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Effect of Sodium Chloride on Establishment of Callus and Organogenesis in *Brassica napus* L.

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Abstract: In order to produce salt tolerant canola (*Brassica napus* L.) plants hypocotyl segments of its that were excised from 7 days old-seedlings cultured in MS medium with various concentrations of PGRs (NAA, IBA, 2, 4-D, KN and BA) and sodium chloride. The best media for induction and growth of callus (6 mg L^{-1} 2, 4-D and 2 mg L^{-1} BA), production of adventitious shoots (1 mg L^{-1} IBA and 1 mg L^{-1} BA) and roots (1 mg L^{-1} NAA and 0.5 mg L^{-1} KN) were determined. Then explants cultured in these media plus 0-273.53 mM L^{-1} sodium chloride. Explants in these media established and in some of concentrations of sodium chloride (68.37-102.56 mM L^{-1}) produced calli and adventitious shoots and roots better than the same media but free from sodium chloride. This finding suggests that somaclonal variation can yield stable-tolerant canola plants.

Key words: Canola, resistance salinity, somaclonal variation, tissue culture

INTRODUCTION

Canola (*Brassica napus* L.) is a member of Brassicaceae that genetically alterded from of rapeseed with low erucic acid, a 22-carbon chain fatty acid that is used in a variety of polymer and lubricant products (Starner *et al*., 1996). Interest in canola is increasing steadily among health-conscious Iranian consumers due to its lowest content of saturated fatty acids among major oil seeds. Salinity stress, which usually occurs in arid and semiarid regions, is a major environmental constraint to crop productivity (Dasgan *et al*., 2002). Because plants respond to salinity by activating a complex set of defense pathways that ultimately culminate in tolerance or susceptibility, the breeding of salinity-tolerance crops has been difficult (Zhu, 2002). Cell culture techniques have proved useful in many areas of plant research (Noaman *et al*., 2004). One of the areas in which the *in vitro* selection approach has been used effectively is plant breeding (Noaman *et al*., 2004). Selection process can be applied at either the cell population level or on regenerated plants from cell cultures and followed by selection in conventional field plots (Barakat and Abdel-Latif, 1996; Barakat and Al-Haris, 1998). In fact, plant cell and tissue culture techniques allow screening of a very large population of cells and regenerated plants in a small space and in a much more controlled environment than in conventional field trials (Bressan *et al*., 1981; Harms and Oerli, 1985; Sabbah and Tal, 1990; Borkid *et al*., 1991; Barakat and Abdel-Latif, 1995a, b; El-Haris and Barakat, 1998; Jain, 2001; Barakat *et al*., 2002). Because saline soil and saline irrigation waters present potential hazards to canola production (Fowler, 1991) and we could increase resistance of canola by tissue cultures methods, therefore the purpose of this study is establishment for improved performance canola under saline condition by tissue culture methods step by step. In the first step we examined effect of media with different concentrations of plant growth regulators and sodium chloride in induction and growth of callus, rooting and shooting in canola.

MATERIALS AND METHODS

This study conducted in Iranian Research Institute of Plant Protection, Tehran, Iran in May 2005-July 2006.

Plant material: One canola (*Brassica napus* L.) cultivar, Pionner was used. In order to establish a stock of plants, seeds that were obtained from Oilseed Research and development Company were sterilized in a commercial sodium hypochlorite solution (with 5% available chlorine) for 8 min and were rinsed 4 times with sterile water. Then sterile seeds were cultured in MS (Murashig and Skoog, 1962) medium without PGRs. Seven days later hypocotyls of (4-6 mm) seedlings were used as explant.

Culture medium, incubation conditions, evaluation procedure and statistical analysis: The basal medium that included the (MS formulation), sucrose 3% (w/v) and agar agar 1% supplemented with PGRs (2, 4-D, NAA, IBA, KN, BAP) and sodium chloride.

The media were adjusted to pH 5.8 and autoclaved at 121°C for 20 min. Cultures were maintained in 16 h photoperiod (for induction and growth of regenerated shoots) and dark (for induction and growth of calli and regenerated roots) at 25°C±1. After establishment, cultures were subcultured at 5-6 weeks intervals on fresh media. And 2 months later diameter of calli and the means number of regenerated shoots and roots per explant were evaluated. Experiment were set up in completely randomized design and repeated 4 times. Treatments has 4 replications. Data were subjected to SD.

RESULTS

Approximately two weeks after of establishment hypocotyl explants inducing of callusing, rooting and shooting were begun. The best medium for induction and growth of callus was MS medium with 6 mg L^{-1} 2, 4-D and 2 mg L^{-1} BA. In medium with 1 mg L^{-1} IBA and 1 mg L^{-1} BA white calli produced regenerated shoots and earlier these results reported by Chamandoosti and Majd (2006). One milligram per liter NAA and 0.5 mg L^{-1} KN was the suitable medium for production of regenerated roots on explants after a short period of callusing (very light yellow calli). After this primary selection, hypocotyl explants cultured in above media plus (0, 68.37, 102.56, 136.75, 170.94, 205.12, 239.31 and 273.50 mM L^{-1}) sodium chloride.

