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IN VITRO REGULATION AND ENHANCEMENT OF ORGANOGENESIS IN SIX CANOLA (*Brassica napus*, L.) GENOTYPES

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ABSTRACT: This study was designed to investigate the effect of some factors affecting on *in vitro* propagation of six canola genotypes (Serw-6, Pactol, Wan-you 25, RG-4514, Serw-4 and AD-201/Gi/ 51). During establishment stage, different medium types (MS and B5) and cytokinin types (BA, Kin and 2ip) were investigated. MS medium supplemented with BA at 1.0 mgl^{-1} proved to be the best treatment for all tested genotypes during this stage. Also, different explant types (shoot tip, internode and hypocotyl), BA concentrations (0.0, 1.0, 3.0 and 5.0 mg l^{-1}) were examined during multiplication stage. Results indicated that while explant type had no effect on shoot induction and growth, BA proved to be the best cytokinin especially at 1.0 mgl^{-1} since it produced the maximum number of shoots/ explant for the most tested genotypes. During rooting stage, effects of MS medium strength (full and half) and auxin type (IAA, IBA and NAA) at the concentration of 1.0 mgl^{-1} were evaluated. Generally, using of full MS medium supplemented with 1.0 mgl^{-1} IBA stated to be the most suitable treatment for root induction and growth of the most investigated genotypes. Obtained plantlets were successfully acclimatized (60-80% survivability) in peat moss and sand (1:1, *V/V*) medium in the greenhouse.

Key words: *Brassica napus*, shoot induction and multiplication, plant growth regulators (PGRs), media composition, murashige and skoog medium (MS), gamborg medium (B5), *in vitro* root formation.

INTRODUCTION

Canola (*Brassica napus* L.) is belonging to the family Brassicaceae and ranks the second largest oilseed crop after soybean in global oil production (Ghnaya *et al.*, 2008; Maheshwari *et al.*, 2011; Borjian and Arak, 2013). Its oil is among the best types of edible oils used in human feeding especially in Northern Europe, United States, Canada, and China. Its high importance regarding to the lowest saturated fat content compared to all edible oils that contains 6% of saturated fatty acids and 94% non-saturated fatty acids and very low level of low density lipids (Cholesterol) and also contains Omega compounds that beneficial to human health (Al-Naggar *et al.*, 2008). It is a widely and globally used for cooking, salad oil, and making margarine. In addition, canola oil is using in lubricants and hydraulic fluids especially when there is a significant risk of oil leaking to waterways or into ground water. As well, it used in the manufacture of inks, biodegradable grease, in pharmaceuticals and cosmetics (Cardoza and Stewart, 2004; Hussain *et al.*, 2014).

In 2015, Egypt spent about 1.925 billion US dollars on importation of vegetable oils combined FAO STAT (2015). The United States Department of Agriculture, division of Foreign Agriculture Service (USDA-FAS), reported in 2013-2014 that Egypt's total oilseeds planted area only covers 3-5% of Egypt's total edible oil consumption so canola has a bright future to

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contribute in reducing oil deficiency gap between production and consumption of edible oil (El-Howeity and Asfour, 2012; Khalil *et al.*, 2015). Canola is grown in Egypt as a winter crop and one of the agricultural opportunities to increase canola production is expanding it into the new reclaimed regions because it competes with clover and wheat in old Delta. Thus, growing canola in salt-affected soils or in less-fertile soils may become successful if it could produce a relatively high economic yield with low level of inputs of nitrogen fertilizer (Moghaieb *et al.,* 2006 ; Al-Naggar *et al.,* 2008).

In recent years, a great deal of effort has gone into improving the quality of *Brassica napus* using classical breeding, several tissue culture and genetic engineering techniques. It is among the first crops to be genetically modified and transformed for improving its production and quality at very significant levels in commercial production. Cardoza and Stewart (2004) reported that various canola cultivars have been developed over the time, but only those that respond to *in vitro* regeneration can be used for biotechnological improvement. For that, various types of explants such as cotyledons (Cheng *et al.,* 2005; Huang 2006; Bennett *et al.,* 2008; Maheshwari *et al.,* 2011), petioles, hypocotyls, leaves (He *et al.,* 2006; Ghnaya *et al.,* 2008; Liu *et al.,* 2008; Maheshwari *et al.,* 2011), stem cuttings, root cuttings, shoot buds (Eapen and Georg, 1997), and pollen grains or microspores (Shi *et al.,* 2007; Cao *et al.,* 2010) have been used for *in vitro* culture. Among these, hypocotyl explants have been proposed as one of the best explant types for *in vitro* regeneration and transformation experiments in canola (Zihang and Bhalla, 2004 ; Burbulis 2010).

Regarding the effect of medium type on shoot initiation and multiplication, Zihang and Bhalla (2004) regenerated plants of *B. juncea* and *B. napus* on MS and B5 media. Of the two basal media, the better response was observed on MS medium with 22.20% regenerated plants while on B5 medium the percentage was 4.24%. In addition, MS medium was used often on shoot initiation and multiplication as stated by Moghaieb *et al.* (2006), Chamandoosti (2006), Burbulis *et al.* (2010) and Maheshwari *et al.* (2011). While, Kennedy *et al.* (2005), Dai *et al.* (2009) and Cristea *et al.* (2013) were used B5 medium basal salts with vitamins for canola shoot regeneration. On another hand, Khalil *et al.* (2015) used MS medium with B5 vitamins on canola regeneration. The effect of medium strength on canola root formation was studied by Chamandoosti *et al. (*2006), Burbulis *et al.* (2010) and Hussain *et al.* (2014) who stated that full MS medium was better than half MS. In contrast, Khalil *et al.* (2015) found that half MS was the best strength for canola root formation.

