IN VITRO SCREENING OF SALT TOLERANT GENOTYPES FOR FURTHER GENE EXPRESSION ANALYSIS IN TOMATO

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Extended Summary

High salinity is one of the major stress factors among the abiotic stresses. In the world, about 400 million hectares of land are affected by high salinity. In Bangladesh about 1 million hectares of land are affected by high salinity in the coastal regions and it is increasing day by day with the expansion of shrimp culture. Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. As saline soils and saline waters are common around the world, great effort has been devoted to understanding physiological aspects of tolerance to salinity in plants, as a basis for plant breeders to develop salinity-tolerant genotypes. In spite of this great effort, only a small number of cultivars, partially tolerant to salinity, have been developed. Further effort is necessary if the exploitation of saline soils and saline waters that are not currently usable is to be achieved. Salinity affects yield quality and quantity, so that yield characters must be taken into account when breeding for salinity tolerance. But not only yield-related characters are important. As salinity affects almost every aspect of the physiology and biochemistry of the plant, the enhancement of crop salt tolerance will require the combination of several to many physiological traits (Cuartero *et al.*, 2006; Flowers and Yeo, 1995; Cuartero and Ferna´ndez-Mun˜oz, 1999), not simply those directly influencing yield. As salinity in soils is variable and plant tolerance depends on the stage of plant development, plants should be phenotyped at several salinity concentrations and at the most sensitive plant stage(s).Tomato (*Solanum lycopersicum* L.) is one of the most important solanaceous vegetable crops in the world in terms of both production and harvested area (FAOSTAT, 2005). Tomato is a favorable food crop for *in vitro* studies due to its low chromosome no i.e., 2n=2x=24 and due to comprehensive knowledge of tomato genetics. Plant tissue culture techniques are recognized as useful instruments in tomato improvement. Several *in vitro* investigations have been conducted on tomato in different applications i.e., production of virus free plants (Moghaieb *et al.*, 2004), genetic transformation (Park *et al.*, 2003) and studies about the effect of variety and plant growth regulators on callus proliferation and regeneration (Chaudhry *et al.*, 2007). Most of the reports about adventitious regeneration in tomato deal with induction of regeneration in hypocotyls or cotyledon explants (Moghaieb *et al.*, 2004, Brichkova *et al.*, 2002, Raiziuddin *et al.*, 2004). Shoot formation from different explants as apical meristem, cotyledons, stems

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leaves, anthers and inflorescences has been reported in tomato (Afroz *et al.*, 2010; Jatoi *et al.*, 1999, 2001; Young *et al.*, 1987; Branca *et al.*, 1990; Compton & Veilleux 1991). The genetics of physiological characters together with other tolerance components related to metabolic defences against salinity have to be studied in order to advance the breeding of tomato genotypes tolerant to salinity. If progress is made on the identification of genes involved in the process of salt tolerance, the isolation of genes from sources of variation, genetic transformation could be possible which is a powerful tool in the breeding of complex character like high salinity. Gene transfer could also be achieved through the crossing programs. Despite the present limitations, it is foreseeable that our ability to design the future breeding programmes based on genetic transformation will be strengthened with the data obtained from ongoing projects. To identify and isolate different salt inducible genes, it is necessary to screen different salt tolerant genotypes. The present study is conducted to explore the bioassay so as to establish a reproducible protocol for screening of different genotypes of tomato in different concentrations of NaCl. The objective of this study is to optimize the protocol for growing of tomato seedlings under different salt concentrations, to identify the genotypes with suitable performance under salt stress for future breeding program and to screen the genotypes for identification and isolation of salt inducible gene. Under this study the seeds of fourteen genotypes of tomato (*Solanum lycopersicon* L) were collected from Bangladesh Agricultural Research Institute, Joydevpur, Gazipur and from local market. The genotypes used in this study were, BARI-2, BARI-11, BD-7260, BD-7290, BD-7295, BD-7286, BD-7269, BD-7258, BD-7289, BD-7292, BD-7291, BD-7302, BD-7301 and BD-7762. The experiment is being conducted at the Molecular Genetics laboratory of Sher-e-Bangla Agricultural University, Dhaka-1207. Seeds were surface-sterilized in 1% (v/v) "Clorox" bleach (Sodium hypochlorite) for 15 minutes and rinsed three times (5 min each) with autoclaved distilled water (Franklin & Dixon, 1993). The sterilized seeds were placed separately in four sterilized Petri dishes containing filter paper (Whatman No.1). Seeds were inoculated in test tubes containing half strength MS medium (1962). Cultures were kept at 25 ± 1°C under 16 h photoperiod at 50 μmol/ m^2/s^{-1} (supplied by white fluorescent lamp). Germinated seedlings of four days served as experimental materials. The salt tolerance assay was performed as described by Zeba, 2009. Four days old germinated seedlings were transferred in a liner order on MS medium supplemented with 0, 50, 100 and 150 mM NaCl. The culture environment included, 25°C, 60% relative humidity, and a 16-h photoperiod from white fluorescent lamps (200 μmol photons/m^2/s^{-1}). After five days, root assay and fresh weight assay of all plants were performed. Until now up to 150 mM of NaCl is not enough stress for some of the fourteen genotypes. So higher concentrations, 200 mM NaCl will be provided for screening. After that statistical analysis and final conclusion will be drawn.