

## Development of Some Canola Genotypes to Salinity Tolerance Using Tissue Culture Technique

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**T**HIS WORK was carried out during 2011- 2015 in the laboratories of Cell Research Department (CRD), Field Crops Research Institute (FCRI), Agricultural Research Center (ARC), Giza, as well as, in the greenhouse of Agronomy Department, Faculty of Agriculture, Ain Shams University, Egypt, to study the variation among canola varieties in response to salt tolerance through tissue culture propagation. This work was aimed to establish a regeneration protocol for canola genotypes, production of salt-tolerance plants through selection of salt-tolerance callus *via* somaclonal variation and subsequent regeneration of plantlets from such callus. Four genotypes of canola were used for establishment a regeneration protocol, Bingo and Torpe were used as highly salt tolerant genotypes while, Conny and Siberian as susceptible genotypes. The results indicated that MS medium supplemented with 2 mg/l 2,4-D was the successive medium for callus formation and shoot regeneration was obtained from MS medium supplemented with 5 mg/l BA + 0.05 NAA, while, 1mg/l IBA was the better growth regulator for rooting.

For *in vitro* selection of salt tolerant calli, Torpe (genotype overcomes the others in calli survival percentages under 8000, 12000 and 16000 ppm NaCl concentrations. The results of shoot frequency from tolerant calli cleared that Torpe genotype recorded the highest value of shoot percentage followed by Bingo genotype, whereas, Conny followed by Siberian genotypes were recorded the lowest values. At 16000 ppm NaCl concentration, only tolerant calli of Torpe genotype was able to initiate shoot under this concentration. For acclimatization after regeneration from the tolerant calli, only five salt tolerant plantlets developed from Torpe genotype and eight salt tolerant plantlets developed from Siberian genotype that succeeded to complete their life cycle and reached maturity stage and produced seeds. These regenerated genotypes were referred as T1, T2, T3, T4 and T5, the five regenerated plants developed from Torpe genotype that tolerate NaCl concentration up to 16000 ppm, as well as, S1, S2, S3, S4, S5, S6, S7 and S8, the eight regenerated plants developed from Siberian genotype that tolerate NaCl concentration up to 12000 ppm.

**Keywords:** *Brassica napus*, Canola, Regeneration, *in vitro* selection, Salt stress.

### Introduction

As a matter of fact, it is worth to know that Egypt is suffering from great shortage in edible oils, where the total local consumption of oils is about 2,788,847 tons while the total local production is about 230110 tons. This clearly indicates that there is a gap of about 91.75% (USDA, 2014). The government pays more than 556.5 million dollars

annually to cover this shortage and to fulfill the requirements of local market. Accordingly, expansion of oil crops cultivated area in Egypt and find out new oil crops are national demands (FAO, 2009). This expansion will occur in new lands (for the limited acreage of oil crops) which are suffering from a biotic stresses, such as salinity, drought, extreme temperatures and chemical toxicity are serious threats to agriculture and

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the natural status of the environment. Increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within the next 25 years, and up to 50% by the year 2050 (Wang et al., 2003; Munns, 2002 and FAO, 2005). Therefore, breeding for drought and salinity stress tolerance in crop plants should be given high research priority in plant biotechnology programs. This indicated that salt tolerance of the crop genotypes is an important factor in the arid and semi-arid conditions.

Canola, which is most often *B. napus*, has received much attention worldwide and may soon be the most popular oilseed crop (Cardoza & Stewart, 2004). *Brassica napus* L. is an amphidiploid ( $2n=38$ ) and is believed to have arisen by interspecific hybridization between the diploid species *B. rapa* ( $2n=20$ ) and *B. oleracea* ( $2n=18$ ) and it is predominantly self-pollinated with about 30% out crossing (Diers & Osborn, 1994).

Canola (*Brassica napus* L., Brassicaceae) is considered as the most important oil crop worldwide, it is a specialty crop in Canada and ranks as the first place (63% of consumption) that was grown about 9.6 million acres in 2002 (Statistics Canada 2003), followed by soybean oil (24%) and sunflower oil (4%), while, in the USA grown about 1.5 million acres in the same year (USDA, 2003). Also, canola ranks the second, in cultivated area (25 million- hectare) following soybeans (FAO, 2005), the third largest source of edible oil following soybean and palm oil (Nowlin, 1991), and the fifth, in economic importance, following rice, wheat, maize and cotton. In Egypt, canola is still in the research phase and not commercially grown till now in spite of the wide gap. It is cultivated in winter and can grow in the new reclaimed lands (New Vally), where salinity and drought are major concerns affecting crop growth and productivity. Therefore, no competition would occur with the major winter crops (clover and wheat) and so, we must produce genotypes of canola more salt-tolerant. Therefore, it can contribute to cover the oil gap in oil production in Egypt.

Canola is classified as moderately tolerant to salinity as per (Mass & Hoffman, 1977) salt tolerance classification table. Saline soil and saline irrigation water present potential hazards to canola production (Fowler, 1991). Therefore,

it ranks first among field oil crops that withstand stressed conditions and by using tissue cultures we could increase salt tolerance of canola by induction of somaclonal variation (Larkin & Scowcroft, 1981).

Cell and tissue culture relating to variability and selection efficiency are to essential components of molecular breeding. Genetic variation in canola is required to breed cultivars that are high yielding, resistant to several biotic and abiotic stress conditions. It is well known that the improvement of plants through conventional breeding methods is slow, time-consuming and labor-intensive. Non-conventional genetic improvement programs based on tissue culture and molecular genetics are essential to complement standard breeding (Lichtenstein & Draper, 1985). The role of explant, genotype, medium composition and culture conditions in the efficiency of callus induction and regeneration in canola was reported by many investigators (Shanti et al., 2001; Bhalla & Weerd, 1999 and Chamandosti et al., 2006).

Shanti et al. (2001) used hypocotyls and cotyledons collected from 6-8 days old seedlings were inoculated aseptically on MS medium containing 2,4-D (1.0, 3.0, 5.0 or 6.0 mg/l) and colchicine (2.0 mg/l). They found that callus formation from cotyledon and hypocotyl implanted on MS medium fortified with 5.0 and 3.0 mg/l 2, 4-D. Majd et al. (2006) reported that hypocotyl explants were more useful for somatic embryogenesis than other explants. On MS medium containing 1 mg/l 2,4-D, 2 mg/l NAA, 2 mg/l BAP, explants produced somatic embryos. The somatic embryos germinated on medium had the same formulation as above and produced shoots. In this context, Kamal et al. (2007) reported that cotyledons possessed higher regeneration ability in comparison to hypocotyls. They obtained 100% shoot regeneration when cotyledonary explants were cultured on a medium containing 1.0 mg/l NAA, 0.8 mg/l BAP and 3.0 mg/l IBA and observed that the frequency of best regeneration of higher genotype, reached 68.8%. Mandal et al. (1989) reported that callus from an unselected cotyledon-derived callus culture was exposed to 175 mM sodium sulphate for 2 or 3 weeks. They found that 9-15% of the population survived and well grew after its transfer to salt-free medium.