Concerning the influence of plant growth regulators on canola shoot induction and multiplication, Zihang and Bhalla (2004) reported that BAP was the most effective stimulator for shoot regeneration and 2.47 shoots per cotyledon explants were obtained with the combination of BAP (3.0 mgl^{-1}), NAA (0.2 mgl^{-1}), and GA_3 (0.1 mgl^{-1}). Chamandoosti *et al.* (2006) stated that callus after approximately one month of culture have been shooted with 2.0 mgl^{-1} BA which produced 89% shoot induction. Moreover, Moghaieb *et al.* (2006) used different concentrations of BA (0.0, 2.25, 4.50, 7.25 and 9.0 mgl^{-1}) and they indicated that adventitious shoots emerged from the embryonic callus in the presence of 4.5 mgl^{-1} BA. Ghnaya *et al.* (2008) evaluated four combinations of BA and NAA (1.0 mg l^{-1} BA + 0.1 mg l^{-1} NAA, 2.0 mg l^{-1} BA + 0.2 mg l^{-1} NAA, 3.0 mg l^{-1} BA + 0.3 mg l^{-1} NAA and 4.0 mg l^{-1} BA + 0.4 mg l^{-1} NAA). The best rate of plant regeneration was obtained with the combination of 3.0 mg l^{-1} BA with 0.3 mg l^{-1} NAA for all genotypes. Burbulis *et al.* (2010) noted that the combination of 4.0 mg l^{-1} BA with 0.5 mg l^{-1} NAA was significantly improved shoot formation frequency. In addition, Hussain *et al.* (2014) stated that medium with 5.0 mgl^{-1} BAP with 0.5 mgl^{-1} IAA was used for shoot regeneration. Khalil *et al.* (2015) showed that the best direct shoot organogenesis was achieved on medium fortified with 0.45 mgl^{-1} TDZ + 3.0 mgl^{-1} BA in the presence of 0.1 mgl^{-1} NAA which gave shoot induction percentage (88.3%) with 10.5 shoot number/explant. Furthermore, the effect of plant growth regulators on canola root formation was reported by Zihang and Bhalla (2004) who evaluated three combinations of PGRs (0.5 mgl^{-1} BA + 0.1 mg l^{-1} NAA), (0.5 mgl^{-1} BA+ 0.2 mgl^{-1} NAA) and (1.0 mgl^{-1} BA + 0.1 mgl^{-1} NAA) and they

noticed that the highest number of roots with tallest roots (9.51 and 4.7 cm, respectively) were observed with the last combination. Also, Chamandoosti *et al.* (2006) studied five treatments (control, 1.0 mgl^{-1} NAA, 2.0 mgl^{-1} NAA, 1.0 mgl^{-1} IBA and 2.0 mgl^{-1} IBA) and they found that 1.0 mg l^{-1} IBA was observed the highest frequency of shoots with rooting percentage (90%). Burbulis *et al.* (2010) reported that proliferated shoots were rooted with 0.1 mgl^{-1} NAA. However, Ismail (2012) tested different concentration of IBA (0, 0.1, 0.2, 0.3, 0.4, or 0.5 mgl^{-1}) and found that 0.3 mgl^{-1} IBA gave the highest percentage (62.9% and 45% for Bactol and Sarow-4, respectively), followed by 0.4 IBA (51.8% and 30%, for Bactol and Sarow-4, respectively. Hussain *et al.* (2014) cleared that the combination of 0.125 mgl^{-1} IAA + 0.250 mgl^{-1} IBA was the best for canola root formation. Thus, Khalil *et al.* (2015) noticed that healthy shoots of Canola (*Brassica napus* L. var. Pactol) were rooted with 0.1 mg l^{-1} IBA.

The aim of the present study was to regulate and enhance the organogenesis therefore improving plantlet growth and morphogenesis of six canola genotypes using different explant sources, plant growth regulators and media composition under aseptic conditions as a first key of biotechnological improvement for improving its production and quality.

MATERIALS AND METHODS

Plant Material and Explant Sterilization

Six canola genotypes (Serw-6, Serw-4, Pactol, Wan-you 25, RG-4514 and AD-201/Gi/51) were used in this experiment. The first two genotypes are Egyptian, the 3rd genotype is French, the 4th genotype is Chinese and the last two genotypes are German. Seeds were obtained from Agricultural Research Division, National Research Center, Egypt. At plant tissue culture lab., Faculty of Environmental Agricultural Sciences, Arish University and during the period from 2013 to 2017, The seeds were submerged in tap water with a few drops of detergent soap in a flask and shacked well for 10 minutes then rinsed under running tap water to remove the soap. Under aseptic condition in laminar air-flow hood, seeds were surface-sterilized with 70 % (*V/V*) ethanol for 2 minutes and subsequently surface sterilized by 20% Clorox (with 5% sodium hypochlorite) for 20 min explants were thoroughly rinsed three times with sterile distilled water after each previous step.

