Journal of Food Technology 1 (3): 139-141, 2003

Studies on Shoot Regeneration of *Brassica juncea* Var. Poorbiraya Under Salt Stress Conditions

Athar Hussain, Mubashrah Munir and Sumaira Abbas
Department of Botany ,Government College University Lahore, Pakistan

Abstrct: Different salts NaCl, $CaCl_2.2H_2O$, $MgSO_4.7H_2O$ and their combination, NaCl + $CaCl_2$ + $MgSO_4.7H_2O$ were employed to establish different equimolar strengths i.e. 50mM, 100mM, 150mM and 200mM to study their effect on *Brassica Juncea* shoot regeneration from leaf explant. Best shoot regeneration in percentage (80%), number (8), length (10mm) was recorded in 50mM of NaCl + $MgSO_4.7H_2O$. + $CaCl_2$. $2H_2O$. $CaCl_2$ proved to be more deleterious in comparable concentration as that of NaCl and $MgSO_4.7H_2O$. No age and position factor was observed for shoot regeneration however it was comparatively more towards lower halves of leaf lamina.

Key words: Regeneration, *Brassica juncea* and stress conditions

Introduction

Brassica oil seed crop occupy over 11 million hectors of world agricultural land and provides 8% of the major oil crops under varied climatic conditions (Downey, 1983). Rapidly cycling nature of *Brassica* species have shaped them as a model plants for research in genetics, molecular biology and physiology (William and Hills, 1986).

Salinity and drought are the two major environmental stresses that are world wide in distribution and limiting the growth and productivity of plants community (except halophytes) including *Brassica* (Kavi kishore *et al.*, 1995; Rudulier *et al.*, 1984; Skirver and Mundy, 1990). The rich agricultural lands of Pakistan have no exception in facing the great danger of salinity. Therefore it is desirable to obtain high frequency of plant generation, which can grow under these stress conditions. *Brassica* can grow under saline conditions, which includes many important oil yielding vegetables and forage crop (Gangopadhyay *et al.*, 1997).

A great number of factors are reported to be involved in plant regeneration of *Brassica* spp. from cotyledons (George and Rao, 1980) peduncle (Stringham, 1977) and leaf disc explants (Eapen and George, 1996; Naqvi *et al.*, 1995).

Regeneration system characterized by a high frequency of shoot formation would be useful in allowing the study of biochemical events associated with shoot induction and wide spread application of gene transfer techniques for crop improvement. In spite of being a major oil seed crop of Indo-Pak subcontinent, little work has been done on *Brassica juncea* in reference to osmotic stress (Kumar, 1984).

It has been shown that *Brassica Juncea* controls glucose metabolism and reduces lipid peroxidation as well as level of oxygen radicals, ameliorating the damage caused by oxidative stress in diabetes (Young *et al.*, 2003). Further more *Brassica juncea* have shown varying degree of hypoglycaemic and antiglycemic activity (Grover *et al.*, 2002). These abilities have enhanced studies in *Brassica Juncea*.

Materials and Methods

The seeds collected from NARC (National Agricultural Research Council) Islamabad were aseptically grown in MS medium (Murashige and Skoog, 1962) supplemented with 3.0 mg/l NAA and 1.0 mg/l BAP. Plumules were grown on the same medium for three weeks to get leaf explants. Sugar used was 3 % in all experiments and all the media were solidified with 1.25% agar. Routinely 30 ml of solidified media were dispensed into 250 ml culture flasks plugged with non-absorbent cotton covered in one layer of muslin cloth. All cultures were maintained at 26 ± 1 °C fewer than 3 k lux cool florescent light for fifteen hours photoperiod. Callus was sub-cultured after 5 weeks. All the MS media were supplemented with 50, 100, 150 and 200 mM of NaCl, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$ either separately or equimolar combinations. A method of constant salt concentration instead of stepwise approach was used to get salt adapted calli. The explant was treated in five replicate per treatment. The electroconductivity of saline solutions used is given in Table 1.