## Materials and Methods

This work was carried out during 2011- 2015 at the laboratories of Cell Res. Dept. (CRD), Field Crops Res. Institute (FCRI), Agric. Res. Center (ARC), Giza, Egypt and Greenhouse of Horticulture Dept, Fac. Agric., Ain Shams Univ. to study the variation among rape genotypes in its salt tolerance through tissue culture propagation.

### *Establishment of canola regeneration protocol*

#### *Plant materials*

Four genotypes of canola (*Brassica napus* L.) were selected according to their salinity tolerance from twenty two genotypes after evaluation them for salinity tolerance during germination stage (Morsi et al., 2015). Two high salt tolerant genotypes (Bingo and Torpe) and two low salt susceptible ones (Conny and Siberian). These genotypes were used for establishing a regeneration protocol for canola.

#### *Seed sterilization and germination*

Canola seeds were surface sterilized by dipping in 70% ethanol for 1 min, followed by immersion in 3% sodium hypochlorite in addition to 2 drops of tween-20 as wetting agent. The solution was kept on shaker for 15 min and subsequently rinsed five times with autoclaved sterilized distilled water to remove excess of the chemical- under aseptic conditions in laminar flow hood. Seeds were blot dried on sterilized Whatman filter paper and germinated aseptically on MS (Murashige & Skoog, 1962) medium free hormone containing 0.8% (w/v) agar as solidified agent. Medium was autoclaved at 121°C and 1.2 kg.cm<sup>2</sup> for 20 min. Then it poured into heat-sterilized glass jars; each contained about 50 ml solidified medium. Then medium was incubated at 25°C under a 16/8 hr's light/dark photoperiodic regime (1000-Lux) and 70% humidity.

#### *Callus induction*

Two sources of explants: hypocotyls and cotyledonary leaves were excised from 7 day-old seedlings, for each genotype from the selected four canola genotypes. Explants were cultured on MS-medium, which containing B5 vitamins, 3% (w/v) sucrose as carbon source, 8% (w/v) agar and supplemented with three concentrations of 2,4 dichlorophenoxy acetic acid (1.0, 1.5 and 2.0 mg/l 2,4-D as well as control) to enhance callus initiation. The pH of the medium was

adjusted at 5.7 to 5.8 with 1M KOH or HCl before autoclaving. Medium was autoclaved at 121°C and 1.2 kg.cm<sup>2</sup> for 20 min. Then 50 ml were poured into heat-sterilized glass jars.

Ten replicates were used for each concentration with explants of each genotype. Each replicate was represented in a jar with ten explants. After culturing of explants, jars were incubated in the dark for one week at 25°C then moved to a light growth chamber under a 16/8 hr's (light/dark) photoperiodic regime (1000-Lux). The percentage of callus induction frequency was measured for each treatment in each genotype.

#### *Regeneration potential*

This experiment was carried out on the induced callus from the explants to determine the best protocol for canola regeneration including the best combination of growth regulators. The combinations of hormones were as followed: MS medium without any growth regulators (control), MS medium + 1.0 mg/l BA + 1.0 mg/l Kinetin (Kin), MS medium + 3.0 mg/l Benzyl adenine (BA) + 0.5 mg/l Indole acetic acid (IAA), MS medium + 4.0 mg/l BA + 0.5 mg/l Naphthalene acetic acid (NAA) and MS medium + 5.0 mg/l BA + 0.05 mg/l NAA.

Ten jars of each treatment (regeneration medium) were used; each jar contained one callus inoculum of 200 mg fresh weight. Cultures were incubated for shoot initiation in the same controlled environmental conditions as for callus formation (mentioned previously). For shoot elongation, shoots (5 mm in length) were transferred to growth regulator free medium and when reached 3.0 cm (after about 6 weeks) in length shoots were transferred to the rooting medium.

#### *Root formation*

Three centimeters in length regenerated shoots were transferred to different rooting media which were contained half strength MS-medium with B5 vitamins, 1.5% (w/v) sucrose, 0.6% (w/v) agar and supplemented with 1.0 mg/l IBA or 0.5 mg/l NAA or hormone free MS medium. Seven replicates for each treatment (rooting medium) were used; each replicate was contained one shoot and was incubated under the same conditions as for shooting stage. Plant regeneration frequency was estimated.

#### *Plant acclimatization*

The plantlets with five to six leaves that showed a well-developed root system (through five to six weeks) were transferred to sterilized mixture of peatmoss and vermiculite (1:1) in 25 cm plastic pots after washed them under running tap water to remove agar traces for 2 min then irrigated with Hoagland nutrients solution (Hoagland & Arnon, 1950) For hardening the plantlets they were covered with transparent polyethylene bags (to keep constant humidity about 90%) and were placed in a humid chamber 25°C under a 16/8 hr's light/dark cycle. After acclimatization for about three weeks bags were removed, the adapted plantlets were transferred to the greenhouse conditions until flowering and seed maturity stages. The seeds of each plant were harvested and considered as an independent line to be used for comparative agent with their mother genotypes.

#### *In vitro selection of salt tolerant calli*

Calli of four canola genotypes were increased in mass through five subcultures and consequently were used to select the most salt tolerant ones. Healthy callus was used for each replicate and transferred to a freshly prepared MS medium supplemented with 2 mg/l 2,4-D and contained five different concentrations of NaCl (0, 4000, 8000, 12000 and 16000 ppm), which were repeated ten times and were incubated under the same environmental conditions used previously for regeneration (mentioned above). The callus survival and shoot frequency were measured for each salt concentration after two weeks and six weeks, respectively. At final, plantlets were transferred to greenhouse for acclimatization process, under the same conditions mentioned previously.

Data were recorded on R0 at harvest time on plant height (cm), first branches height/plant (cm), number of branches/plant, number of pods/plant, weight of straw/plant (g), seed weight/plant (g) and seed index (100 seed weight). R<sub>1</sub> seeds were planted under greenhouse conditions on salt stress and control conditions.

#### *Statistical analysis*

Data were exposed to proper statistical analysis of a completely randomized design (CRD) and were subjected to two-factor of analysis of variance (ANOVA) as published by Gomez & Gomez (1984) and were transformed by the square root transformation method according to Steel & Torrie (1980). The treatment means

were compared by Duncan multiple range test at 0.05 confidence level by the use of MSTAT-C computer programs (Waller & Duncan, 1969).

## **Results and Discussion**

#### *Establishment of canola regeneration protocol*

Canola (*Brassica napus* L., Brassicaceae) is considered as the most important oil crop worldwide, and is a promising crop to participate in the reduction of oil gap in Egypt. Tissue culture technique and establishment of an efficient system for shoot regeneration in some canola genotypes were mainly a prerequisite for canola improvement to be more tolerant for environmental stress such as salinity and drought. Based on the results of the seedling selection under salinity data, four canola genotypes namely, Bingo, Torpe, Conny and Siberian were chosen to achieve this aim.

#### *Callus formation*

After germination, 7-days old, cotyledon and hypocotyl explants of the four studied canola genotypes were cultured on different MS medium supplemented with different concentrations of 2,4-D (2,4-dichlorophenoxyacetic acid).

