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REGENERATION OF PLANTS IN EMS TREATED LOCAL MUNG BEAN (*VIGNA RADIATE* L. WILCZEK) UNDER SALT STRESS

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ABSTRACT

The regeneration of EMS treated seeds of two local varieties of mung bean was investigated under salt stress conditions. Three explants e.i. cotyledon, leaf and shoot apical meristem of mung bean obtained from *in vitro* grown EMS treated seeds of varieties NM-92 and Khalood were inoculated on MS medium containing various concentrations of 2, 4-D and BAP and IBA. The highest callus proliferation (98.3%) was obtained in EMS treated leaf of NM-92 followed by 96.5% in shoots of NM-92 control on MS medium supplemented with 1.8µM 2, 4-D and 3.56µM BAP. The increase in callus fresh weight was higher in leaf explants of both treated varieties. Similarly 57.3±1.3 callus cultures of treated leaf explants of NM-92 were survived on media containing 50mM NaCl. Similarly the highest regeneration (65.5±2.1%) was observed in NaCl selected callus cultures raised from shoots of EMS treated seeds.

INTRODUCTION

Mung bean [*Vigna radiate* (L) Wilczek] of family 'fabaceae' is a warm season annual food legume. It is traditionally cultivated mainly for its edible seeds to dry areas especially under irrigated conditions throughout tropical, subtropical and temperate zones of Asia including Pakistan, India, Bangladesh, Sri Lanka, Nepal, China, Japan, Korea and Thailand. Mung bean has short life cycle of 60-70 days, cultivation improve the soil condition and increase the income of small landholders (Fernandez and Shanmugasundaram, 1988). In Pakistan, mung bean seeds are split in mill and cooked as dal. It is also used in making bread, curries, noodles, soups and sweets. Mung bean seeds roasted with spices are also commonly used. The seeds contain 22-24% proteins, which are easily digestible.

In Pakistan, mung bean is grown under both rainfed and irrigated agro-ecology zones. It is cultivated on an area of 140,800 hectares with 93,000 tons production in 2011-12 (Economic Survey of Pakistan 2011-12). In Sindh province, mung bean is cultivated on an area of 4,800 hectares and 2300 tons annual production. Mung bean can be cultivated twice in a year due to favorable climatic conditions of Pakistan. The average yield of mung bean in Sindh is about 550kg per hectares, during last twenty years, which is very low as compared to other countries. The main reason of low yield is narrow genetic bases and low yield potential of the land races. Some phytotoxic chemicals also adversely affected cultivation of crop country wide (Aslam et al., 2010, Moustakas et al., 1994). Similarly, poor establishment of mung bean crop limit the production (Kirchhof et al., 2000, Naseem et al., 1997) and high crop production is associated with early vigour (Kumar et al., 1989).

Mutagenesis through physical and chemical mutagens has been proved to create variations in crops. Mutation induction is used to produce desired traits that cannot found in nature or have been

lost during offers the possibility of inducing desired attributes that either cannot be found in plants naturally or have been lost with passage of time. Chemical mutagens like EMS, MMS, and NaN3 etc. affect seed germination, shoot and root lengths. The reduction in germination of mutant seeds is due to delayed biological and physiological processes needed for germination including hormonal imbalance, enzyme activity and inhibition of mitotic process (Ananthaswamy et al., 1971, Sato and Gaul, 1967).

The mutagenesis in crops is based on principles genetic variation and selection. The development of plant tissue culture from last three decades has made it possible to transfer part of the breeding work from field to laboratory conditions. Tissue culture and regeneration of plants open new potentials for improving the crops (Kita et al., 2007, Hildebrand et al., 1991). The somatic embryogenesis resulting into regeneration can produce large number of plants in very short time duration (Wu et al., 2007). The somatic embryogenesis was reported in few species of *Vigna* (Amitha and Reddy, 1998, Girija et al., 2000). The development of shoots from different explants of mung bean and callus has been reported such as from cotyledon (Gulati and Jaiwal, 1990), cotyledonary node (Avenido and Hattori 2001), immature cotyledons (Tivareker and Eapen 2001), petioles of primary leaf (Mahalakshmi *et al*., 2006) and shoot tip (Gulati and Jaiwal 1992). The aim of the present investigation was to study the regeneration via somatic embryogenesis in EMS treated local mung bean, which can be used for genetic improvement.

