In vitro selection and molecular characterization of salt tolerant canola plantlets

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ABSTRACT

In this study, a system for in vitro selection of salt tolerant canola clones (plantlets) was developed. The developed system relies on somaclonal variation and the high regenerative capacity of hypocotyl explants of the Egyptian canola cultivar Serw 4 via direct and indirect shoot organogenesis. The optimized regeneration system involves callus formation from hypocotyl explants of 4 day-old seedlings on MS medium supplemented with 1.5 mg/l 2,4-D and shoot proliferation from proliferated microcalli colonies on MS + 4.0 mg/l BA. Shoot organogenesis frequecny ranged from 35 to 40%. This system was used for in vitro selection of salt tolerant plantlets from hypocotyl explants of seedlings germinated on salt containing medium. Under selection pressure, shoot organogenesis frequencey dropped to 5% for selected cell lines and zero for non-selected cell lines onto 0.3% NaCl. The biochemical and molecular characterization of the selected and non-selected clones were performed using: proline accumulation, total protein content, SDS-PAGE and RAPD analysis. The salt-tolerant plantlets were characterized by improved growth on saline media, rapid accumulation of proline, high content of total protein, synthesis of new set of high molecular weight proteins (38 and 94 KD) and unique RAPD banding profiles. The criteria of the selected clones outlined above may be a part of biochemical make up performed by these canola clones to tolerate extreme salt stress.

Key words: *Brassica napus, canola, regeneration, salt stress, proline, SDS-PAGE, RAPD.*

INTRODUCTION

It is well-known that Egypt is suffering from a great shortage in edible oils. The gap between production and consumption is estimated by about 90% (FAO, 2004). In this context, canola is considered as the most important oil crop worldwide. It ranks the third among the oil crops, following palm oil and soya oil and the fifth among economically important crops, following rice, wheat, maize and cotton. From the nutritional point of view canola oil is the best for human consumption because it contains not more than 6 % of saturated fatty acids and about 96 % of its content is non-saturated fatty acids; accordingly it contains very low level of low denisty lipids (Cholesterol). Recently, canola was introduced to Egypt as a new non-traditional oil crop. Unfortunately, it is impossible to expand canola cultivated area in old Delta because it competes with clover and wheat. Therefore, the only realistic startegy to increase canola cultivated area is to grow

canaola plants in new reclaimed lands (New Vally), where salinity and drought are major concerns affecting crop growth and productivity.

In addition to conventional breeding methods, biotechnological approaches for developing salt-tolerant cell lines have been reported for several crops. Several methods of selection for enhanced salt- tolerant genotypes have been developed (Tal and Dehan, 1977). In this context, Dix and Street (1975); McCoy (1987); Hassan and Wilkins (1988); Kumar and Sharma (1989) and Olmos *et al.* (1995) described *in vitro* selection as an alternative method for improvement of salt tolerance. This trait is associated with biochemical and physiological adjustments. It is often assumed that the altered phenotypes of cells with enhanced ability to survive and grow in the presence of high levels of NaCl is associated with biochemical and physiological adjustments which involve alteration in gene expression (Singh *et al.*, 1985). Several research groups have reported variation in SDS-PAGE protein patterns of plants and cell cultures in response to salinity. Those variations include the novel expression; over-expression and repression of some proteins (Ericson and Alfinito, 1984; King *et al.*, 1986; Ramagopal, 1987; Xu and Yu, 1990 and Lusardi *et al.* ,1991).

The synthesis of stress induced proteins, accumulation of non-toxic osmolytes (such as proline) and biochemical and physiological make up of certain cell lines provide an evolutio-nary value to the cell survival under adverse conditions. The accumulation of proline in response to water and salt stress is investigated by many workers, among them, Stewart and Lartha (1980); Pandy and Ganapathy (1985); Chandler and Thorpe (1987) and El Bahr *et al.* (1993).

Successful *in vitro* selection of canola cell lines and clones with enhanced tolerance to salinity and drought was reported (Gangopadhyay *et al.*, 1997). The selected cell lines showed superior growth under salinity and drought stress conditions. The biochemical analysis indicated that a new isomer for acid phosphatase was detected in salt tolerant cell lines only. Survey of available literatures dealing with canola biotechnology also indicated that plant cell cultures were used successfully to develop new hybrid seeds in the genus *Brassica* (Bhalla and De Weed, 1999) and production of transgenic canola plant (Babic *et al.*, 1999, Fukuoka *et al.*, 1998 and Khan *et al.*, 2002 and El Mergawy, 2007).

This report described an effective and rapid method for *in vitro* selection of salt-tolerant canola plantlets. In order to characterize the selected line, variations in SDS- PAGE protein patterns, proline accumulation, RAPD banding patterens and total protein content of the selected and non-selected cell lines were compared.

MATERIALS AND METHODS

Selection of salt tolerant cell line

Seeds of canola (*Brassica napus* L. cv. Serw 4) were surface-sterilized and germinated on basal MS medium containing increasing levels of NaCl (0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 %). Seedlings germinated and survived on 0.2% NaCl were used as a source of explants. Cotyledonary leaf and hypocotyl explants were cultured on MS medium (Murashige and Skoog, 1962) containing B5 vitamins (Gamborg *et al.*, 1968); 1.5 mg/l 2,4-D and 0.2% NaCl. After two weeks of cultivation under dark conditions, explants with microcalli colonies were transferred to the same medium but with 0.3% NaCl. Within 4 weeks, explants proliferated callus tissue. The actively growing callus was selected and subcultured twice on regeneration MS medium (4 mg/l BA) supplemented with 0.3% NaCl. This type of

callus and plantlets was designated as a selected line, while the callus and plantlets proliferated on salt-free medium and derived from explants excised from seedlings germinated on salt-free medium were designated as a non-selected line. Both lines were subjected to salt-free and salt containing media (0.3-1.2% NaCl). Growth measure-ements, biochemical and molecular comparisons were carried out after six weeks of cultivation on media.