#### *Callus initiation from cotyledon explants*

After four weeks of culture, the number of Embryogenic callus was recorded for each genotype. Distinguish between Embryogenic and non-Embryogenic callus was carried out on the basis of callus external aspect. Embryogenic calli were glossed aspect, compact, characterized by their tallow color and their globular structure, while non-Embryogenic calli were of wet aspect, translucent and color more brownish (Gandonou et al., 2005) and expressed as percentages of embryogenic calli per total number of calli.

Data in Table 1 show the mean callus initiation of cotyledon explants. Effects of media, genotypes and their interaction on callus initiation were tested as follows:

#### *Effect of media*

The effect of media on callus initiation from cotyledons was presented in Table 1. Data indicate that there were significant differences among different media. It is clear that the highest value of callus frequency percentage was obtained from, the best medium, 2.0 mg/l 2,4-D concentration (73.25% equal to 1.103) and the lowest value of callus frequency percentage was obtained from

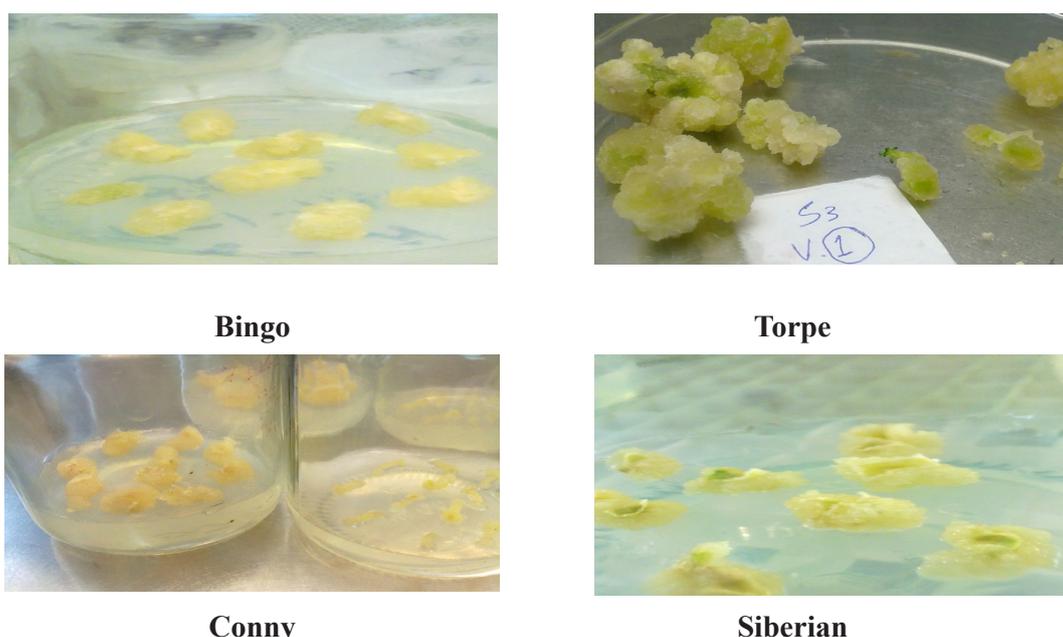
0.5 mg/l 2,4-D (59.75% equal to 1.041) (Fig. 1). While, in the absence of growth regulator, all genotypes failed to produce callus tissue from

cotyledon explant where, the cotyledonary leaves after culturing were increased very much in size with burning the edges of all explants (Fig. 2).

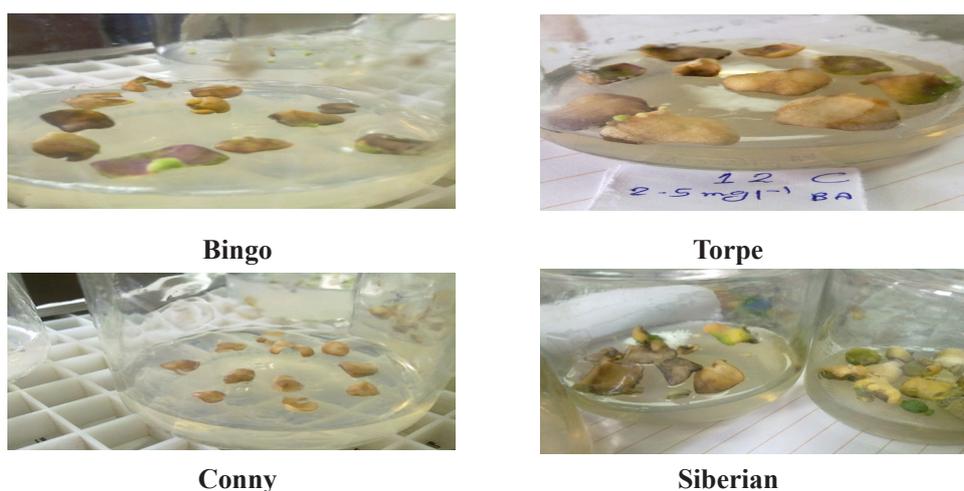
**TABLE 1. Effect of cotyledon explant and different media on callus initiation of the four canola genotypes**

2,4-D mg/l	Bingo	Torpe	Conny	Siberian	Mean
Control	0.710h	0.710h	0.710h	0.710h	<b>0.710c</b>
0.5	1.024e	1.160ab	0.879g	1.100cd	<b>1.041b</b>
1.5	0.970ef	1.167ab	0.960f	1.143bc	<b>1.060b</b>
2.0	1.087d	1.200a	0.968f	1.159ab	<b>1.103a</b>
Mean	<b>0.948c</b>	<b>1.059a</b>	<b>0.879d</b>	<b>1.028b</b>	

Data are transformed according to Steel & Torrie (1980).



**Fig. 1. Callus induction from cotyledon explants of four canola genotypes cultured 21-days on MS-medium supplemented with 2 mg/l 2,4-D.**



**Fig. 2. Increasing of cotyledon explants in size with burning the edges for the four genotypes on control medium 21-days.**

### Effect of genotypes

In all genotypes callus induction for cotyledons was observed in 10 days. Respecting genotypes, Torpe genotype recorded the best frequency (89.00% equal to 1.059) followed by Siberian genotype (64.00% equal to 1.028). On the other hand, the lowest callus initiation percentage was (38.33% equal to 0.879) for Conny genotype (Fig. 1).

### Effect of interaction

The interaction between genotypes and media was significant in callus initiation from cotyledon explants (Table 1). The highest value of callus initiation percentage was (95% equal to 1.200) for Torpe genotype by adding 2 mg/l 2,4-D medium, While, Conny genotype gave the lowest percentage on 0.5 mg/l 2,4-D medium. With respect to interaction effect of callus initiation from cotyledon explants, the control treatment

did not have any callus for all genotypes (Fig. 2).

### Callus initiation from hypocotyl explants

With respect to callus formation, callus proliferation started within two weeks from cut ends of the hypocotyl explants. Data in Table 2 shows the mean callus initiation percentages of hypocotyl explants.