Culture Medium and Conditions

The sterile seeds were cultured on MS basal salt mixtures including vitamins (Murashige and Skoog, 1962) free PGRs in jars containing 50 ml medium, supplemented with 3% (*W/V*) sucrose, 0.8% (*W/V*) agar and 0.1 gl^{-1} myo-inositol. Medium pH was adjusted to 5.6 - 5.8 before gelling and then autoclaved at 121°C for 20 min. then, 10 seeds were cultured in each jar, the cultures were put in the dark for 7 days and they were maintained in an air conditioned incubation room at 22 ± 2°C under 16 hr./day photoperiod which provided by cool white fluorescent lamps (light intensity 2500 Lux.).

Shoot Bud Induction and Proliferation

After 3 weeks of seed germination, the *in vitro* shoot tip, axillary node and hypocotyl explants (0.5–1.0 cm length) were prepared from seedlings of each genotype and cultured (4 explants/jar) on MS or Gamborg (B5) (Gamborg *et al.*, 1968) media supplemented with 3% (*W/V*) sucrose, 0.8% (*W/V*) agar and 0.1 gl^{-1} myo-inositol in the presence of 1.0 mgl^{-1} of different cytokinins. The used cytokinins were 6-Benzyladenine (BA), Kinetin (Kin) and 2-isopentenyl adenine (2ip). After 4 weeks from culture, the induced shoot buds were transferred to MS medium supplemented with 3% (*W/V*) sucrose, 0.8% (*W/V*) agar and 0.1 gl^{-1} myo-inositol in the presence of different concentrations (0.0, 1.0, 3.0 and 5.0 mgl^{-1}) of BA for shoot proliferation. Shoot induction percentage, number of shoots/ explant, shoot length, number of leaves/shoot and callus formation percentage were the studied characteristics during this stage.

In vitro Rooting

The proliferated shoots (0.5-1 cm length) of canola genotypes were used as explants and were cultured firstly on free MS medium for a week then were transferred to full or half strengths MS medium. The medium was

supplemented with 3% (*W/V*) sucrose and 0.8% (*W/V*) agar in the presence of 1.0 mgl^{-1} of indole butyric acid (IBA), indole acetic acid (IAA) and α-Naphthalene acetic acid (NAA) for 4 weeks to monitor the initiation and quality of adventitious roots on the regenerated shoots.

Acclimatization of Plantlets

The well-developed healthy *in vitro* rooted plantlets after 4 weeks were washed thoroughly under tap water and hardened for *ex vitro*. The plantlets were planted in pots containing a mixture of peat moss and sand in the ratio of 1:1 (*V/V*) then placed in a plastic tunnel and wetted with tap water and covered with transparent plastic bags to maintain humidity. After 3 weeks, the established plants were transplanted to polyethylene bags containing garden soil and farmyard manure for further growth. The survival rate of *in vitro* propagated plantlets after 6 weeks of transplanting was 60-80% and they grew as normal plants.

Statistical Analysis

All the experiments were set up in completely randomized design (CRD) with five replicates per treatment and with four explants per replicate (jar). Data were subjected to analysis of variance (ANOVA) and the statistical difference among the means was analyzed by Duncan's multiple range test (DMRT) Duncan (1995) at 1% level of probability using statistical products and service solutions software for windows, version 17.0 (SPSS Inc., 2007).

RESULTS AND DISCUSSION

Establishment Stage

Effect of medium type on shoot induction of six canola genotypes

Results presented in Table 1 point out that in most cases, medium type had no significant effect on shoot induction percentage, except in the case of Serw-4 and AD-201/Gi/81 genotypes since B5 medium was better for the former one, while MS medium was better for the later one. The lowest shoot induction percentage was recorded with Wan-you-25 genotype without significant difference between both media in this regard. Regarding the effect of interaction between medium type and canola genotype on shoots number/explant, the highest values of shoot number were obtained with Pactol genotype without significant difference between both media. Generally, medium type did not significantly affect on this character except with AD-201/Gi/81 genotypes since MS medium significantly surpassed B5 medium and this is may due to the genotypic effects or due to the medium composition.

Concerning the effect of interaction between medium type and canola genotype on shoot length, the longest shoot (4.72cm) was observed with Serw-4 cultured on B5 medium followed by the same genotype with MS medium which recorded 3.78 cm. Medium type did not significantly affect on shoot length of Pactol, Wan-you-25 and AD-201/Gi/81 genotypes. However, MS medium was better than B5 for Serw-6 and RG-4514 genotypes. On the other side, B5 gave taller shoot than MS for Serw-4 genotype. Results presented in Table 1 clear that the highest values of leaf number/explant were recorded with Pactol genotype either with MS or with B5 media without significant difference between them. Medium type had no significant effect on number of leaves/shoot for all genotypes except for Serw-6 and AD-201/ Gi/81 where MS medium proved to be better than B5 in this regard according to Zihang and Bhalla (2004). In conclusion, presented results clear that we can use both of MS or B5 media for canola shoot induction.

Callus formation is a significant problem for plant micropropagation. In this experiment, it was observed with all studied treatments but at different percentages. The highest percentages of callus formation were appeared with Pactol genotype on either MS or B5 media.