Protein extraction and assay

One gram fresh tissue was homogenized in 1 ml of sodium phosphate buffer (pH 6.8); centrifuged for 10 min at 10000 rpm and the supernatant was transferred to a new tube for assay. Protein concentration was determined according to the method of Bradford (1976).

Protein electrophoresis

For SDS-PAGE, proteins extracted in Na-Phosphate were used and electrophoresis was performed according to Laemmli (1970) using 10 % acrylamide in the separating gel and 3 % in the stacking gel. Protein samples (20-30 μg) in a total volume of 16 μl (8 μl of sample in extraction buffer + 8 μl 2x denaturing buffer) were denatured for 3 min in boiling water bath; cooled; centrifuged and a sample of 15 μl was applied. Electrophoretic separation was carried out using EC mini gel unit at 60 volt for four hr. After electrop-horesis, gel was stained with Coomassie brilliant blue (R-250) and destained with high methanol solution. Molecular weights of polypeptide bands were calculated from a calibration curve of low molecular weight marker kit of Pharmacia.

Proline determination

Free proline was measured colourim-etrically in fresh callus tissue according to Bates *et al.* (1973).

DNA extraction and RAPD analysis

DNA isolation was performed using the CTAB method. RAPD was performed as described by Williams *et al.* (1990) with minor modifications. Briefly, PCR amplification was performed in 20µl reaction mix containing 20 ng genomic DNA, 0.5 unit Taq polymerase, 200 µM each of dATP, dCTP, dGTP, dTTP, 10 p mole random primer (Operon) and appropriate amplification buffer. The mixture was assembled on ice, overlaid with a drop of mineral oil. Amplification was performed for 45 cycles using Biometera Uno thermal cycler, as follows: One cycle at 92 °C for 3 min and then 45 cycles at 92 °C for 30 sec, 35 °C for 60 sec and 72 °C for 2 min (for denaturation, annealing and extension, respectively). Reaction was finally incubated at 72 °C for 10 min and further 10 min at 62 °C. Eight decamer of oligonucleotide random primers (OPA1, OPA2, OPA3, OPB1, OPB2, OPB3, OPZ1 and OPBG1) were used for RAPD analysis based on their ability to amplify canola genome and producing reproducible amplification patterns.

Agarose electrophoresis

The amplification products were analyzed by electrophoresis in 1.5% agarose in TAE buffer, stained with 0.2 µg/ml ethidium bromide and photographed under UV light. Two markers were used, i.e. 1Kbp DNA ladder (Gibco) and 100 bp DNA ladder (BIRON).

RESULTS

Regeneration

The regeneration process involved the culturing of hypocotyl explants of one-week-old seedlings onto MS medium supplemented with 1.5 mg/l 2, 4-D or 4 mg/l BA for two weeks under dark conditions and then transferred to MS medium supplemented with 4.0 mg/l BA, for indirect and direct shoot

organogenesis, respectively. Within three weeks, shoot primordia were proliferated through indirect shoot organogenesis (after a phase of callus formation). Direct shoot organogenesis from hypocotyl explants took place onto 4 mg/l BA after five weeks of cultivation. Transferring the proliferated shoots (direct or indirect) to basal MS medium, led to shoot elongation and rooting. The obtained plantlets could be easily acclimatized for *ex vitro* conditions. Fig. (1) shows the different steps of direct and indirect shoot organogenesis from hypocotyl explants of Egyptian canola cultivar (Serw 4).

Selection program and responses of the two lines to NaCl salinity

The selection program detailed in materials and methods section is easy to perform and allows selection at different stages, i.e. selection during seed germination on saline media; selection during callus proliferation and selection during subsequent subculturing and regeneration on media containing increasing levels of NaCl. Comparative studies on the behavior of the two types of calli (selected and non-selected) were conducted (Table 1). The obtained data indicate clearly that the capability of both types of calli (selected and non-selected) to proliferate shoots under selection pressure is decreased, as a result of salt stress. However, the ability of the selected callus to proliferate shoot onto 0.3% NaCl is 5% and zero for the non-selected line at the same level of salt stress (Table 1). The two types of calli were further subjected to increasing levels of NaCl (0.0-1.2 %). Data of callus growth, expressed as fresh and dry weight yield and tolerance ratio, are presented in Table (2). The selected line was evidently capable of tolerating the addition of NaCl to the culture medium at 3.0, 0.6 and 0.9 %. Both fresh weight and dry weight yield of the selected cell line and the highest yield of fresh weight (2.8 g) and dry weight (200 mg) were achieved at 0.6% NaCl. For the non-selected callus, the highest yield of fresh and dry weights (2.5g and 186 mg, respectively) was observed on salt-free medium. It was noted that the tolerance ratio of the two lines behaved in a similar fashion to fresh weight yield (Table 2). Fig. (2) shows the growth and differentiation of selected and non selected lines on salt containing media.