Result

Leaf explants, 5 to 6 days old showed curling and callusing at the points touching the media resulting compact and non-watery calli which were less vigorous and healthy than the control one. Browning of the callus was observed with an increase in age and salt concentration of the medium. Regenerated shoots were appeared when callus was 3-5 weeks old.. Regenerated shoots from all the media had rammo-colour (off-white to bright green), however yellowing and whitening of the leaves (older) from the regenerated shoots did

Table 1: The range of Electroconductivities of saline solutions

Medium	50mM	100mM	150mM	200mM
NaCl	5.5×10^4	5.5×10^4	6.855×10^4	6.98×10^4
$CaCl_2.2H_2O$	6.38×10^4	6.81×10^4	7.72×10^4	6.55×10^4
$MgSO_4.7H_2O$	5.93×10^4	6.19×10^4	6.54×10^4	8.06×10^4
$NaCl + CaCl_2$	6.86×10^4	6.95×10^4	8.39×10^4	11.47×10^4

Table 2: Shoot regeneration response under different salt stress condition

	Concentration															
Character studied	NaCl (mM)				$CaCl_2.2H_2O$ (mM)				$MgSO_4.7H_2O$ (mM)				$NaCl + CaCl_2.2H_2O + MgSO_4.7H_2O$ (mM)			
	50	100	150	200	50	100	150	200	50	100	150	200	50	100	150	200
Callusing	+ + +	+ + +	+ +	+ +	+ +	+ +	+	+	+ + +	+ +	+ +	+ +	+ + +	+ + +	+ +	+ +
% of shoot regeneration	80	65	60	40	15	10	5	5	55	45	45	40	80	80	40	40
Mean number of regenerated shoots	4	3	3	2	3	1	1	1	4	3	2	2	8	4	2	1
Mean length of regenerated shoot (mm)	6	4	4	3	8	5	3	3	5	2	2	2	10	5	3	2

+/+ +/+ + + = Slow, moderate and rapid callus growth respectively
3 mg/l NAA and 1 mg/l BAP were supplemented in all these media

appear in the saline media of higher concentration (150-200mM) 200mM equimolar concentration of NaCl + $MgSO_4.7H_2O$ + $CaCl_2.2H_2O$, gave highest percentage of shoot regeneration (80% in two cultures) and mean number of regenerated shoots (8) among all the same concentrations of these salts separately. $CaCl_2$ proved to be more deleterious in comparable concentration as that of NaCl and $MgSO_4.7H_2O$ (Table 2). No age and position factor was observed for shoot regeneration however it was comparatively more towards lower halves of leaf lamina.

Discussion

Decline in the callus growth due to salt concentration (150 - 200 mM) observed in present study is a usual phenomenon in *Brassica juncea.* (Sharma *et al.*, 1991) and in other plants as well (Cushman *et al.*, 1990, Greenway and Munns, 1980; Reddy and Vaidyanath, 1986). This retardation of growth may be due to the fact that certain amount of the total energy available for tissue metabolism is channelled to resist the stress (Cushman *et al.*, 1990). Several reports of plant regeneration from seedling explants of Brassica species have been published during the past decade. However there is a considerable variation on the observation of different groups even when dealing with the same species (Lazzeri and Dunwell, 1986 and Murata and Orton, 1987). Equimolar concentration of NaCl + $CaCl_2$ $2H_2O$ + $MgSO_4.7H_2O$ were applied as a source of salinity. It is believed that responses to this combination may be more representative of a generalized plant response to calamity (He and Cramer, 1992). Maximum shoot regeneration at this combination may be attributed to the stimulatory effect of sulphate in the medium (Eapon and George, 1996) and particularly to some regulatory substance (s) emanating from lamina (Sharma *et al.*, 1991) and greater leaf turgor pressure (Wright *et al.*, 1995) in *Brassica juncea.* This study found salt stress, beneficial for regeneration which is in accordance with previous reports (Yoshida *et al.*, 1983, Reddy and Vaidyanath, 1986) as salt tolerance is considered nature of embryonic cells that tolerate a higher level of salt stress than the other cell types (Binh and Heszky, 1990).

Proline is another factor, which can account for stimulating regeneration upto 80% (Rout *et al.*, 1995) because it has been found significantly due to an increase in salt concentration (Chandler and Thrope 1987, Jains *et al* 1991b, Kumar and Sharma 1989, Watad *et al.*, 1983, Gangopadhyay *et al.*, 1997).

These results indicate beyond the genetic effect as shoot formation give rise to the question of cellular totipotency and of correlation influence. This system for efficient regeneration of *Brassica juncea* var. poorbiraya from leaf explant can be applied to improve cultivar selection for environmental stress at the cell levels.

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