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Effect of exogenous proline on *in vitro* regeneration of *Sorghum bicolor* (L. moench) under induced salt stress

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Salt stress is a major global constraint that limits the agricultural productivity of crops, specifically in arid and semi- arid regions. The study was conducted to evaluate the effect of exogenous proline on *in vitro* regeneration of *Sorghum bicolor* under induced salt stress conditions by using shoot apex explants. Initially, the effect of sodium chloride as a stress factor on *in vitro* regeneration of *Sorghum bicolor* was studied by adding sodium chloride at a concentration range of 20-100mM to callus induction medium containing Murashige and Skoog (MS) medium supplemented with 2.5mg/L of 2,4-dichlorophenoxy acetic acid and 0.25mg/L of kinetin and further, to regeneration medium containing Murashige and Skoog (MS) medium supplemented with 4mg/L of benzyl aminopurine. Finally, the effect of exogenous proline on maximum salt stress induced by 100mM sodium chloride on callus induction and regeneration was studied by the application of proline at different concentrations ranging from 200-3000mg/L, of which 1000mg/L of proline was observed to be efficient for ameliorating the adverse effects of salt stress on *in vitro* regeneration. These findings can provide an insight for developing salt tolerant lines of sorghum through *in vitro* studies.

Keywords: Exogenous proline, salt stress, sodium chloride, *Sorghum bicolor*, *in vitro* regeneration

Introduction

Sorghum bicolor (L. Moench) is an important agronomical cereal crop, ranking fifth in terms of global production among cereals. It is a dryland crop highly tolerant to drought and widely adapted for cultivation in arid, semi-arid tropical and sub tropical regions of Asia and Africa. Sorghum is used as food, fodder, fiber and also as a potential source of biofuel across a wide range of environments and production scenarios (Kresovich *et al.*, 2005). The global climatic change with dramatic changes in temperature and water availability

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has subsequently increased the attractiveness towards the cultivation of dryland crops such as sorghum (Songul *et al.,* 2009).

Salt stress in arid and semi-arid regions is one of the major stresses that can severely limit plant growth and its net primary productivity (Allakhverdiev *et al.,* 2000; Koca *et al.,* 2007). It is estimated that salinity affects 20% of the irrigated land in the world (Yamaguchi and Blumwald, 2005). Hence, improving the agricultural productivity and sustainability by combating salt stress has become the major concern in these regions (Meloni *et al*., 2004). The reduction in the growth of plants under salt stress may occur due to (a) an osmotic stress due to lowering of the external water potential, or (b) effects of specific ions on metabolic processes ranging from the absorption of nutrients to enzyme activation or inhibition (Carvajal *et al.,* 2000). The mechanisms of salt tolerance typically involve ion regulation and osmoregulation that can be brought about by accumulation of various compatible solutes by the salt stressed plants (Karley *et al.,* 2000; Vera-Estrella *et al.,* 2004). Further, sugars, sugar alcohols and many other molecules such as amino acids, organic acids or inorganic ions have also been reported to be involved in improving salt tolerance mechanisms (Munns, 2005). *Sorghum bicolor* is characterized to be moderately tolerant to salinity (Igartua *et al.,* 1995) and so sorghum has the potential as a crop for salt affected areas (Igartua *et al.,* 1994). Earlier studies have also reported that sorghum exhibits a large genotypic variation for tolerance to salinity (Maiti *et al.,* 1994).

Proline is a compatible osmolyte that accumulates under salt stress in many crops (Munns and Tester, 2008). Proline serves to protect the plant cells against the negative effects of salt by maintaining the osmotic balance, stabilizing subcellular structures, such as membranes and proteins and scavenging reactive oxygen species (ROS) (Ashraf and Foolad, 2007). Exogenously supplied proline can also be osmoprotective (Tal and Katz, 1980) and cryoprotective (Withers and King, 1979) to higher plant cells. Several reports are available confining to the effective role of exogenous proline in osmoregulation of various plant species under salt stress (Lone *et al*., 1987; Wyn *et al.,* 1977). Further, exogenous proline was found to effectively enhance the activities of catalase and peroxidase to detoxify hydrogen peroxide in tobacco under salt stress (Hoque *et al.,* 2007).

In sorghum, with increase in salinity, there was a decline in all the *in vitro* characters including callus induction and regeneration frequency (Kusuma, 2012). Of the various strategies currently used for combating salt stress in plants, improvement in salt tolerance of crops through exogenous application of different types of chemicals including plant growth regulators, osmoprotectants and inorganic nutrients seems to be an efficient, economical

and shot-gun approach (Ashraf *et al.*, 2008). Studies also have reported that reduction in germination, growth and chlorophyll contents induced by salt stress was improved by exogenous application of proline in sorghum (Khalid *et al.*, 2010). However, much limited information is currently available, pertaining to the role of exogenous proline on *in vitro* culture of sorghum under salt stress. So the current study was initiated to examine the effective role of exogenous proline on callus induction and regeneration in sorghum under induced salt stress.