Proline accumulation

Proline content of the two lines cultured for six weeks on increasing levels of NaCl presented in Table (2) reveals clearly that the increment in proline content is parallel with the increment of salt concentrations in the culture media. However, the non-selected line accumulated less proline, compared with the selected line. Moreover, the selected cell line maintained high levels of proline (4.5 μ mole/ gFW) when cultured on salt-free medium, compared with 3.5 μ mole / gFW) for non-selected line (Table 2).

Protein content

The effect of salt stress on total protein content of the two lines is presented in Table (2). A gradual increase in protein content is observed in response to increment in NaCl concentration in the culture media. The selected cell line maintained higher protein content at all levels of NaCl tested, even on salt-free medium, compared with the non-selected line. The two lines contained maximum protein levels (7.1 and 5.1 mg protein / gram fresh callus), for selected and non-selected callus, respectively, on 1.2 % NaCl (Table 2).

Table (1): Callus survival and shoot frequency of selected and non-selected hypocotyl derived callus on increasing levels of NaCl.

NaCl (%)	Non-selected		selected	
	*Callus survival frequency	**Shoot frequency	*Explant survived	**Shoot frequency
0.0	100	40	100	35
0.1	95	20	100	40
0.2	60	1	100	20
0.3	10	0	50	5
0.4	5	0	15	2

* Callus survival = (No. of survived explants / Total No. of explants) X 100

**Shoot frequency = (No. of explants proliferate shoots / Total No. of explants) X 100

Table (2): Response of selected and non-selected lines to NaCl salinity.

NaCl (%)	Non-selected					Selected				
	FW (g±SE)	DW (mg)	Tolerance ratio*	Proline content**	Protein content***	FW (g±SE)	DW (mg)	Tolerance ratio*	Proline content**	Protein content***
0.0	2.5±0.70	186	100	3.5	3.3	2.0±0.20	149	100	4.5	4.5
0.3	2.46±0.24	185	98	4.0	3.7	2.4±0.09	190	120	9.0	5.5
0.6	1.4±0.11	100	56	6.0	4.5	2.8±0.10	200	140	12.0	6.0
0.9	0.5±0.70	30	20	5.8	4.6	2.6±0.15	195	130	13.0	7.0
1.2	0.4±0.26	25	16	5.0	5.1	1.8±0.40	100	90	11.0	7.1

FW = Fresh weight DW = Dry weight

*Tolerance ratio = [FW on saline medium / FW on salt free medium (control)] x 100

** μmole proline/gFW of callus

*** mg protein / gFW of callus

SDS-PAGE protein patterns

SDS-PAGE protein patterns of the two lines are presented in Fig. (3). These results showed clearly that, when cultures of the two lines were transferred to media containing NaCl, a polypeptide band with a molecular weight of 38 KD was over-expressed in the selected line, even on salt free-medium (Fig. 3). The most significant differences however, were found in the novel-expression of 94 KD polypeptide band in the case of the selected line. The levels of the novel-expressed and the over-expressed proteins remained constant at salt free and salt containing medium.

RAPD analysis

The genetic variability among the selected and non selected lines on salt containing and salt free media was analyzed using RAPD molecular marker technique. Of the total 8 random decamer primers (OPA1, OPA2, OPA3, OPB1, OPB2, OPB3, OPZ1 and OPBG1), 2 primers gave polymorphic bands in RAPD analysis. A total of 56 RAPD bands were obtained. On average each primer produced 7 bands. The amplification products ranged from 100 bp to 1000 bp. The primer OPB1 (Fig. 4) produced maximum (7) bands, out of which two were polymorphic. The primer OPG2 produced two polymorphic bands from a total of four bands. The rest of the primers gave amplification products ranged from 7 to 8 without any polymorphism (Fig. 4). Intense non-parental bands were obtained among the selected salt tolerant lines (500 and 600 bp) following PCR amplification using the primer OPB1. Similarly, the RAPD profile, generated by PCR amplification using the primer OPG2 revealed genetic polymorphism among the selected salt tolerant and control non-selected lines (Fig. 4).