Effects of media, genotypes and their interaction on callus initiation were tested as follows:

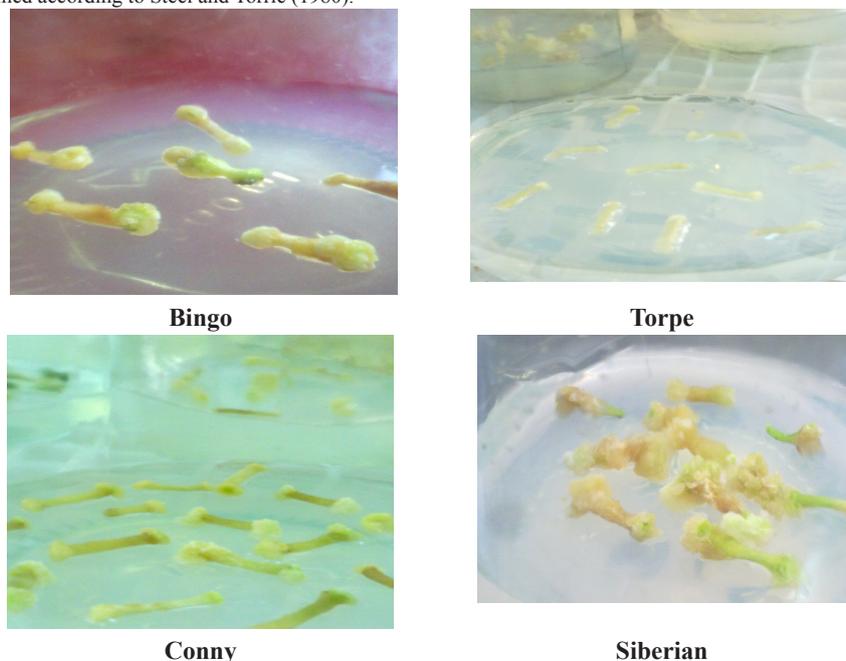
### Effect of media

The effect of media on callus initiation from hypocotyls was presented in Table 2. A high percentage of medium formed callus (49.00% equal to 1.094) for 2.0 mg/l 2,4-D concentration whereas, 1.5 mg/l 2,4-D concentration produced the lowest percentage (28.75% equal to 0.874) (Fig. 3).

**TABLE 2. Effect of hypocotyl explant and different media on callus initiation of the four canola genotypes.**

2,4-D mg/l	Bingo	Torpe	Conny	Siberian	Mean
Control	0.975 <sup>e</sup>	0.748 <sup>ghi</sup>	1.076 <sup>bc</sup>	1.123 <sup>abc</sup>	<b>0.980<sup>b</sup></b>
0.5	1.060 <sup>cd</sup>	0.722 <sup>hi</sup>	0.985 <sup>de</sup>	0.803 <sup>g</sup>	<b>0.892<sup>c</sup></b>
1.5	0.910 <sup>ef</sup>	0.716 <sup>i</sup>	0.797 <sup>gh</sup>	1.073 <sup>bc</sup>	<b>0.874<sup>c</sup></b>
2.0	1.151 <sup>ab</sup>	0.891 <sup>f</sup>	1.176 <sup>a</sup>	1.160 <sup>a</sup>	<b>1.094<sup>a</sup></b>
Mean	<b>1.024<sup>a</sup></b>	<b>0.769<sup>b</sup></b>	<b>1.008<sup>a</sup></b>	<b>1.040<sup>a</sup></b>	

Data are transformed according to Steel and Torrie (1980).



**Fig. 3. Callus initiation from hypocotyl explants of the four canola genotypes cultured 21-days on MS-medium supplemented with 2 mg/l 2,4-D.**

*Effect of genotypes*

Callus induction for cotyledons was observed in three weeks in all genotypes. It is clear that callus so depressed for Torpe genotype (4.25% equal to 0.769) compared to other genotypes: Bingo, Conny and Siberian (48.75, 44.00 and 53.50% equal to 1.024, 1.008 and 1.040, respectively). Generally, it very obvious from Fig. 3 that the initiated calli for all genotypes arrived to a fixed size (1-3 mm) and stop its growth thereafter, where, it has not given enough calli for regeneration shoot.

*Effect of interaction*

Data in Table 2 showed that the response of all genotypes adding 2,4-D concentrations specially Torpe genotype was recorded lowest value with 1.5 mg/l 2,4-D medium (1% equal to 0.716) and all enhanced calli did not give any increase in its mass after that (Fig. 3). For all genotypes, callus induction from cotyledons was faster than hypocotyls and recorded the best frequencies (95% equal to 1.200) with MS medium supplemented with 2 mg/l 2,4-D as presented in Table 2. From Tables 1 and 2, it is obviously clear that callus induction was greatly enhanced with

using cotyledons more than hypocotyls of the four different genotypes on all different media and this phenomenon which pronounced specially in Torpe genotype.

*Shoot regeneration*

Establishment of an efficient system for shoot regeneration in the selected canola genotypes was mainly a prerequisite for canola salt tolerance. After 21-days, the explants-derived calli were transferred into the regeneration media. To induce shoot regeneration system from canola callus, four combinations of growth regulators in addition to control treatment were used.

*Effect of media*

The regeneration frequencies of the hypocotyl derived calli from four canola genotypes are listed in Table 3. The data indicated that the best regenerated shoot medium was achieved by the supplementation of 5 mg/l BA + 0.05 NAA (41.67% equal to 0.944), while, control medium and MS-medium supplemented with 1 mg/l BA + 1.0 mg/l Kin recorded the lowest values of shoot induction (11.67% and 25.84% equal to 0.779 and 0.861), respectively.

**TABLE 3. Mean regenerated shoots from callus of the four canola genotypes cultured on MS-medium supplemented with four combinations of growth regulators.**

Media mg/l	Bingo	Torpe	Conny	Siberian	Mean
Control	0.770 <sup>hi</sup>	0.827 <sup>fighi</sup>	0.750 <sup>i</sup>	0.770 <sup>hi</sup>	<b>0.779<sup>d</sup></b>
1 BA + 1.0 Kin	0.830 <sup>efghi</sup>	0.921 <sup>bcdef</sup>	0.807 <sup>ghi</sup>	0.884 <sup>cdefg</sup>	<b>0.861<sup>c</sup></b>
3 BA + 0.5 IAA	0.884 <sup>cdefg</sup>	1.000 <sup>ab</sup>	0.827 <sup>fighi</sup>	0.972 <sup>abcd</sup>	<b>0.921<sup>ab</sup></b>
4 BA + 0.5 NAA	0.867 <sup>defgh</sup>	0.941 <sup>bcde</sup>	0.810 <sup>fighi</sup>	0.901 <sup>bedfg</sup>	<b>0.880<sup>bc</sup></b>
5 BA + 0.05 NAA	0.884 <sup>cdefg</sup>	1.054 <sup>a</sup>	0.850 <sup>efghi</sup>	0.989 <sup>abc</sup>	<b>0.944<sup>a</sup></b>
Mean	<b>0.847<sup>b</sup></b>	<b>0.949<sup>a</sup></b>	<b>0.809<sup>b</sup></b>	<b>0.903<sup>a</sup></b>	

Data are transformed according to Steel and Torrie (1980).