Effect of cytokinin type on shoot induction of six canola genotypes

Results presented in Table 2 clear that in most cases there were no significant differences among different cytokinin types or control treatments concerning shoot induction percentage either within or between different genotypes. The highest value of shoot number (2.23) was achieved with Pactol genotype cultured on medium supplemented with BA. It is

Table 1. Effect of medium type on shoot induction of six *Brassica napus* L. genotypes after 4 weeks from culture during establishment stage

Canola genotype	Medium type	Shoot induction (%)	No. shoots/exp.	Shoot length (cm)	No. leaves/shoot	Callus formation (%)
Serw-6	MS	98.43^{ab}	1.36^{bcd}	3.02^{bc}	7.03^{bc}	25.00^{bc}
	B5	100.00^{a}	1.16^{de}	1.51^{ef}	5.06^{d-g}	19.27^{bcd}
Pactol	MS	98.43^{ab}	1.71^{a}	2.44^{cde}	9.20^{a}	42.70^{a}
	B5	97.91^{ab}	1.48^{abc}	2.04^{def}	7.78^{ab}	33.85^{ab}
Wan-you-25	MS	82.91^{d}	1.15^{de}	1.66^{ef}	4.56^{fg}	3.33^{e}
	B5	82.91^{d}	1.09^{e}	1.29^{f}	3.93^{g}	9.16^{de}
RG-4514	MS	100.00^{a}	1.30^{b-e}	2.90^{bcd}	6.50^{b-e}	15.83^{cde}
	B5	93.33^{abc}	1.21^{de}	1.23^{f}	4.95^{efg}	3.75^{e}
Serw-4	MS	88.33^{cd}	1.36^{bcd}	3.78^{b}	6.76^{bcd}	21.54^{bcd}
	B5	95.83^{ab}	1.25^{cde}	4.72^{a}	5.78^{c-f}	24.93^{bc}
AD-201/Gi/81	MS	99.58^{a}	1.53^{ab}	2.87^{bcd}	6.94^{bc}	20.41^{bcd}
	B5	91.25^{bc}	1.08^{e}	2.09^{c-f}	4.67^{fg}	8.75^{de}

1.0 mgl^{-1} BA was supplemented to the studied media and shoot tip was the explant that used during this experiment. Means in each column followed by the same letters are not significantly different according to Duncan's multiple range test at 1% level of probability.

Table 2. Effect of cytokinin type at 1.0 mg/l on shoot induction of six *Brassica napus* L. genotypes after 4 weeks from culture during establishment stage

Canola genotype	Cytokinin	Shoot induction (%)	No. shoots/exp.	Shoot length (cm)	No. leaves/shoot	Callus formation (%)
Serw-6	BA	97.91^{a}	1.60^{bc}	1.84^{d-g}	9.09^{bc}	38.54^{bcd}
	Kin	98.95^{a}	1.18^{def}	2.48^{c-g}	5.13^{fgh}	22.91^{d-h}
	2ip	100.00^{a}	1.23^{def}	2.78^{c-f}	5.48^{fgh}	22.91^{d-h}
	Control	100.00^{a}	1.02^{f}	1.95^{d-g}	4.48^{gh}	4.16^{hi}
Pactol	BA	100.00^{a}	2.23^{a}	1.37^{fg}	13.66^{a}	63.95^{a}
	Kin	96.04^{ab}	1.57^{bc}	2.31^{d-g}	7.66^{cde}	45.00^{bc}
	2ip	98.33^{a}	1.41^{cd}	2.80^{c-f}	6.95^{def}	30.83^{c-f}
	Control	98.33^{a}	1.16^{def}	2.48^{c-g}	5.68^{e-h}	13.33^{f-i}
Wan-you-25	BA	92.50^{abc}	1.39^{cd}	1.22^{g}	6.14^{efg}	14.16^{f-i}
	Kin	69.16^{d}	1.03^{ef}	1.64^{efg}	3.67^{h}	0.83^{i}
	2ip	83.33^{c}	1.03^{ef}	1.55^{efg}	3.57^{h}	7.50^{hi}
	Control	86.66^{bc}	1.04^{ef}	1.49^{efg}	3.60^{h}	2.50^{i}
RG-4514	BA	99.16^{a}	1.74^{b}	2.08^{d-g}	9.46^{bc}	27.50^{c-g}
	Kin	95.00^{ab}	1.18^{def}	2.25^{d-g}	4.86^{fgh}	10.83^{ghi}
	2ip	99.16^{a}	1.10^{def}	2.59^{c-g}	4.57^{gh}	0.83^{i}
	Control	93.33^{abc}	1.00^{f}	1.33^{fg}	4.00^{gh}	0.00^{i}
Serw-4	BA	83.33^{c}	1.82^{b}	4.62^{a}	9.70^{b}	54.12^{ab}
	Kin	94.16^{ab}	1.24^{def}	3.85^{abc}	5.36^{fgh}	35.50^{cde}
	2ip	93.33^{abc}	1.05^{ef}	4.32^{a}	5.05^{fgh}	3.33^{i}
	Control	97.50^{a}	1.14^{def}	4.23^{ab}	4.97^{fgh}	0.00^{i}
AD-201/Gi/81	BA	97.50^{a}	1.73^{b}	1.43^{efg}	8.37^{bcd}	34.16^{cde}
	Kin	95.83^{ab}	1.35^{cde}	3.31^{a-d}	6.01^{efg}	19.16^{e-i}
	2ip	95.83^{ab}	1.13^{def}	2.91^{b-e}	4.69^{gh}	0.83^{i}
	Control	92.50^{abc}	1.02^{f}	2.28^{d-g}	4.14^{gh}	4.16^{hi}

Shoot tip was the explant that used during this experiment. Means in each column followed by the same letters are not significantly different according to Duncan's multiple range test at 1% level of probability.

clear that BA was the best cytokinin that induced the highest number of shoots compared with other cytokinins or control treatments. This result is in agreement with Zihang and Bhalla (2004), Chamandoosti *et al*. (2006), Moghaieb *et al.* (2006) and Thiyagarajan and Venkatachalam (2012) who mentioned that BA was more effective than other tested cytokinins for shoot initiation and development. The preference to use BA as cytokinin because its degradation is slow and it can be autoclaved without losing its activity.