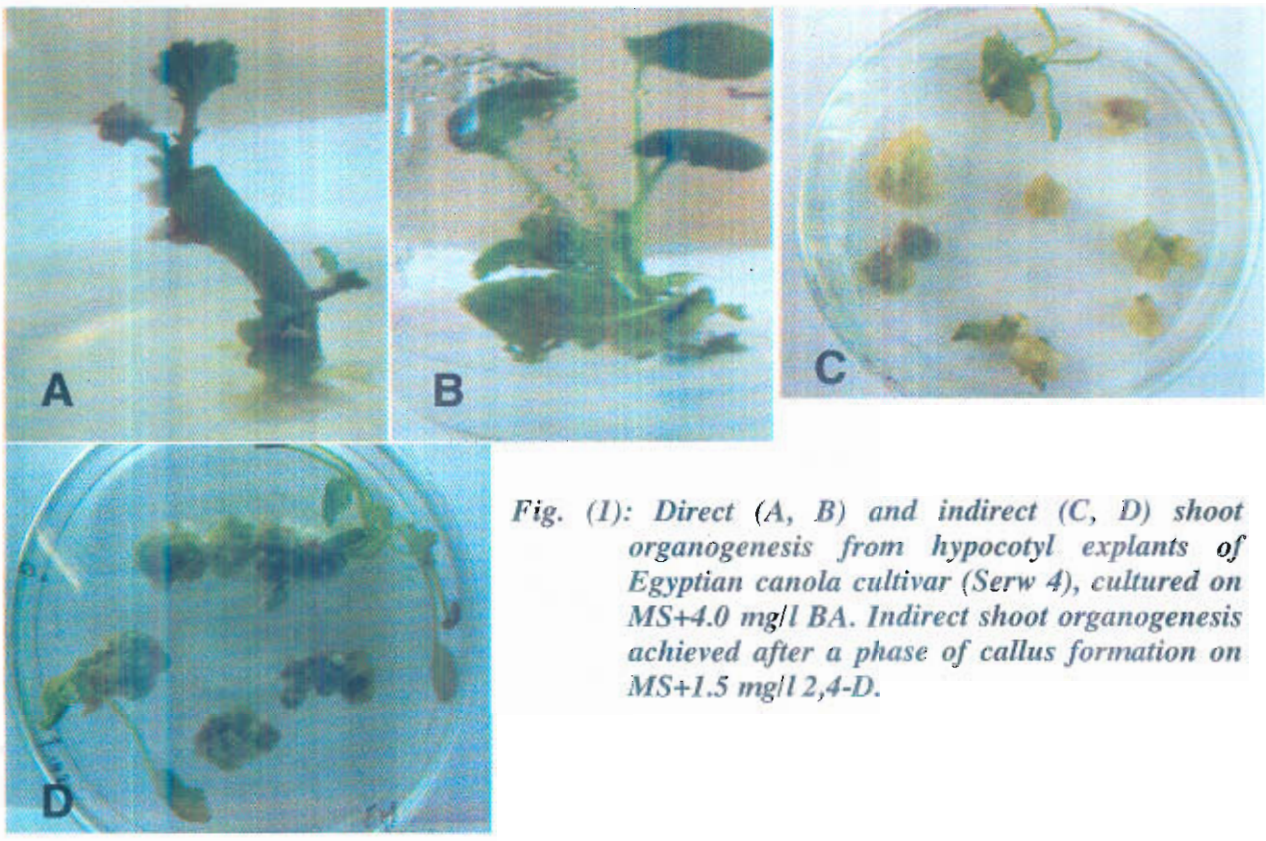

Fig. (1): Direct (A, B) and indirect (C, D) shoot organogenesis from hypocotyl explants of Egyptian canola cultivar (Serw 4), cultured on MS+4.0 mg/l BA. Indirect shoot organogenesis achieved after a phase of callus formation on MS+1.5 mg/l 2,4-D.

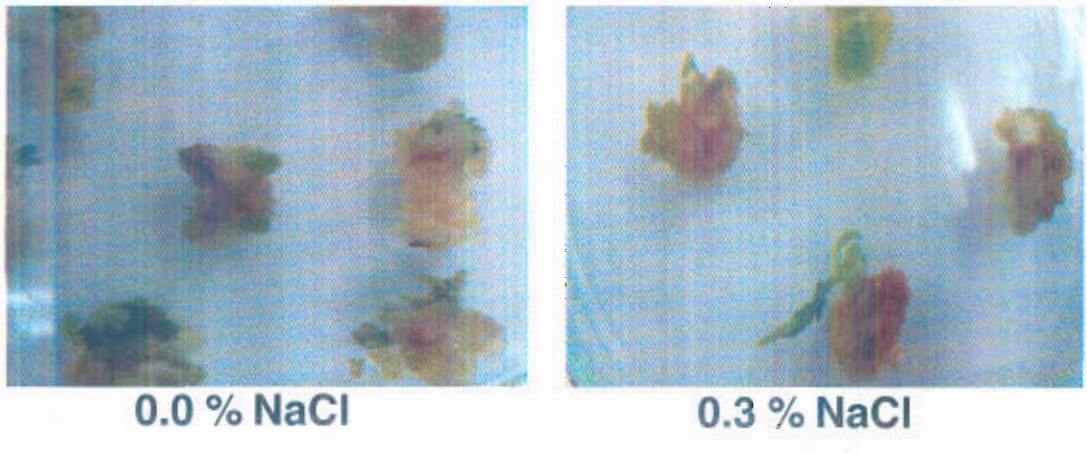

Fig. (2): Growth and differentiation of the selected line on salt free and salt containing media.