Effect of sodium chloride on induction and growth of calli: After establishment, hypocotyl explants produced calli. Theses calli were compact, yellowish, nonorganogen and were similar to calli in medium with the same kind and concentration of PGRs but free from salt (sodium hypochlorite) (Fig. 1a). 0-68.37 mM L^{-1} sodium chloride had a positive effect on growth of calli, so that in 68.37 mM L^{-1} sodium chloride diameter of calli was the maximum (3.3125±0.4716) (Fig. 1b). The reducing effect of sodium chloride was in 102.56 mM L^{-1} sodium chloride and higher. In 170.90-273.50 mM L^{-1} sodium chloride diameter of calli was zero (Fig. 1c and Table 1).

Effect of sodium chloride on the means number of regenerated roots per explants: Approximately two weeks after establishment of explants in media containing

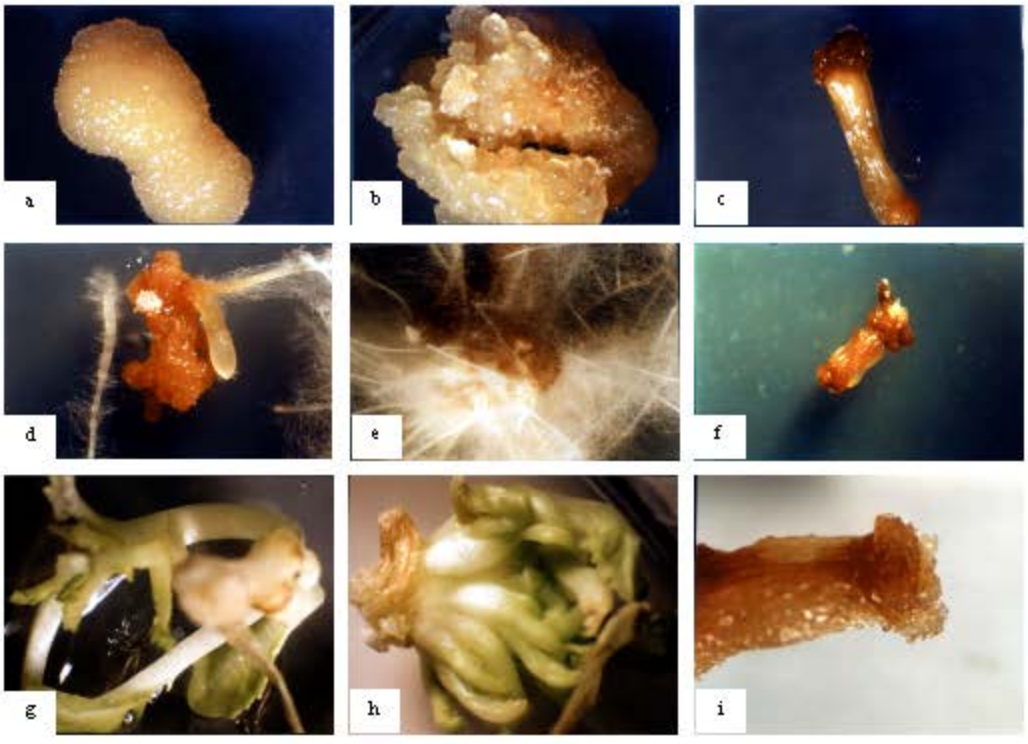

Fig. 1: Effect of sodium chloride on callusing, rooting and shooting of *Brassica nacus* L. Effect of sodium chloride on induction and growth of calli in 0 mML^{-1} (a), 68.37 mM L^{-1} (b), 273.50 mM L^{-1} (c), the mean numbers of regenerated roots in 0 mM L^{-1} (d), 102.56 mM L^{-1} (e), 273.50 mM L^{-1} (f), the mean number of regenerated shoots in 0 mM L^{-1} (g), 68.37 mM L^{-1} (h) and 273.50 mM L^{-1} (i)

Table 1: Effect of NaCl on diameter of calli in *Brassica napus* L.

NaCl (mM L^{-1})	Diameter of calli (mm)
0.00	2.9375±0.2577
68.37	3.3125±0.4716
102.56	2.4375±0.2772
137.75	2.3125±0.6240
170.94	0
205.12	0
239.31	0
273.50	0

Data represent means of four replicates. There is no significant difference between treatments and control at $p<0.01$

Table 2: Effect of NaCl on regenerated roots (Mean±SD) in *Brassica napus* L.

NaCl (mm L^{-1})	Regenerated roots
0	4.2125±0.2680
68.37	4.1625±0.5921
102.56	7.2125±1.9049
137.75	2.8500±0.6801
170.94	1.6500±0.3594
205.12	2.2500±0.4784
239.31	1.2000±0.2160
273.50	0

Data represent means of four replicates. There is significant difference between treatments and control at $p<0.01$

1 mg L^{-1} NAA and 0.5 mg L^{-1} KN plus different concentrations of sodium chloride very light yellow and compact calli on explants were appeared. Theses calli were able to produce regenerated roots very well. Several kinds of roots were seen on explants. The difference of roots related to length and diameter of them. Also roots were always produced in one of the ends of explants.