Generally, shoot length did not significantly response to supplementation the media with any tested cytokinin type. The tallest shoot lengths were recorded with Serw-4 genotype regardless providing the medium with any type of cytokinin or not.

The highest values of leaf number/explant were recorded with Pactol genotype supplemented with BA which attained 13.66 leaves/shoot followed by the same cytokinin with Serw-4 which gave 9.70 leaves/shoot then the same cytokinin on RG-4514 genotype which observed (9.46) after that, the same cytokinin on Serw-6 that observed (9.09). As for the single-effect of cytokinin on number of leaves/explant, using BA achieved the highest values for all studied genotypes. In addition, callus formation was observed with all studied treatments at different percentages except with control treatment on RG-4514 and on Serw-4 genotypes. The highest percentage of callus formation was appeared on Pactol genotype with BA followed by using BA on Serw-4.

Multiplication Stage

Effect of explant type on shoot induction of six canola genotypes

Results presented in Table 3 point out that explant type did not affect on shoot induction percentage for any of investigated genotype except in the case of Wan-you-25 genotype since hypocotyl explant gave the lowest value of shoot induction (%) compared with other explant types. Also, explant type had no effect on number of proliferated shoots on explant. There were no wide differences among genotypes in this respect.

Concerning the effect of interaction between explant type and canola genotypes on shoot length, the longest shoot (6.82 cm) was observed on shoot tip explant of Serw-4 followed by axillary node explant of the same genotype which recorded 3.90 cm. Results presented in Table 3 cleared that the highest values of leaf number/shoot were recorded with Pactol genotype by using shoot tip, axillary node or hypocotyl explant which recorded 8.94, 8.78 and 7.74 leaves/shoot, respectively without significant differences among these explants.

Generally, there was no significant difference between axillary node and hypocotyl explants concerning shoot length of all investigated genotypes except Serw-4. Almost always it could be observed that explant type did not significantly affect on number of leaves/shoot of different tested genotypes.

Callus formation is a significant problem for plant micropropagation. In this experiment, it was observed with all studied treatments but at different percentages. The highest percentages of callus formation were appeared on Pactol genotype with different explant sources.

Effect of benzyl adenine concentration on shoot proliferation and growth of six canola genotypes

In order to induce multiple shoots, *in vitro* regenerated shoots were cultured on full strength MS medium supplemented with different concentrations (0.0, 1.0, 3.0, and 5.0 mgl^{-1}) of BA. Results shown in Table 4 indicate the interaction effect of different BA concentrations on morphogenic response of single-shoot explant of the studied canola genotypes. Most investigated genotypes produced the maximum number of shoots/explant when medium was fortified with 1.0 mgl^{-1} BA. While, Wan-you-25 genotype gave the highest number of shoots/explant by amending the medium with 3.0 mgl^{-1} BA. These findings are in agreement with Moghaieb *et al.* (2006) Also, Similar findings have been reported with various plant species including *Eclipta alba* (Dhaka and Kothari, 2005), *Quercus euboica* (Kartsonas and Papafotiou, 2007), *Ulmus parvifolia* (Thakur and Karnosky, 2007), *Stevia rebaudiana* (Ahmad *et al.,* 2011 ; Aman *et al.,* 2013) and *Sacostemma brevistigma* (Thomas and Shankar, 2009).

Table 3. Effect of explant type on shoot induction of six *Brassica napus* L. genotypes after 4 weeks from culture on MS medium supplemented with 1.0 mgl^{-1} BA during multiplication stage

Canola genotype	Explant type	Shoot induction (%)	No. shoots/exp.	Shoot length (cm)	No. leaves/shoot	Callus formation (%)
	Shoot tip	100.00^{a}	1.19^{cde}	2.98^{bcd}	6.29^{bc}	13.28^{cde}
Serw-6	**axillary node**	100.00^{a}	1.31^{b-e}	1.88^{ef}	5.83^{bc}	21.87^{bcd}
	Hypocotyl	97.65^{a}	1.28^{b-e}	1.92^{def}	6.02^{bc}	31.25^{abc}
	Shoot tip	100.00^{a}	1.61^{ab}	2.90^{b-e}	8.94^{a}	35.93^{ab}
Pactol	**axillary node**	97.03^{a}	1.69^{a}	2.39^{c-f}	8.78^{a}	44.37^{a}
	Hypocotyl	97.50^{a}	1.48^{abc}	1.44^{fg}	7.74^{ab}	34.53^{ab}
	Shoot tip	85.62^{c}	1.12^{de}	1.99^{def}	4.45^{cd}	6.87^{de}
Wan-you-25	**axillary node**	92.50^{abc}	1.23^{cde}	1.71^{fg}	5.34^{c}	10.62^{de}
	Hypocotyl	70.62^{d}	1.01^{e}	0.73^{g}	2.94^{d}	1.25^{e}
	Shoot tip	98.12^{a}	1.30^{b-e}	2.32^{c-f}	6.17^{bc}	11.87^{cde}
RG-4514	**axillary node**	95.00^{ab}	1.32^{b-e}	2.41^{c-f}	6.14^{bc}	11.25^{de}
	Hypocotyl	96.87^{a}	1.14^{cde}	1.47^{fg}	4.86^{cd}	6.25^{de}
	Shoot tip	95.00^{ab}	1.32^{b-e}	6.82^{a}	6.80^{abc}	13.59^{cde}
Serw-4	**axillary node**	93.75^{abc}	1.38^{a-d}	3.90^{b}	6.77^{abc}	33.34^{ab}
	Hypocotyl	87.50^{bc}	1.22^{cde}	2.04^{def}	5.24^{c}	22.78^{bcd}
	Shoot tip	97.50^{a}	1.38^{a-d}	3.25^{bc}	6.52^{bc}	11.25^{de}
AD-201/Gi/81	**axillary node**	93.12^{abc}	1.23^{cde}	2.55^{c-f}	5.22^{c}	20.00^{b-e}
	Hypocotyl	95.62^{a-d}	1.32^{b-e}	1.65^{fg}	5.67^{bc}	12.50^{cde}