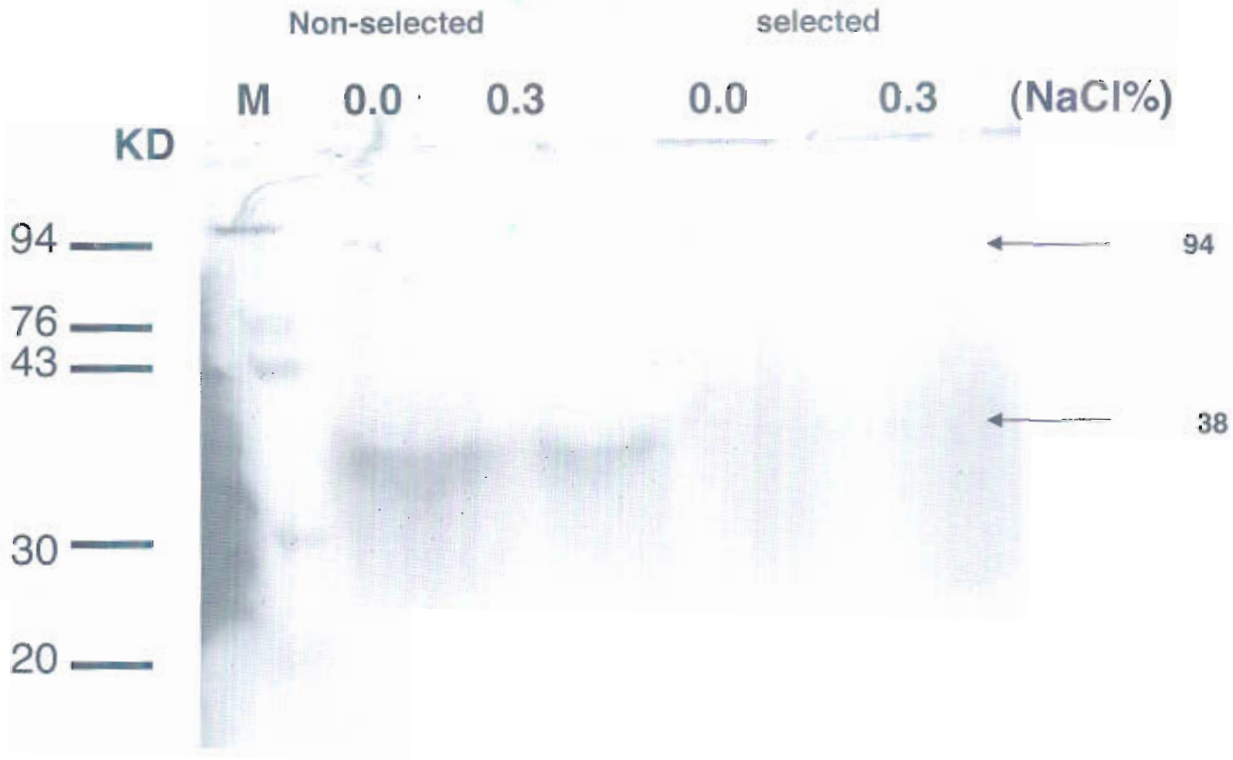

Fig. (3): SDS-PAGE patterns of proteins extracted from selected and non-selected canola shoots grown on salt-free and salt containing media. M is low molecular weight marker (LMW) of Pharmacia. Arrows point to polypeptide bands overexpressed or newly expressed as a result of salt stress.

DISCUSSION

Tissue culture techniques have been applied for the selection of cell lines of some crops with enhanced ability to tolerate high salt concentration. The selected cell line obtained through the selection program described herein, maintained high growth rate on all levels of NaCl tested. These results confirm the suggestions of Stavarek and Rains (1984), that salt tolerance performed at cellular level allows the isolation and establishment of salt tolerant derivatives capable of growth on high levels of salinity. Most of previously published reports indicated that the selected cell lines maintained higher rate of growth on saline media e.g. Hasegawa *et al.* (1980) for tobacco; Hassan and Wilkins (1988) in tomato and Olmos *et al.* (1995) in pea. In a few cases as in alfalfa, the NaCl selected cell lines grew optimally when salt was added to the medium (Croughan *et al.*, 1978).

Data of the present study revealed that the selected cell line accumulates more proline than the non-selected cell line. It has been assumed that the evaluation of proline levels in salt stressed plants may be related to their ability to tolerate extreme salinity (Stewart and Lartha, 1980). In this connection, Pandy and Ganapathy (1985) confirmed earlier hypothesis suggested that, proline may be a cytosolute which plays a role in osmoregulation (Stewart and Lee, 1974) and / or act as a protective agent for cytoplasmic and cellular