*Effect of genotypes*

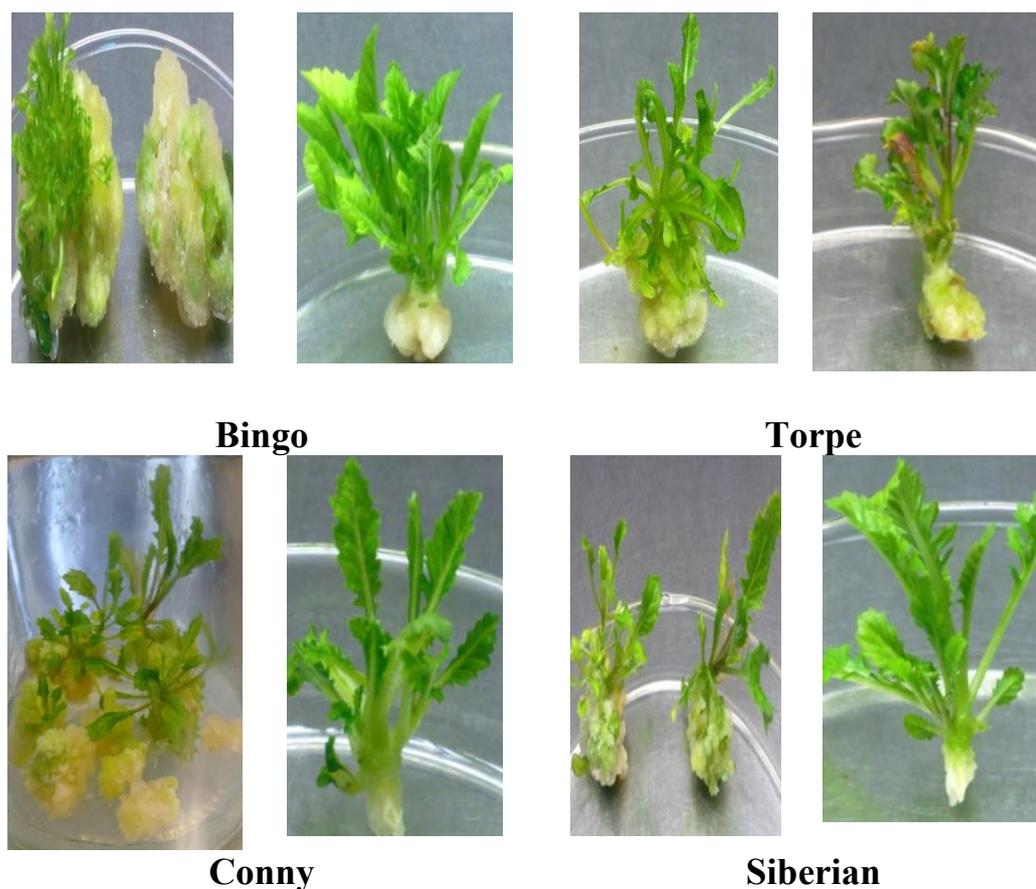
Data in Table 3 showed that genotypes different in their response, e.g. Torpe genotype show the highest regeneration ability (42.67% equal to 0.949) followed by Siberian and Bingo genotypes and the lowest percentage obtained from Conny genotype (16.67% equal to 0.809) (Fig. 4).

*Effect of interaction*

Table 3 shows that in absence of growth regulators (control medium), the lowest regenerated shoot was observed with all genotypes.

The best results of regenerated shoot percentage occurred when calli tissue of Torpe genotype were grown on MS-medium supplemented with 5 mg/l BA + 0.05 NAA, where recorded (63.33% equal to 1.054) followed by the same genotype with 3 mg/l BA + 0.5 IAA (53.33% equal to 1.000).

Generally, it is noted that, regenerated shoot percentage was varied depending on the canola genotypes and the combination of growth regulators added to the regeneration medium, also noted that increasing the concentration of benzyl adenine increased the regeneration ability of all genotypes.



**Fig. 4. Shoot formation from calli of the four canola genotypes cultured 45-days on MS-medium supplemented with 5 mg/l BA + 0.05 NAA.**

In the present work, frequency of plantlet regeneration was obtained from calli derived from cotyledons and hypocotyls explants of canola when using 2,4-D. The poor response of canola callus induction of all genotypes on MS-free auxins medium may reflect requirement of growth regulators. On the other hand, numerous workers had reported that, auxins such as 2,4-D allowed callus initiation of cotyledon and hypocotyl explants, these results agree with those obtained by Turgut et al. (1994), Bogunia & Przywara (2000), Martin & Mohanty (2002) and Chamandoosti & Azad (2012).

#### *Root formation*

Shoots that regenerated on MS media supplemented with growth regulators were transformed to another rooting media with or without growth regulator. Data in Table 4 show that in both rooting media, rooting efficiency was between 57.14 and 100% equal to 0.711 and 0.714, respectively. MS medium supplemented with 1 mg/l IBA was better and faster than the

other media in the root percentage (85.71% equal to 0.713) as shown in in Fig. 5. Torpe genotype recorded the highest frequency of root induction percentage, while Conny genotype recorded the lowest value (61.90% equal to 0.711), respectively. Generally, the differences among genotypes or media were not significant. Similar results were obtained by Ono et al. (1994) and Majd et al. (2006)

#### *In vitro selection of salt tolerate calli*

This experiment was conducted to select *in vitro* canola calli tolerant to increased levels of NaCl in cultures relies on somaclonal variation with the ultimate objective to regenerate such tolerant calli into complete tolerant canola plants to salinity conditions. Calli of four canola genotypes were increased in mass through five subcultures and consequently on 2 mg/l 2,4-D medium supplemented with different concentrations of NaCl (0, 4000, 8000, 12000 and 16000 ppm) were used to select the most salt tolerant ones. Healthy callus was used for each

replicate and transferred to a freshly prepared MS medium supplemented with 2 mg/l 2,4-D and contained five different concentrations of NaCl (0, 4000, 8000, 12000 and 16000 ppm) and callus

survival% and shoot frequency were recorded for each salt concentration after two weeks and six weeks, respectively.

**TABLE 4. Root formation on the shoot 45-days of the four canola genotypes cultured on three different MS-medium.**

Media mg/l	Bingo	Torpe	Conny	Siberian	Mean
Control	0.712 <sup>a</sup>	0.712 <sup>a</sup>	0.711 <sup>a</sup>	0.712 <sup>a</sup>	<b>0.712<sup>a</sup></b>
1 IBA	0.713 <sup>a</sup>	0.714 <sup>a</sup>	0.712 <sup>a</sup>	0.713 <sup>a</sup>	<b>0.713<sup>a</sup></b>
0.5 NAA	0.712 <sup>a</sup>	0.713 <sup>a</sup>	0.711 <sup>a</sup>	0.712 <sup>a</sup>	<b>0.712<sup>a</sup></b>
Mean	<b>0.712<sup>a</sup></b>	<b>0.713<sup>a</sup></b>	<b>0.711<sup>a</sup></b>	<b>0.712<sup>a</sup></b>	

Data are transformed according to Steel & Torrie (1980).