Effect of sodium chloride on the means number of regenerated roots per explants was very clear. From 0-102.56 mM L^{-1} effect of sodium chloride was increasing. (In 102.56 mM L^{-1} sodium chloride the means number of regenerated roots was 7.2125±1.9049), (Fig. 1d and e). In concentrations 102.56 mM L^{-1} the reducing effect of salt (sodium chloride) caused that number of regenerated roots were gradually reduced and in 273.50 mM L^{-1} sodium chloride no rooting were seen (Fig. 1f and Table 2).

Effect of sodium chloride on the means number of regenerated shoots per explants: In media suitable for producing regenerated shoots (1 mg L^{-1} IBA and 1 mg L^{-1} BA), explants produced calli at first, similar earlier cases. These calli were white to light yellow and able to regenerating shoots (Fig. 1g). The means number of regenerated shoots related to the concentration of sodium chloride. 68.37 mM L^{-1} sodium chloride and 273.50 mM L^{-1} of this salt had the high level of increasing effect (Table 3) and reducing effect on the means number of regenerated shoots per explants, respectively (Fig. 1h and i).

Table 3: Effect of NaCl on regenerated shoots (Mean±SD) in *Brassica napus* L.

NaCl (mm L^{-1})	Regenerated shoots
0	2.2500±0.3862
68.37	3.0250±0.4442
102.56	1.3000±0.2380
137.75	0.9000±0.2380
170.94	1.1000±0.2380
205.12	0
239.31	0
273.50	0

Data represent means of four replicates. There is significant increase in 100 mm L^{-1} and significant reduction in 200 and 250 mm L^{-1} at $p<0.01$

Growth of this regenerated shoots was letter than to regenerated shoots in media with the same concentrations of PGRs but free from salt (sodium chloride) but similar to these shoots, rooted in MS medium with 1 mg L^{-1} IBA and potted successfully.

DISCUSSION

There are many reports about effect of salt stress on growth and constituent of plants in tissue culture systems. For example Omar *et al.* (1993) conducted to determine the effects of sodium chloride cellular content of sunflower callus culture as the first step towards using such technique for production of salinity tolerant sunflower. These researcher resulted that sodium chloride caused reduction in callus fresh weight. They stressed that reduction in callus fresh weight might be a result of reduced water availability in the culture medium due to increase sodium chloride concentration. Mercado *et al.* (2000) used of growth of apical stem sections and adventitious organogenesis to evaluate salinity tolerance in cultivated tomato (*Lycopersicon esculentum* L.) and resulted that this approach may not be a reliable tool to evaluate salt tolerance in tomato because the lack of agreement between the results obtained with the *in vitro* tests, adventitious shoot formation and growth of apical stem section. However, Rus *et al.* (2000) studied salt responses induced by long-term callus culture in leaf callus tissue of the cultivated tomato species and its wild salt-tolerant relative (*L. pennellii* Correl Darcy). They concluded that the salt responses varied according to the precedence of calli (from control to saline medium). Selection a proper scale for evaluating salinity by tissue culture is very important, because breeding for water stress tolerance by the traditional methods is time consuming procedure (Droffling *et al.*, 1993). In this research we used diameter of calli and the means number of adventitious shoots and roots in saline media and resulted that this approach is a suitable tool to evaluate effect salt in canola. Present results demonstrated that

hypocotyl explants of canola not only can live in saline media but also in some concentrations of salt (68.37-102.56 mM L^{-1}), diameter of calli, also the means number of adventitious roots and shoots per explant were higher. In fact salt stress caused that to induce a variation in these reactions. As we know variation observed in plants regenerated from tissue culture termed somaclonal variation (Larkin and Scowcroft, 1981). Induction of resistance or tolerance in canola for salt stress (75 mM L^{-1}) need to gene transfer (Srivastava *et al*., 2004). These researchers for the first time reported that the constitutive expression of a pea PR10 gene in *Brassica napus* enhances their germination and growth in the presence of 75 mM L^{-1} sodium chloride. PR10 proteins are encoded by a gene family and have been characterized from various plant species (Liu *et al*., 2003). In this research resistance in canola in 68.37-102.56 mM L^{-1} sodium chloride has been induced by somaclonal variation. It seems that there is a positive correlation between our results and results of transgenic plant production. We can find that variation induced in regenerated roots and shoots, also in prolonged cells (callus) were lead to increasing the means number of adventitious root and shoots and diameter of calli. In this experiment regeneration of roots and shoots were by passing phase callus culture and regenerated organs (roots and shoots) originated from calli. So far somaclonal variation could induced new characteristic such as enhancement salinity tolerance, on the other hand new organs (roots and shoots) were completely regenerated. It is interesting that according to our findings effect of sodium chloride on rooting are similar to effect of this salt on somatic embryogenesis. Effect of sodium chloride on somatic embryogenesis reported earlier (Majd *et al*., 2006). The similarity between somatic embryogenesis with induction of adventitious roots has been earlier reported (Neumann, 1995).

Present findings suggest that somaclonal variation can yield stable, salt-tolerant plants. We decides that produce whole regenerated plants in the next researches.

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