Means in each column followed by the same letters are not significantly different according to Duncan's multiple range test at 1% level of probability.

Table 4. Effect of different concentrations of BA on morphogenic responses of single-shoot explant of six *Brassica napus* L. genotypes after 4 weeks from culture during multiplication stage

Canola genotype	BA concentration (mgl^{-1})	No. shoots / explant	Shoot length (cm)	No. leaves / shoot
Serw-6	**0.0**	1.00 ^{f}	1.51 $^{d-h}$	5.75 ^{ij}
	1.0	3.12 ^{a}	1.67 $^{c-h}$	21.50 ^{a}
	3.0	1.37 ^{ef}	1.56 $^{d-h}$	12.12 ^{bc}
	5.0	2.25 ^{bcd}	1.35 ^{fgh}	20.87 ^{a}
Pactol	**0.0**	1.00 ^{f}	3.97 ^{a}	6.00 ^{hij}
	1.0	1.25 ^{ef}	1.22 ^{gh}	7.12 $^{g-j}$
	3.0	1.75 ^{cde}	2.18 $^{b-f}$	7.50 $^{f-j}$
	5.0	1.62 ^{def}	1.40 $^{e-h}$	7.87 $^{e-j}$
Wan-you-25	**0.0**	1.00 ^{f}	2.51 ^{bc}	4.87 ^{j}
	1.0	1.25 ^{ef}	1.27 ^{gh}	9.50 $^{c-g}$
	3.0	2.37 ^{bc}	1.72 $^{b-h}$	9.62 $^{c-g}$
	5.0	1.87 $^{b-e}$	1.60 $^{d-h}$	11.00 ^{cde}
RG-4514	**0.0**	1.25 ^{ef}	2.33 ^{bcd}	7.50 $^{f-j}$
	1.0	2.37 ^{bc}	1.60 $^{d-h}$	14.87 ^{b}
	3.0	1.87 $^{b-e}$	1.07 ^{gh}	11.37 ^{cd}
	5.0	1.50 ^{ef}	1.00 ^{gh}	8.12 $^{d-j}$
Serw-4	**0.0**	1.00 ^{f}	2.22 $^{b-e}$	6.00 ^{hij}
	1.0	1.75 ^{cde}	1.85 $^{b-g}$	10.87 $^{c-f}$
	3.0	1.50 ^{ef}	1.41 $^{e-h}$	9.37 $^{c-h}$
	5.0	1.50 ^{ef}	1.10 ^{gh}	8.75 $^{c-i}$
AD-201/Gi/81	**0.0**	1.00 ^{f}	2.53 ^{b}	7.00 $^{g-j}$
	1.0	2.50 ^{ab}	1.66 $^{c-h}$	10.87 $^{c-f}$
	3.0	1.75 ^{cde}	1.25 ^{gh}	10.62 $^{c-f}$
	5.0	1.87 $^{b-e}$	0.95 ^{h}	15.37 ^{b}

Means in each column followed by the same letters are not significantly different according to Duncan's multiple range test at 1% level of probability.

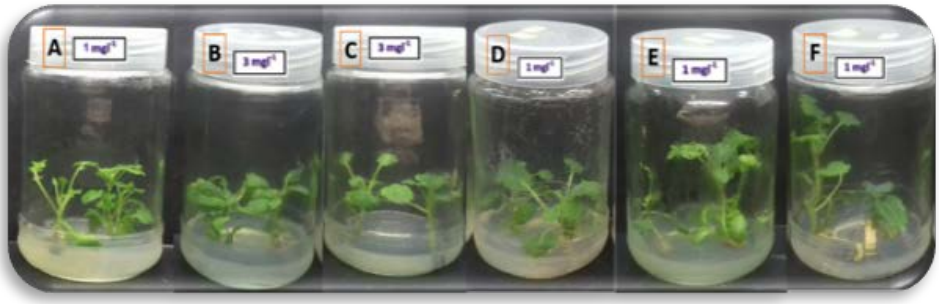

Fig. 1. Influence of different concentrations of BA on studied canola genotypes

Serw-6 with 1.0 mgl^{-1} (A), Pactol with 3.0 mgl^{-1} (B), Wan-you-25 with 3.0 mgl^{-1} (C), RG-4514 with 1.0 mgl^{-1} (D), Serw-4 with 1.0 mgl^{-1} (E), AD-21/Gi/81 with 1.0 mgl^{-1} (F)

In most cases, providing the medium with BA at any of applied concentrations significantly suppressed shoot elongation. The highest values of shoot length were belonged to control (without BA) treatments. The tallest shoot length (3.97 cm) was obtained when Pactol genotype was cultured on MS medium without BA.