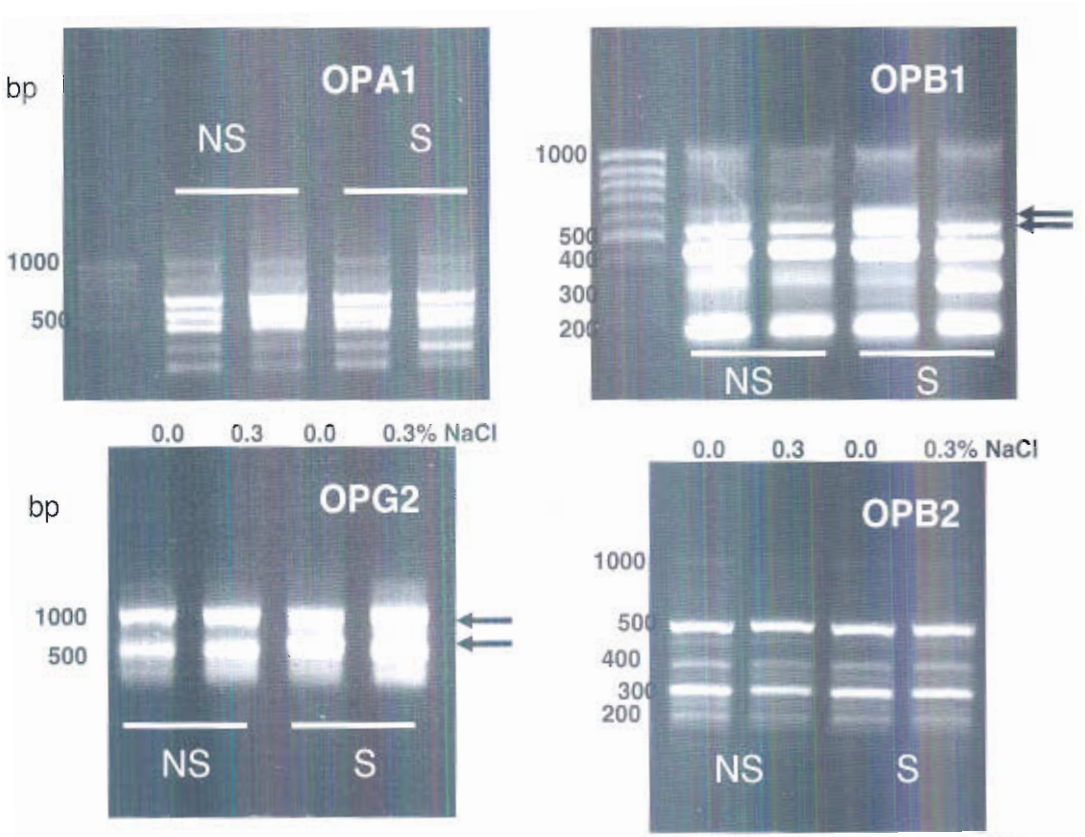

Fig. (4): Salient RAPD banding patterns of DNA extracted from selected (S) and non-selected (NS) canola shoots grown on salt-free and salt containing media. M is 50 bp DNA ladder. Arrows point to polymorphic bands.

structure. Accumulation of proline in salt stressed cells was also reported by Rains *et al.* (1986); Jam *et al.* (1987) and Chandler and Thorpe (1987). In the present study, a protective value for proline may be accepted and evidences can be taken from its rapid accumulation by salt-tolerant cell line, compared with the non-selected one.

The most striking observation in the present study is the increase of total protein content in selected and non-selected lines in response to salt stress. It is well-documented that protein synthesis in plants growing under saline conditions is adversely affected. The marked increase in protein content detected herein may be due to the synthesis of a new set of proteins (osmoprotectant proteins) or inactivation of proteolytic (hydrolytic) enzymes as proposed by Dubey (1994). Similar observations were reported by Johansen *et al.* (1991) on pea. Also, this increment in protein content may be attributed to the high levels of pre-existing proteins in the initial explant.

SDS-PAGE profile of selected and non-selected cell lines growing on various levels of NaCl revealed that salt adapted cells accumulate high levels of 38 KD protein, in addition to the novel-expression of 94 KD protein. The results outlined above are in agreement with Ericson and Alfinto (1984) and Lusardi *et al.* (1991) in elucidating the expression of new proteins in response to salt stress. Similar results were observed by

Harrington and Alm (1988). They detected polypeptide bands of molecular weights ranging from 15 to 115 KD in response to salt stress and heat shock of cultured tobacco cells. Expression of high molecular weight proteins (46-80 KD) in response to salt stress was detected in mung bean by Gulati and Jaiwal (1994) . Although salt induced proteins lend a protective role to salt tolerant cell lines against the deleterious effects of salt stress, the exact physiological functions of these proteins are not fully understood. It has been assumed that salt induced proteins act as osmoprotectants or regulatory proteins (Key *et al.*, 1982 and Sachs and Ho, 1986).

Detection of variants at the DNA level is of immense importance in order to utilize *in vitro* selected lines in crop improvement. The variants may have genetic or epigenetic basis therefore, early detection of these is of prime importance. It is rather difficult to detect the genetic variation from morphological features. Among the different molecular techniques, RAPD is widely used to study the variation at the DNA level among the variants (Rout *et al.*, 1998; Soniya *et al.*, 2001 and Bennici *et al.*, 2003). The detection of some polymorphic bands in the selected line, only reported in this study, are in conformity with the study by Saif *et al.* (2001) for the detection of genetic variation using RAPD technique among the irradiated and salt stressed (200mM NaCl) calli, In the present study, RAPD analysis of genetic variation among the *in vitro* selected salinity tolerant genotypes suggests, that the variation can be detected at the stage of regeneration even before hardening in the green house. It is therefore clear that *in vitro* selection conditions have induced varied amounts of genetic changes among the selected salt tolerant lines. The reported variation at the DNA level can be attributed to somaclonal variations. Cellular and molecular mechanisms behind the variations are mitotic irregularities leading to chromosomal instability, occurrence of gene amplification or deletion, gene inactivation or reactivation of silent genes, transposition and somatic crossing over, DNA methylation in case of epigenetic variation and point mutations (Larkin and Scowcroft, 1981; Muller *et al.* 1990).

Finally, it could be concluded that the selected canola lines obtained herein are characterized by: (1) improved growth on saline media; (2) rapid accumulation of proline; (3) high protein content, (4) novel-expression of high molecular weight (94KD) proteins, in addition to the over-expression of 38 KD proteins and detection of non-parental polymorphic DNA bands in the selected line. These criteria may be a part of physiological, biochemical and genetic make up performed by this cell line to tolerate extreme salt stress.

REFERENCES

Babic,V.; Datla,R.;Scolles,G. and Keller, W.(1999). Development of an efficient *Agrobacterium*- mediated transformation system for *Brassica carinata*. Plant Cell Reports,17:183-188.

Bates, L.S.; Waldren, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water stress studies. Plant and Soil 39: 205-207.

Bennici, A., Anzidei, M. and Vendramin, G.G. (2003). Genetic stability and uniformity of *Foeniculum vulgare* Mill. regenerated plants through organogenesis and somatic embryo-genesis. Plant Sci. 33 1-7.

Bhalla, P. and De Weed, N.(1999) . *In vitro* propagation of cauliflower,*Brassica oleracea* var. botrytis for hybrid seed production. Plant Cell,Tissue and Organ Culture, 56:89-95.

Bradford, M.M. (1976). A rapid and sensitive method for the qualification of microgram quantities of protein utilizing the principle of protein dye binding. Annals of Biochem, 72:248-254.