**Bingo**



**Torpe**



**Conny**



**Siperian**

**Fig. 5. Root formation on shoot of canola genotypes cultured 45-days on MS-medium supplemented with 1 mg/l IBA.**

*Callus survival percentage**Effect of salinity*

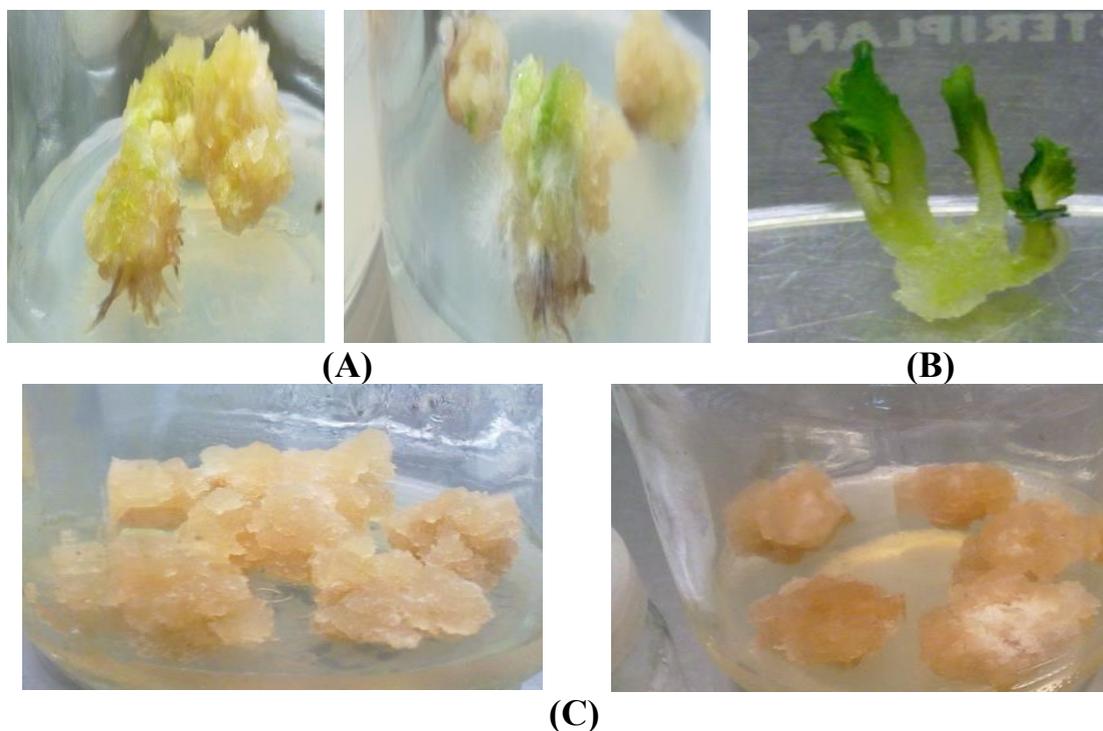
Table 5 shows that the effect of increasing levels of NaCl in culture medium on callus survival percentage. The highest NaCl concentration was the greater the reduction. Reduction in callus survival percentage varied

according to the concentration of NaCl were the maximum reduction when 16000 ppm NaCl was applied as shown in Fig. 6 and 7. Similar results were reported by Chandler et al. (1986), Raldugina and Peskova (1988) and Mandal et al. (1989)

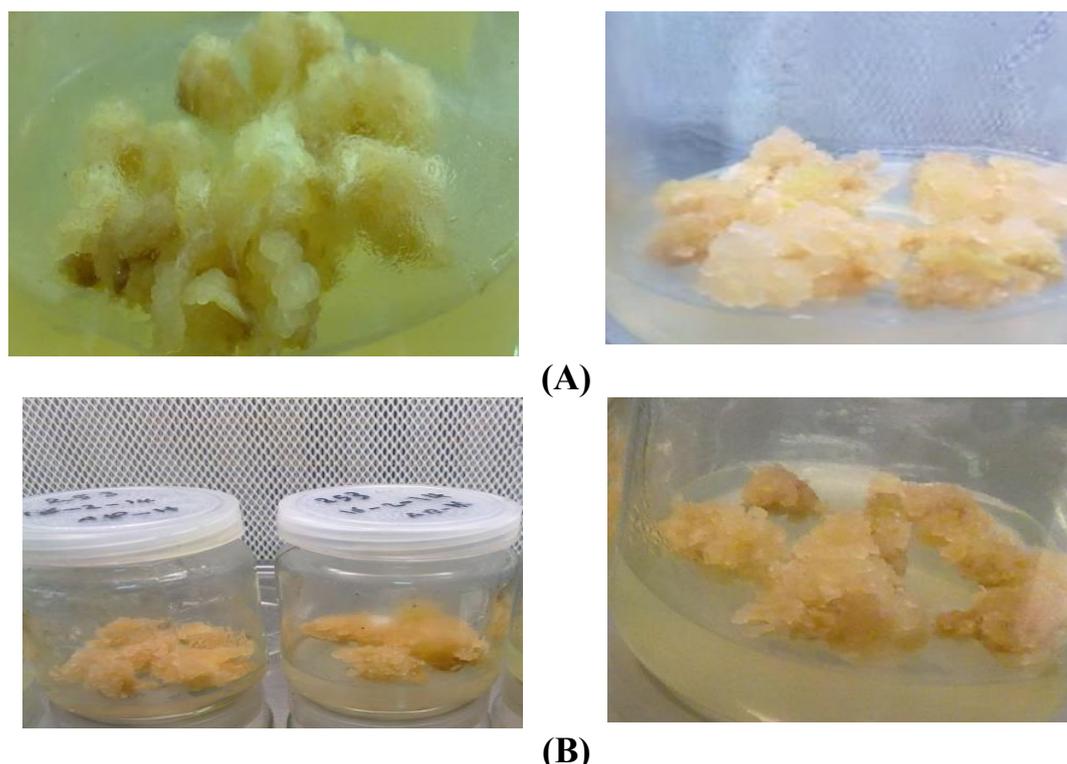
**TABLE 5. Callus survival of the four canola genotypes under different NaCl concentrations after two weeks of culturing.**

NaCl (ppm)	Bingo	Torpe	Conny	Siberian	Mean
0	1.220 <sup>a</sup>	1.220 <sup>a</sup>	1.220 <sup>a</sup>	1.220 <sup>a</sup>	<b>1.220<sup>a</sup></b>
4000	1.220 <sup>a</sup>	1.220 <sup>a</sup>	1.220 <sup>a</sup>	1.220 <sup>a</sup>	<b>1.220<sup>a</sup></b>
8000	1.127 <sup>abc</sup>	1.173 <sup>ab</sup>	0.967 <sup>def</sup>	1.127 <sup>abc</sup>	<b>1.098<sup>b</sup></b>
12000	0.900 <sup>ef</sup>	1.070 <sup>bcd</sup>	0.843 <sup>fg</sup>	0.900 <sup>ef</sup>	<b>0.928<sup>c</sup></b>
16000	0.710 <sup>g</sup>	1.013 <sup>cde</sup>	0.710 <sup>g</sup>	0.843 <sup>fg</sup>	<b>0.819<sup>d</sup></b>
Mean	<b>1.035<sup>ab</sup></b>	<b>1.139<sup>a</sup></b>	<b>0.992<sup>c</sup></b>	<b>1.062<sup>b</sup></b>	

Data are transformed according to Steel & Torrie (1980).