Results presented in Table 4 clear that while fortifying the medium with BA had no effect on number of leaves/shoot of Pactol genotype, this character significantly enhanced in the other genotypes by enriching the medium with BA. Also, results show that while increasing of BA concentration did not affect on this character in Serw-4 and Serw-6 genotypes, the ultimate concentration of BA gave the highest values of this character with Wan-you-25 and AD-201/Gi/81 genotypes. Concerning RG-4514 genotype, medium and high concentrations (3 and 5 mgl^{-1}) of BA recorded the highest number of leaves/shoot without significant difference between both concentrations.

In vitro Rooting Stage

Effect of auxin type and medium strength on root formation of six canola genotypes

As shown from results in Table 5, generally, providing the medium with different investigated auxins significantly improved root formation percentage. In most cases, IBA and IAA proved to be more effective than NAA for root induction of Serw-6, Wan-you-25, RG-4514 and Serw-4 genotypes. On the other side, there were no significant differences among different tested auxins in this regard on AD-21/Gi/81 genotype, while IBA was the best for root initiation on Pactol genotype shoots. Almost always, medium strength had little or no effect on root formation percentage.

Generally, the ultimate number of roots/ shoot were produced when the medium was amended with IBA. This auxin stated to be the most effective auxin among different tested auxins in this regard in all investigated genotypes. These results are in agreement with those of Chamandoosti *et al.* (2006), Kaewpoo and Te-chato (2009), Ismail (2012) and Khalil *et al.* (2015). In contrast, Sujatha and Mukta (1996) and Kalimuthu *et al.*, (2007) found in their studies that IAA was more suitable hormone than IBA for root formation whilst, Zihang and Bhalla (2004) and Burbulis *et al.* (2010) stated that proliferated shoots were rooted with NAA.

Serw-6, Wan-you-25, Serw-4 and AD-201/Gi/81 genotypes had the maximum numbers of roots/ shoot (8.25, 9.00, 9.00 and 9.00, respectively) when full medium strength was provided with IBA without significant differences among these genotypes.

Root length followed the similar trend of number of roots/shoot, since the tallest roots of all examined genotypes were detected when shoots were cultured on full strength MS medium supplemented with IBA without significant differences among tested genotypes in this regard.

Table 5. Influence of auxin type at 1.0 mgl^{-1} and medium strength on root formation of six *Brassica napus* genotypes after 4 weeks from culture during rooting stage

Canola genotype	MS strength	Auxin type	Root formation (%)	No. roots/ shoot	Root length (cm)
Serw-6	Full	Control	0.00 ^{d}	0.00 ^{l}	0.00 ^{i}
		IAA	75.00 ^{ab}	2.50 $^{e-j}$	1.66 $^{c-h}$
		IBA	100.0 ^{a}	8.25 ^{a}	5.81 ^{a}
		NAA	50.00 ^{bc}	1.12 $^{h-l}$	0.68 ^{ghi}
	Half	Control	0.00 ^{d}	0.00 ^{l}	0.00 ^{i}
		IAA	12.50 ^{cd}	0.75 ^{jkl}	0.37 ^{i}
		IBA	75.00 ^{ab}	2.25 $^{f-k}$	1.00 $^{f-i}$
		NAA	50.00 ^{bc}	1.12 $^{h-l}$	0.50 ^{hi}
Pactol	Full	Control	0.00 ^{d}	0.00 ^{l}	0.00 ^{i}
		IAA	25.00 ^{cd}	1.00 $^{i-l}$	0.50 ^{hi}
		IBA	100.0 ^{a}	5.75 ^{b}	6.00 ^{a}
		NAA	100.0 ^{a}	3.00 $^{e-h}$	2.00 $^{c-f}$
	Half	Control	100.0 ^{a}	2.50 $^{e-j}$	1.25 $^{d-i}$
		IAA	100.0 ^{a}	4.00 $^{b-f}$	2.00 $^{c-f}$
		IBA	100.0 ^{a}	3.25 $^{d-g}$	0.57 ^{hi}
		NAA	0.00 ^{d}	0.00 ^{l}	0.00 ^{i}
Wan-you-25	Full	Control	0.00 ^{d}	0.00 ^{l}	0.00 ^{i}
		IAA	100.0 ^{a}	3.75 $^{c-g}$	2.00 $^{c-f}$
		IBA	100.0 ^{a}	9.00 ^{a}	6.75 ^{a}
		NAA	75.00 ^{ab}	3.25 $^{d-g}$	2.25 ^{cde}
	Half	Control	0.00 ^{d}	0.00 ^{l}	0.00 ^{i}
		IAA	100.0 ^{a}	2.25 $^{f-k}$	1.00 $^{f-i}$
		IBA	75.00 ^{ab}	3.00 $^{e-h}$	0.57 ^{hi}
		NAA	25.00 ^{cd}	0.75 ^{jkl}	0.50 ^{hi}
RG-4514	Full	Control	100.0 ^{a}	3.75 $^{c-g}$	2.62 ^{c}
		IAA	100.0 ^{a}	2.00 $^{g-k}$	1.87 $^{c-g}$
		IBA	100.0 ^{a}	5.00 ^{bcd}	6.37 ^{a}
		NAA	100.0 ^{a}	4.00 $^{b-f}$	1.62 $^{c-h}$
	Half	Control	0.00 ^{d}	0.00 ^{l}	0.00 ^{i}
		IAA	100.0 ^{a}	2.25 $^{f-k}$	1.87 $^{c-g}$
		IBA	100.0 ^{a}	3.25 $^{d-g}$	1.87 $^{c-g}$
		NAA	0.00 ^{d}	0.00 ^{l}	0.00 ^{i}
Serw-4	Full	Control	25.00 ^{cd}	1.00 $^{i-l}$	0.25 ^{i}
		IAA	100.0 ^{a}	2.75 $^{e-i}$	1.12 $^{e-i}$
		IBA	100.0 ^{a}	9.00 ^{a}	6.25 ^{a}
		NAA	100.0 ^{a}	3.75 $^{c-g}$	2.37 ^{cd}
	Half	Control	0.00 ^{d}	0.00 ^{l}	0.00 ^{i}
		IAA	100.0 ^{a}	2.25 $^{f-k}$	1.87 $^{c-g}$
		IBA	75.00 ^{ab}	3.00 $^{e-h}$	2.25 ^{cde}
		NAA	25.00 ^{cd}	0.50 ^{kl}	0.25 ^{i}
AD-201/Gi/81	Full	Control	0.00 ^{d}	0.00 ^{l}	0.00 ^{i}
		IAA	100.0 ^{a}	4.25 $^{b-e}$	2.37 ^{cd}
		IBA	100.0 ^{a}	9.00 ^{a}	6.25 ^{a}
		NAA	100.0 ^{a}	5.25 ^{bc}	3.75 ^{b}
	Half	Control	0.00 ^{d}	0.00 ^{l}	0.00 ^{i}
		IAA	75.00 ^{ab}	5.00 ^{bcd}	2.12 $^{c-f}$
		IBA	100.0 ^{a}	5.00 ^{bcd}	2.50 ^{c}
		NAA	100.0 ^{a}	4.00 $^{b-f}$	2.37 ^{cd}