Chandler, F.S. and Thorpe, A.T. (1987). Proline accumulation and sodium sulfate tolerance in callus cultures of *Brassica napus* L. CV. Westar. Plant Cell Reports 6: 176-179.

Croughan, T.P.; Stavarek, S.J. and Rains, D.W. (1978). Selection of a NaCl tolerant line of cultured alfalfa cells. Crop Sci.,18:959.

Dix, P.J. and Street, H.P. (1975). Sodium chloride resistant cultured cell lines from *Nicotiana sylvestris* and *Capsicum annum.* Plant Sci., 5:321-137.

Dubey, R. (1994). Protein synthesis by plants under stressful conditions. In "Handbook of Plant and Crop Stress", Mohommad, P. (ed), Marcel, Dekker, New York pp: 277-299.

El-Bahr, K.M.; Ghanem, A.S. Abo-Shady, A.; Hamoud, A.M. and Saker, M. M. (1993). Salt tolerance in tomato tissue cultures. I: Selection of NaCl tolerant line of cultured cells. Egypt. J. Hort. 20: 323-336.

EI-Mergawy, R. Gamal. (2007). Molecular genetic analysis of salt stress in canola plants. M.Sc. Thesis, Fac. Agriculture, Cairo Univ., Egypt.

Ericson, M.C. and Alfinito, S.H. (1984). Proteins produced during salt stress in tobacco cell cultures. Plant Physiol., 74:506-509.

FAO (2004). Imports and exports of selected agriculture commodities, Group III(Table c.18) (http:\www.fao.org\ag).

Fukuoka, H.; Ogawa,T. and Matsuoka,M.(1998). Direct gene delivery into isolated microspores of rapeseed (*Brassica napus* L.) and the production of fertile transgenic plants.Plant Cell Reports,17:323-328.

Gamborg, O.L.; Miller, R.A. and Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Research, 50: 148-151.

Gangopadhyay,G.; Basu,S. and Gupta,S. (1997). *In vitro* selection and physiological characterization of NaCl- and mannitol-adapted callus lines in *Brassica juncea.* Plant Cell,Tissue and Organ Culture, 50:161-169.

Gulati, A. and Jaiwal, P.K. (1994). Salt induced polypeptides in two lines of *Vigna radita* L. wilczek which differ in salt resistance. Nat. Academy Science Letters-India 16: 287-292.

Hasegawa, P.M.; Bressan, A. and Handa, A.K. (1980). Growth characteristics of NaCl selected and non-selected cells of *Nicotiana tabaccum* L. Plant cell Physiol., 21: 1347-1350.

Hassan, S.N. and Wilkins, A.D. (1988). *In vitro* selection for salt tolerant lines in *Lycopersicon peruvianum.* Plant Cell Reports 7: 463-465.

Jam, K. R.; Dhawan, R.S.; Sharma, D.R. and Chowdyury, J.B. (1987). salt tolerance and proline accumulation: a comparative study in salt tolerant and wild type cultured cells of eggplant. Plant Cell Reports 6: 3 82-284.

Johansen, C.; Saxena, N.; Chaahan, Y.; Subbarao, G.; Pundix, R.; Kuma, J. and Jana, M.(1991). Genotypic variations in salinity response of chikpea and pigeonpea. Proc. Mt. Cong. Plant Physiol., Singha, 5K.; Sane; P.V.; Bahargava, S.C. and Agrawel, P.K. (eds), New Delhi, India pp: 977-983.

Key, J.L.; Cylin, K.; Geglarz, E. and Scholfft, I. (1882). The heat shock response in plants. In "Heat Shock from Bacteria to Man", Schlesinger, Mi.; Mashburner, A.; Tissieres, A. (eds), Cold Spring Harbor, New York pp: 329- 336.

Khan, M.; Rashid, H. and Quraishi, A. (2002). High frequency shoot regeneration

from hypocotyls of canola (*Brassica napus* L.) cv.Dunkled. Plant Tissue Culture 12(2):131-138.

King, G.L.; Hussay, C.R. and Turner, V.A. (1986). A protein induced by NaCI in suspension cultures of *Nicotiana tabaccum* accumulates in whole Plant roots. Plant Mol. Biol., 7:441-449.

Kumar , V. and Sharma, R. (1989). Isolation and characterization of sodium chloride resistant callus cultures of *Vigna radiata* L. I. Exp. Bot., 40:143-147.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head bacteriophage T4. Nature 224: 680-685.

Larkin, P.J. and Scowcroft, W.R. (1981). Somaclonal variation – a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet., 60: 197-214.

Lusardi, MC.; Locatell. F.; Stadler,J. and Lupotito. E. (1991). *In vitro* characterization of salt selected maize genotype S. J. Genet. and Breed., 45: 285-292.

McCoy, T. J. (1987). Tissue culture evaluation of NaCI tolerance *versus* whole plant response. Plant Cell Reports 6: 31-34.

Muller, E., Brown, P.T.H., Hartke, S. and Lorz, H. (1990). DNA variation in tissue-culture-derived rice plants. Theor. Appl. Genet., 80: 673-679.

Murashige, T. and Skoog. F. (1962). A revised medium for rapid and bioassays with tobacco tissue cultures. Physiol. Plant, 15: 473-497.

Olmos, E.; Piquera, A. and Hellin, E. (1995). *In vitro* selection of salt tolerant cell line from pea calli cultures. In "Current Issue in Plant Molecular and Cellular Biology", Terzi, M.; Cella, R. and Falavigna ,A. (eds), K Academic Publishers pp: 359-368.

