتوية على 1 مجم/لتر بنزيل أدنين، والتي أظهرت 4.7% من الأفرع الناتجة عليها غير متزججة. وأخيراً فقد تم بنجاح أقلمة تلك الأفرع الناتجة ذات النمو الطبيعي في خليط من التربة تضمن 2 بيتموس: 1 رمل (بالحجم) والذي اظهر أعلى نسبة حيوية للنباتات (95%) وأفضل نمو.

Table (2): Effect of different concentrations of BA and NAA or 2,4-D and their combinations on the percentage of callus formation of *Solanum viride* explants after 6 weeks *in vitro*.

Auxin / BA (mg/l)	NAA(mg/l)						2,4-D(mg/l)					
	0.0	0.5	1.0	1.5	2.0	Mean	0.0	0.5	1.0	1.5	2.0	Mean
0.0	0.00 o	18.67 n	33.33 l	55.00 i	28.89m	27.18 e	0.00m	25.33k	31.67j	46.67i	20.33 l	24.80 e
0.5	0.00 o	48.33 j	73.67f	100.0 a	41.64 k	52.73 d	0.00 m	56.67g	63.33f	68.33e	53.00gh	48.27 d
2.0	0.00 o	90.00 c	96.67b	86.00 d	60.00 h	66.53 c	0.00 m	73.11d	80.67c	51.67h	46.67 i	50.42 c
3.5	27.67m	65.66 g	85.67d	89.00cd	79.00 e	69.40 b	27.67k	93.33b	100.0a	82.67c	48.00 h	70.33 b
5.0	53.00 i	87.33cd	81.33e	89.00cd	91.33 c	80.40 a	53.00gh	100.0a	100.0a	100.0a	21.33 l	74.87 a
Mean	16.13 e	62.00 c	74.13b	83.80a	60.18 d		16.13 d	69.69b	75.13a	69.87b	37.87 c	

Table (3): Effect of different concentrations of BA and NAA or 2,4-D and their combinations on callus fresh weight (g) of *Solanum viride* explants after 6 weeks *in vitro*.

Auxins / BA (mg/l)	NAA(mg/l)						2,4-D(mg/l)					
	0.0	0.5	1.0	1.5	2.0	Mean	0.0	0.5	1.0	1.5	2.0	Mean
0.0	0.00 m	0.36 l	0.78 k	0.86 k	0.31 l	0.46 e	0.00 p	0.18 o	0.44 l	0.58 k	0.21 no	0.28 e
0.5	0.00 m	1.14 ij	1.63 h	3.61 a	1.56 h	1.59 c	0.00 p	0.31mn	0.37 m	0.75 j	0.30mn	0.35 d
2.0	0.00 m	1.97 g	2.08 f	2.91 b	2.72 c	1.94 b	0.00 p	0.98 h	1.31 f	1.10 g	0.84 i	0.85 c
3.5	0.94jk	2.05 f	2.72c	2.99 b	2.33 e	2.21 a	0.94 h	1.55 e	2.10 b	1.31 f	0.91 hi	1.36 b
5.0	1.04 j	1.25 i	2.55 d	1.58 h	1.01 j	1.49 d	1.04gh	1.91 d	2.42 a	2.00 c	0.28 n	1.53 a
Mean	0.40 e	1.36 d	1.95 b	2.39 a	1.59 c		0.40 e	0.99 c	1.33 a	1.15 b	0.51 d	

Table (4): Effect of different concentrations of BA and NAA or 2,4-D and their combinations on direct organogenesis of *Solanum viride* after 6 weeks *in vitro*.

*** All obtained shoots were vitrified**

Auxin BA (mg/L)	NAA(mg/L)						2,4-d (mg/L)				
	0.0	0.5	1.0	1.5	2.0	Mean	0.0	0.5	1.0	* All	*
	Shoot number										
0.0	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 h	0.00 h	0.00 h	* All	*
0.5	12.33 b	5.33 d	2.17 f	0.00 g	0.00 g	3.97 b	12.33 b	4.33 d	0.00 h	* All	*
	20.67a	8.43 c	2.50 f	0.00 g	0.00 g	6.32 a	20.67a	5.83 c	4.11 de	* All	*
3.5	3.83 e	2.67 f	0.00 g	0.00 g	0.00 g	1.30 c	3.83 e	2.83 g	0.00 h	* All	*
5.0	3.33e	0.00 g	0.00 g	0.00 g	0.00 g	0.67 d	3.33f	0.00 h	0.00 h	* All	*
Mean	8.03 a	3.29 b	0.93 c	0.00 g	0.00 g		8.03 a	2.60 b	0.82 c	* All	*
	Root number										
0.0	0.00 i	4.33 d	4.67 cd	6.00 b	2.50 fg	3.50 c	0.00 i	0.00 i	0.00 i	* All	*
0.5	6.77 a	3.83 de	6.00 b	0.00 i	2.33 fg	3.79 b	6.77 a	0.00 i	1.67 gh	* All	*
	5.17 c	3.00 ef	4.67 c	4.33 d	2.67 f	3.97 a	5.17 b	0.00 i	1.33 h	* All	*
3.5	3.83 de	2.00 g	3.00 ef	3.33 e	2.33 fg	2.90 d	3.83 d	0.00 i	1.00 h	* All	*
5.0	1.83 g	1.67 g	2.23 fg	2.00 g	1.00 h	1.75 e	1.83 g	0.00 i	0.00 i	* All	*
Mean	3.52 b	2.79 b	4.11 a	3.13 c	2.17 e		3.52 a	0.00 e	0.80 d	* All	*

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