Means in each column followed by the same letters are not significantly different according to Duncan's multiple range test at 1% level of probability.

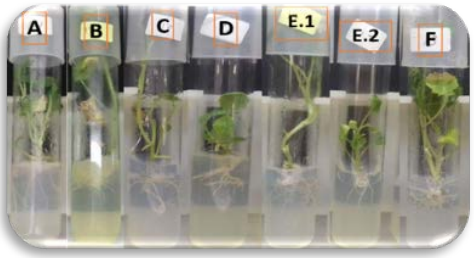

Fig. 2. Influence of auxin type and medium strength on studied canola genotypes

Serw-6 with IBA on full MS (A), Pactol with IBA on full MS (B), Wan-you-25 with IBA on full MS (C), RG-4514 with IBA on full MS (D), Serw-4 with IBA on full MS (E.1), Serw-4 with IBA on half MS (E.2), AD-21/Gi/81 with IBA on half MS (F)

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تنظيـــم وتحســـين التكشـــف العضـــوي معمليـــاً فـــي ســـتة أصنـــاف مـن الكـــانـــولا

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صممت هذه الدراسة لبحث تأثير بعض العوامل التي تؤثر على الاكثار الدقيق لستة أصناف من الكانولا وهي (سرو ٦، باكتول، وان يو ٢٥ ، ارجي ٤٥١٤ ، سرو ٤ ، أه دي ٢٠١/جي اي/٨١)، خلال مرحلة التأسيس، تم دراسة أنواع بيئات مختلفة وهي (موراشيجي وسكوج ، جامبورج) وكذلك تأثير أنواع سيتوكينين مختلفة وهي (بنزيل ادينين، كاينيتين، ٢ ايزوبنتينيل ادينين)، ثبت أن استخدام بيئة موراشيجي وسكوج المضاف إليها ١ ملجم/لتر من البنزيل ادينين هي الأفضل لجميع الأصناف تحت الدراسة خلال هذه المرحلة، كذلك تم دراسة تأثير أنواع مختلفة من المنفصلات النباتية وهي (القمة النامية، البرعم الجانبى، الهيبوكوتيل) وايضاً تأثير تركيزات مختلفة من البنزيل ادينين وهي (صفر، ١، ٣ و ٥ ملجم/لتر) خلال مرحلة التضاعف، وأشارت النتائج إلى أنه لم يكن هناك تأثير واضح بين المنفصلات النباتية المختلفة على نشوء ونمو البراعم، كذلك ثبت أن تركيز ١ ملجم/لتر من البنزيل ادينين هو الأفضل حيث أعطى اكبر عدد من البراعم الناشئة على المنفصل النباتي لأغلب الأصناف تحت الدراسة، خلال مرحلة التجذير، تم تقييم تأثير قوة بيئة موراشيجي وسكوج (كاملة القوة وبنصف قوتها) وكذلك تأثير نوع الاوكسين (اندول حامض الخليك – اندول حامض البيوتريك – نفثالين حامض الخليك) بتركيز ١ ملجم/لتر، وبصفة عامة، ثبت أن استخدام بيئة موراشيجي وسكوج بقوتها الكاملة والمضاف اليها ١ ملجم/لتر من اندول حامض البيوتريك هي الأنسب لتحفيز نشوء ونمو الجذور على اغلب الأصناف تحت الدراسة، تم أقلمة االشتلات المتحصل عليها بنجاح وبمعدل بقاء وحيوية (٦٠-٨٠%) في بيئة البيت موس والرمل بنسبة (١:١) في الصوبة البلاستيكية.

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