**Fig. 6. Salt tolerant calli developed from Bingo genotype (A) and initiated shoot (B) at 12000 ppm NaCl concentration, death of Bingo calli at 16000 ppm NaCl (C).**



**Fig. 7.** Salt tolerant calli developed from Conny genotype (A) at 12000 ppm NaCl, death of Conny calli at 16000 ppm NaCl concentration (B).

#### *Response of genotypes*

With respect to callus survival percentage, data revealed significant differences among Torpe, Conny and Siberian genotypes as well as between Bingo and Conny genotypes. The highest reduction was in Conny genotype: 53.33% equal to 0.922, while, Torpe genotype recorded the highest value: 80.74% equal to 1.139.

#### *The interaction effect*

From Table 5, there was no significant differences between all genotypes and 4000 ppm NaCl concentrations as compared with control treatment. Behavior of Bingo genotype was the same behavior of Siberian genotype at 8000 and 12000 ppm NaCl concentrations (77.77 and 33.33% equal to 1.127 and 0.900, respectively) (Fig. 8). Torpe genotype surpassed the others in survival calli percentages under 8000, 12000 and 16000 ppm NaCl concentrations, where recorded (88.89, 59.26 and 55.56% equal to 1.173, 1.070 and 1.013, respectively) (Fig.9). Under the highest concentration 16000 ppm, there was no survival calli produced from Bingo and Conny genotypes, while, callus of Siberian genotype hold out under this concentration despite that

it was within the sensitive group for salinity (22.22% equal to 0.843).

#### *Shoot frequency from tolerant calli*

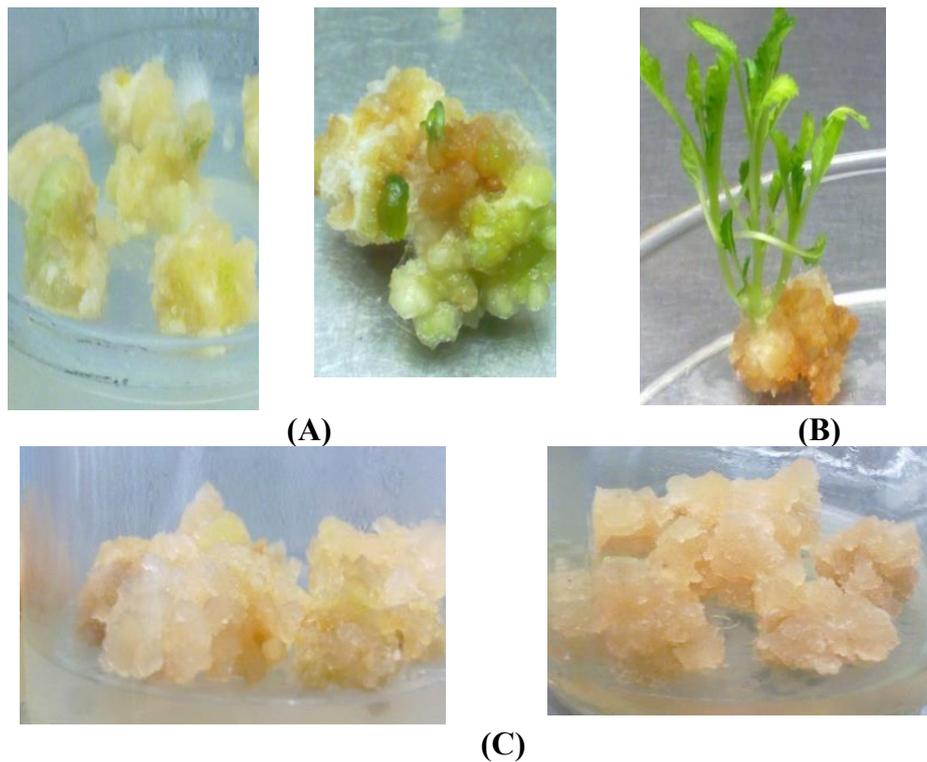
Tolerant calli from previous experiment were cultured on MS-medium supplemented with the best growth regulators combination (5 mg/l BA + 0.05 mg/l NAA) in addition to different concentrations of NaCl and shoot percentage were recorded as showed in Table 6.

#### *Effect of salinity*

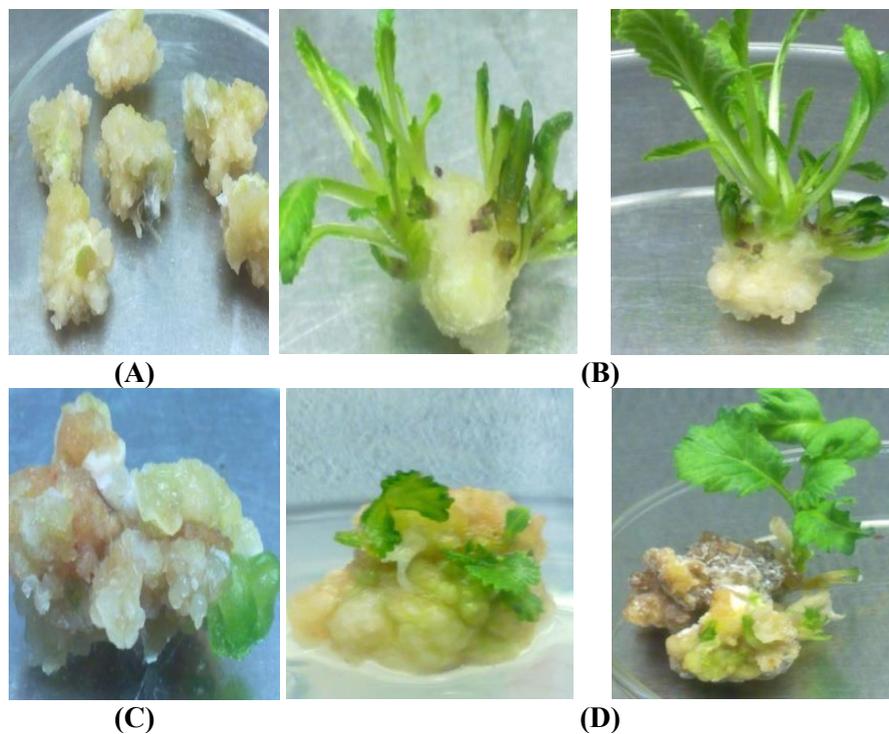
When salt concentrations were increased up to 12000 or/and 16000 ppm NaCl, the shoot percentage was decreased by 22.22 and 5.56% equal to 0.838 and 0,743. Similar results were reported by Raldugina & Peskova (1988) and Al-Naggar et al. (2008).

#### *Response of genotypes*

It was cleared from Table 6 that Torpe genotype recorded the highest value of shoot percentage following by Bingo genotype of: 48.89% equal to 0.983 and 26.66% equal to 0.889, respectively. Whereas, Conny following by Siberian genotypes were recorded the lowest values of: 11.11% equal to 0.862 and 25.00% equal to 0.889, respectively.



**Fig. 8.** Salt tolerant calli developed from Siberian genotype (A) and initiated shoot (B) at 12000 ppm NaCl, death of Siberian calli at 16000 ppm NaCl (C) concentration..