MATERIALS AND METHODS

The seeds of two local mung bean varieties 'NM-92' and 'Khalood' were obtained from Nuclear Institute of Agriculture, Tandojam, Sindh Pakistan. Seeds were first treated with EMS and then inoculated on media to study the somatic embryogenesis and regeneration.

Treatment of Seeds with Ethyl Methane Sulfonate (EMS): About 300 seeds of each variety NM-92 and Khalood were presoaked for one hour in distilled water under aeration conditions. After pre-soaking, excess water was drained off and seeds were subjected to 0.5% ethyl methane sulfonate (EMS) solution one hour under shaking conditions. For controls, about 100 seeds of each variety were soaked in distilled water for same time duration. After treatment, EMS solution was drained off and treated seeds were washed in distilled water for twenty minutes to remove the residual chemical and then dried on blotting paper.

Sterilization and Inoculation of Seeds: The EMS treated seeds were surface sterilized for 15 minutes in 200ml of 50% (v/v) and then rinsed with sterile dist. water three times (five min each wash) NaOCl, inoculated on simple MS media (Murashige and Skoog, 1962) and placed in growth room at 25 ± 2°C and 16/8 hours L/D photoperiod at 2000 lux light intensity. For callus induction, cotyledons, apical meristem and leaf explants were excised from *in vitro* grown plantlets aseptically after seven days and inoculated on MS medium supplemented with 1.81µM 2,4-D and 3.55µM BAP and incubated in growth room. After two weeks, proliferating calli were subcultured on either same fresh medium as well MS medium supplemented with 1.81µM 2,4-D, 3.55µM BAP and 50mM NaCl. The sub-culturing of proliferation calli was done after every two weeks. After six weeks, all calli were shifted on regeneration media containing MS medium additionally

supplemented with 3.40µM IBA. After rooting, regenerated plantlets were shifted in pots containing garden soil and covered with polythene bags to maintain humidity.

RESULTS AND DISCUSSION

Callusing response: The callusing response was observed on MS media supplemented with 1.81µM 2, 4-D and 3.55µM of BAP. Maximum callusing response was observed from leaf and shoot explants of both verities NM-92 and Khalood on MS media supplemented with 1.81µM 2, 4-D and 3.55µM BAP. The highest callusing response (98.3%) was observed in leaf explants of treated NM-92 followed by 96.5% shoots of control Khalood variety. The lowest callusing response (16%) was observed in cotyledons of Khalood (Table-1). The increase in callus fresh weight was higher in leaf derived callus of varieties. The highest callus fresh weight (++++) was detected in leaf explants of both treated varieties while the slowest fresh weight increase (+) was detected in cotyledon derived calli of control NM-92 and Khalood and treated NM-92 varieties after six weeks of inoculation. Similarly callus proliferation response was detected second week in leaf explants followed by in shoots and cotyledons after third week of inoculation (Table-1).

Table: Percentage of callus formation and increase in fresh weight of callus cultures on MS medium supplemented with 1.81µM 2,4-D and 3.56µM BAP

Line	Explants					
	Cotyledon		Shoot		Leaf	
	% Callusing	Callus FW	% Callusing	Callus FW	% Callusing	Callus FW
NM-92 Treated	22.5	+	81.6	+++	98.3	++++
NM-92 Control	53.4	++	96.5	+++	61.5	+++
Khalood Treated	32.2	+	68.3	++	80.4	++++
Khalood Control	16	+	61.2	++	71.3	+++

The survival rate of callus was evaluated on MS medium supplemented with 1.81µM 2,4-D, 3.55µM BAP salinized with 50mM NaCl. Callus initiated from leaf explant of treated variety NM-92 showed the best survival response as 57.3±1.3% callus cultures were survived followed by 51.2±1.4% in cotyledon derived callus of control NM-92 variety after four weeks of NaCl stress. Similarly callus cultures derived from shoots of NM-92 showed the sensitivity as only 23.5±3.2% calli was survived (Table-2).