Pandy, R. and Ganapathy,S. (1985). The proline enigma: NaCI tolerant and NaCl sensitive callus line of *Cicer arietinum*. Plant Sci., 40: 13-17.

Rains, D.W.; Croughan, S.S. and Croughan, T. P. (1986). Isolation and characterization of mutant cell line and plant salt tolerance. In "Cell Cultures and Somatic Cell Genetics of Plants", Vasil, K.l. (ed), Academic press, New York pp: 537-547.

Ramagopal, S. (1987). Salinity stress induced tissue specific proteins in barley seedlings. Plant Physiol., 84: 324-331.

Rout, G.R., Das, P. and Raina, S.N. (1998). Determination of genetic stability of micropropagated plants of ginger using random amplified polymorphic DNA (RAPD) markers. Bot. Bull. Acad. Sin., 39: 23-27.

Sachs, MM. and Ho, D.T. (1986). Alteration of gene expression during environmental stresses in plants. Ann. Rev. Plant Physiol., 37: 363.

Saif-Ur-Rashid, M., Asad, S., Zafar, Y. and Washeed, R.A. (2001). Use of radiation and *in vitro* techniques for development of salt tolerant mutants in sugarcane and potato. IAEA-TECDOC, 1227: 51-60.

Singh, K.N.; Handa, K.A.; Hasegawa, M.P. and Brissan, A.R. (1985). Proteins associated with adaptation of cultured tobacco cells. Plant Physiol., 79:126-137.

Soniya, E.V., Banerjee, N.S. and Das, M.R. (2001). Genetic analysis of somaclonal variation among callus derived plants of tomato. Current Science, 80 (9): 1213-1215.

Stavarek, S.J. and Rains, D.W.(1984). Cell Culture Techniques: Selection and Physiological Studies of Salt tolerance. In "Salinity Tolerance in Plants, Strategies for Crop Improvement", Stajpls, C.R. and Toenniessen, H.G. (eds), Wiley, New york pp: 321-334.

Stewart, R. G. and Lartha, F. (1980). Accumulation of amino acids and related compounds in relation to environmental

stresses. In "The Biochemistry of Plants, Vol.5", Miflin, J. (ed.) Academic Press, New York p. 609.

Stewart, R.G. and Lee, J.A. (1974). The role of proline accumulation in halophytes. Planta 120: 279-289.

Tal, M.; H. and Dehan, K.(1977). Salt tolerance in the wild relatives of the cultivated tomato: Response of callus tissue of *Lycopersicon esculentum*. Z. Pflanzen Physiol. 86(2): 231-240.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Raflaski, J.A. and Tingey, S.V. (1990). DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.

Xu, Y.L. and Yu, S.W. (1990). Changes in protein properties and protein patterns in salt adapted alfafa callus. Phytophysiologia Sinico 18: 246-252.

الملخص العربي

الانتخاب في الأنبوبة والتوصيف الجزيئي لنبيتات الكانولا المتحملة للملوحة

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تعتبر الملوحة من أهم مسببات نقص الإنتاج النباتي وعليه فإن تطوير سلالات أكثر تحملاً للملوحة يعتبر من أهم أهداف مربي لنبات. تم في هذه الدراسة تطوير نظام للانتخاب المعملي لنبيتات الكانولا المتحملة للملوحة، ويعتمد هذا النظام على توظيف التباينات الجسمية والقدرة العالية للاستيلاد من السويقة الجنينية السفلى لصنف الكانولا المصري سرو ٤ عن طريق إعادة التكشف المباشر وغير المباشر للسيقان. يعتمد نظام الاستيلاد الذي تم التوصل إليه على تكوين الكالاس من السويقة الجنينية السفلى على بيئة موارشيج وسكوج مضافاً إليها ١,٥ ملجم/ لتر 2,4-D ثم تكشف السيقان من مستعمرات الكالاس الدقيقة على بيئة MS مضافاً عليها ٤ ملجم/ لتر بنزيل أدينين. تراوح معدل تكشف السيقان من ٣٥ إلى ٤٠% . استخدم هذا النظام في الانتخاب في الأنبوبة لنبيتات متحملة للملوحة ناشئة من السويقات الجنينية السفلى لبادرات نامية على بيئة محتوية على الملح. تحت ضغوط الملح، لوحظ انخفاض معدل التكشف للسيقان إلى ٥% بالنسبة للسلالات الخلوية المنتخبة وإلى صفر% للسلالات غير المنتخبة على بيئة تحتوي ٠,٣% كلوريد صوديوم. تم التوصيف البيوكيميائي والجزيئي لكلا من السلالات الخلوية المنتخبة وغير المنتخبة باستخدام تراكم البرولين ومحتوى البروتين الكلي والتفريد الكهربي للبروتينات SDS-PAGE ولل DNA (RAPD) . اتصفت النبيتات المتحملة للملوحة بنمو أفضل على البيئات المحتوية على الملح وبتجميع أسرع للبرولين وبمحتوى أعلى من البروتينات الكلية وبظهور مجموعة جديدة من البروتينات ذات الوزن الجزيئي العالي (٣٨، ٩٤ كيلو دالتون) وبأنظمة حزم RAPD فريدة. ويمكن اعتبار هذه المعايير للسلالات المتحملة للملوحة كجزء من ميكانيكية بيوكيميائية متكاملة لتحمل تركيزات عالية من الملوحة في الكانولا.