**Fig. 9.** Salt tolerant calli developed from Torpe genotype (A) and initiated shoot (B) at 12000 ppm NaCl, salt tolerant calli developed from Torpe genotype (C) and initiated shoot (D) at 16000 ppm NaCl concentration.

*The interaction effect*

Data in Table 6 indicate that salt stress reduced shoot frequency of all genotypes under study. The reduction percentage in shoot ranged from (33.33 to 22.22% equal to 0.900 to 0.833) in Bingo genotype, from (55.56 to 44.44% equal to 1.023 to 0.967) in Torpe genotype, from (22.22 to 0.00% equal to 0.843 to 0.710) in Conny genotype and from (55.56 to 0.843% equal to 1.023 to 0.843) in Siberian genotype as NaCl levels increased from 4000 to 12000 ppm, respectively. At 16000 ppm NaCl concentration only tolerant calli of Torpe genotype was able to initiate shoot under this concentration of 22.22% equal to 0.843 as shown in Fig. 8. This was similar with Chamandoosti (2007).

*Plant acclimatization*

Plantlets that showed a well-developed root system (through four to five weeks) and became ready to be acclimatized were transferred to

sterilized mixture of beat moss and vermiculite (1:1) in 25 cm plastic pots after washed them under running tap water through green house conditions.

Canola regenerated plantlets at different growth stages up to maturity during the acclimatization process are shown in Fig. 10-14. Only five salt tolerant plantlets developed from Torpe genotype and eight salt tolerant plantlets developed from Siberian genotype succeeded to complete their life cycle and reached maturity stage and produced seeds. These regenerated genotypes were referred as T1R0, T2R0, T3R0, T4R0, T5R0 for the five regenerated plants developed from Torpe genotype that tolerate NaCl concentration up to 16000 ppm, as well as, S1R0, S2R0, S3R0, S4R0, S5R0, S6R0, S7R0, S8R0 for the eight regenerated plants developed from Siberian genotype that tolerate NaCl concentration up to 12000 ppm.

**TABLE 6. Shoot frequency obtained from the select salt tolerant calli of the four canola genotypes under different NaCl concentrations after four weeks of culturing.**

NaCl (ppm)	Bingo	Torpe	Conny	Siberian	Mean
0	0.900 <sup>bcd</sup>	1.117 <sup>a</sup>	0.843 <sup>bcd</sup>	1.023 <sup>ab</sup>	<b>0.971<sup>a</sup></b>
4000	0.900 <sup>bcd</sup>	1.023 <sup>ab</sup>	0.843 <sup>bcd</sup>	1.023 <sup>ab</sup>	<b>0.948<sup>a</sup></b>
8000	0.967 <sup>abc</sup>	0.967 <sup>abc</sup>	0.777 <sup>cd</sup>	0.843 <sup>bcd</sup>	<b>0.888<sup>ab</sup></b>
12000	0.833 <sup>bcd</sup>	0.967 <sup>abc</sup>	0.710 <sup>d</sup>	0.843 <sup>bcd</sup>	<b>0.838<sup>bc</sup></b>
16000	0.710 <sup>d</sup>	0.843 <sup>bcd</sup>	0.710 <sup>d</sup>	0.710 <sup>d</sup>	<b>0.743<sup>c</sup></b>
Mean	<b>0.862<sup>bc</sup></b>	<b>0.983<sup>a</sup></b>	<b>0.777<sup>c</sup></b>	<b>0.889<sup>b</sup></b>	

Data are transformed according to Steel & Torrie (1980).



at the beginning of acclimatization process



After two weeks



After four weeks

**Fig. 10.** Canola regenerated plantlets at different ages during the acclimatization process.



**Fig. 11.** Torpe genotypes at flowering stage.



**Fig. 12. Torpe genotypes at harvesting stage.**



**Fig. 13. Siberian genotypes at flowering stage.**



**Fig. 14. Siberian genotypes at harvesting stage.**

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### تطوير بعض اصناف الكانولا لتحمل الملوحة باستخدام تقنية زراعة الانسجة

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اجريت تجارب هذه الدراسة في معامل قسم بحوث الخلية - معهد بحوث المحاصيل الحقلية - مركز البحوث الزراعية بالجيزة - مصر خلال الاعوام من 2011 حتى 2015 وايضا صوبة قسم البساتين- كلية الزراعة- جامعة عين شمس وتهدف الدراسة إلى تأسيس نظام لزراعة الانسجة لأربعة اصناف من الكانولا (الصنفان Torpe و Bingo متحملان للملوحة، والصنفان Conny و Siberian حساسان للملوحة) ، إنتاج نباتات متحملة للملوحة من خلال انتخاب الكالوس المتحمل للملوحة واستيلاء نباتات من كل كالوس تحت أربعة تركيزات مختلفة من كلوريد الصوديوم 4000، 8000 ، 12000 ، 16000 جزء في المليون بالإضافة إلى معاملة المقارنة. أشارت النتائج أن افضل البيئات المستخدمة لتكوين الكالوس هي بيئة MS مضافا لها 2 ملجم/لتر 2,4-D، وتم استيلاء الجزء الخضرى من بيئة MS مضافا لها 5 ملجم/لتر بنزيل ادنين + 0.05 ملجم/لتر نفتالين اسيتك اسيد، بينما بيئة MS مضافا إليها 1 ملجم/لتر إندول بيوتيرك اسيد كانت الافضل في تكوين الجذور. كما أوضحت النتائج أن استخدام الأوراق الفلقية كمستقطع نباتى كان هو الأفضل والأسرع للحصول على نباتات كاملة ، وأن أفضل الأصناف الصنف Torpe يليه الصنف Siberian في إنتاج الكالوس واستيلاء الجزء الخضرى وتكوين الجذور، بينما سجل الصنف Conny أقل القيم. وبانتخاب الكالوس المتحمل للملوحة وجد ان النسبة المئوية للكالوس الحى وكذلك نسبة الجزء الخضرى المتكون تناقصتا عقب المعاملة بتركيز 8000 جزء في المليون وانخفضت بشدة تحت تركيز 12000 جزء في المليون بينما الصنف Conny لم يعطى اى جزء خضرى، وعند تركيز 16000 جزء في المليون تم الحصول على كالوس حى من الصنفى Torpe و Siberian فقط ولم يعطى الأخير اى جزء خضرى تحت ذلك التركيز. وعند اقلمة النباتات الناتجة من التجربة السابقة، انتج الصنف Torpe خمسة تراكيب وراثية متحملة للملوحة عند تركيز 16000 جزء في المليون وهى T1R0, T2R0, T3R0, T4R0, T5R0 ، وانتج الصنف Siberian ثمانية تراكيب وراثية متحملة للملوحة عند تركيز 12000 جزء في المليون وهى S1R0, S2R0, S3R0, S4R0, S5R0, S6R0, S7R0, S8R0. وعند اكثار سلالات الصنف Torpe أنبتت جميع السلالات ولكن أعطى فقط ثلاثة منهم ثمارا، وكذلك عند اكثار سلالات الصنف Siberian، وذلك خلال اكثار البذور خلال عامى 2015/2016 و 2016/2017.