Table-2: Percentage survival of calli on MS medium supplemented with 1.81µM 2, 4-D, 3.56µM BAP and 50mM NaCl (Mean±SD)

Line	Percentage Survival of Calli		
	Cotyledon	Shoot	Leaf
NM-92 Treated	30.5±0.5	45±2.1	57.3±1.3
NM-92 Control	51.2±1.4	25.5±3.2	42.4±1.8
Khalood Treated	27.1±1.2	34.5±1.1	32.5±0.8
Khalood Control	25.5±1.5	30.5±0.5	28.4±1.1

For regeneration studies, NaCl selected calli were shifted regeneration medium containing MS salts and vitamins, 3.55µM BAP, 3.40µM IBA and 50mM NaCl. The regeneration response was observed in all treated and control calli of both varieties selected on NaCl containing media. The regeneration of callus cultures obtained from controls was higher as compared to EMS treated seeds. The highest embryogenesis and regeneration (65.5±2.1%) was observed in shoots derived callus cultures of NM-92 followed by 57±1.6% in leaf of NM-92 (Table-3). The process of callus formation, somatic embryogenesis and regeneration from leaf and shoot explants under NaCl stress is shown in figures 1 and 2).

Table-3: Frequency of Plantlets formation in different calli on MS media supplemented with 3.56µM BAP, 3.40µM IBA and 50mM NaCl (Mean±SD)

Line	% Regeneration of Callus		
	Cotyledon	Shoot	Leaf
NM-92 Treated	12.3±1.4	46±2.4	42.5±1.2
NM-92 Control	28.5±0.75	65.5±2.1	57±1.6
Khalood Treated	21.5±1.1	31.4±1.8	28.6±0.6
Khalood Control	24.6±0.5	35±2.1	34.5±1.1

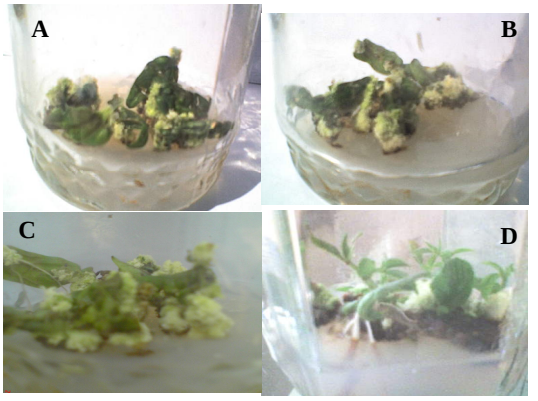

Figure-1: Somatic embryogenesis and regeneration in mung bean through leaf

A & B: Callus induction from leaf explant
C: somatic embryogenesis in leaf callus
D: Callus regeneration under salt stress

Figure-2: Callus regeneration in mung bean from shoots

A & B: Plantlets and roots formation from callus
C: Regenerated plant shifted in pot

The selection genotype during *in vitro* somatic embryogenesis and regeneration is one of the most important factors in success of tissue culture and EMS mutagenesis (Kita et al., 2007, Ko et al., 2004). The somatic embryos formation, establishment of proliferating embryogenic cultures and finally regeneration are three key steps of genotype selection and somatic embryogenesis in plants (Kita et al., 2007). In plants, somatic embryogenesis and regeneration has drawn more attention than other techniques because large number of plants can be produced with in relatively short time duration (Wu et al., 2007). The regeneration of mung bean from different explants was also reported by other researchers. Malik and Saxena, (1992) reported the regeneration in *Phaseolus vulgaris* from cotyledonary nodes and shoot apex on MS medium containing 80µM of BAP. Werner et al., (2003) reported the role of cytokinin in shoot induction. The immature cotyledons of *V. radiata* on BAP and IAA containing medium resulted in green spots and plantlets formation (Tivareker and Eapen, 2001). In our study 2, 4-D was used instead of IAA. These results also confirmed the results of Hossain *et al*., (2007) who reported that calli in different salt treatments induce different growth rates in Chrysanthemum (*C. morifolium*). The medium containing 50mM NaCl showed the highest and uniform growth of callus whereas further increase of salt to 100mM NaCl significantly reduced the callus growth. The reduction in fresh weight was also reduced at higher NaCl concentrations. In the study it was also observed that somatic embryogenesis in callus cultures obtained from EMS treated explants were found higher as compared to untreated (control) cultures but regeneration was higher in control cultures. This may be mutation effect of EMS treatment that reduced the regeneration in somatic embryos. These results also confirmed the study reported by Simmonds and Donaldson, (2000) that the high efficiency embryogenesis usually does not correlate with higher regeneration of plants.

Conclusion: The process of regeneration via somatic embryogenesis is one of the most important steps in tissue culture and chemical mutagenesis. The mutagenic response of EMS in mung bean was observed during embryogenesis and regeneration. Further screening of regenerated plants under field conditions and salt stress may result beneficial attributes.

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