Materials and Methods

Explant preparation

The seeds of *Sorghum bicolor* M35-1 obtained from International Crops Research Institute for the Semi-arid Tropics (ICRISAT), India were used for the study. The seeds were soaked in distilled water for about 2 hours and were then washed in 2 drops of tween-20 for 5 minutes followed by rinsing in distilled water. The seeds were surface sterilized using 70% ethanol for 1 minute and 0.1% mercuric chloride for 5 minutes. The seeds were then washed in sterile distilled water for 3 minutes. The surface sterilized seeds were then inoculated in 90mm petriplates (20-30 seeds per plate) containing Murashige and Skoog (MS) (1962) medium. The pH of the MS media was adjusted to 5.8 using 0.1M NaOH before autoclaving. The petriplates were incubated at 25±2°C in the dark conditions for seed germination. The shoot apices were excised using a sterile scalpel on the 5^{th} day and were used as the source material for callus induction.

Callus induction and regeneration

The shoot apex explants were inoculated in the callus induction medium (CIM) containing MS basal salts supplemented with 3% sucrose, 0.8% agar and the plant growth regulators- 2,4-D at 2.5mg/L and kinetin at 0.25mg/L. The cultures were incubated at 25±2°C under dark conditions with routine inspection. The frequency of callus formation and the nature of callus were examined after 4 weeks of incubation in the dark. The calli formed were transferred after 4 weeks to the regeneration medium (RM) that included MS media supplemented with 4mg/L of benzyl aminopurine (BAP). The cultures were incubated for about 2 weeks at 25±2°C under a 16 h light (2000 lux) and 8 h dark photoperiod. The regeneration frequency and the number of regenerated shoots formed were calculated and recorded after 2 weeks.

Effect of temperature and light on callus induction

A separate experiment was carried out initially to determine the optimal temperature for callus induction under *in vitro* conditions. For this, the shoot apex explants inoculated in the callus induction medium containing MS + 2.5mg/L 2,4-D + 0.25mg/L kinetin, were incubated at two different temperatures of 25±2°C and 30±2°C. The percentage of callus formation (mean) was calculated after 4 weeks of incubation in the dark. Further, the shoot apex explants in CIM were also incubated separately in light (16/8h photoperiod) and dark conditions at 25±2°C to study the effect of light Vs dark for callus induction and the mean percentage of callus formation was observed.

Effect of NaCl on callus induction and regeneration

The effect of salinity on callus induction was studied separately by supplementing the callus induction medium with the following concentrations of sodium chloride (NaCl), viz. 20, 40, 60, 80 and 100mM respectively. The shoot apex explants were inoculated in the above saline media and were incubated for about 4 weeks at 25±2°C in dark. The frequency of callus formation and the nature of callus were observed after 4 weeks. Further, to study the effect of NaCl on regeneration, the regeneration medium containing MS + 4 mg/L of BAP was supplemented with sodium chloride at 20, 40, 60, 80 and 100mM respectively. The calli formed in CIM containing different concentrations of NaCl respectively were then transferred after 4 weeks onto the regeneration medium containing the respective concentration of NaCl (20-100mM). The regeneration frequency and the number of regenerated shoots formed were recorded after 2 weeks of incubation at 25±2°C under a 16 h light (2000 lux) and 8 h dark photoperiod.

Effect of exogenous proline on callus induction and regeneration under induced salt stress

The amino acid proline is an osmoregulator and its role in combating salt stress was studied by the addition of L-proline in the callus induction medium and regeneration medium that contained 100mM NaCl respectively. L-proline was added at a concentration of 200, 500, 1000, 2000, 3000mg/L respectively to determine its effect on callus induction and regeneration in saline MS media. Under these conditions, the *in vitro* response of shoot apex explants in terms of callus formation frequency, nature of callus, regeneration frequency and the number of regenerated shoots were studied at suitable time intervals and the results were recorded.

Data analysis

Each experiment was performed with 20 shoot apex explants and each experiment consisted of three replicates. The explants were incubated in dark for callus induction and under light for regeneration. For callus induction, the data were recorded after 4 weeks and further after 2 weeks, for regeneration. The percentage of callus induction frequency was calculated by the (number of calli formed / total number of explants inoculated) X100. For regeneration, the percentage of regeneration was calculated by (number of calli responded/ total number of explants) X 100 and the mean number of regenerated shoots formed per explant was also calculated. For statistical analysis, data were analyzed using analysis of variance (ANOVA) and Fisher's least significant difference (LSD) (Fisher, 1935; Anthony, 1986) was used to compare the means.

Results and Discussion

Callus induction and regeneration

Callus induction was observed to be higher as of 97% in CIM that contained MS medium supplemented with 2.5mg/L of 2,4-D and 0.25mg/L of kinetin at 25±2°C under dark conditions (Table 2). The calli presented a hard, nodular and compact creamy- white appearance which developed directly from the shoot apex explants (Fig. 4a). Several reports are available, confirming the combined effect of 2,4-D and kinetin in callus induction from different explants of *Sorghum bicolor* (Shyamala and Smith, 1988; Sanjay *et al.,* 2006; Sudhakar *et al.,* 2008; Liming *et al.,* 2010). Further, the calli subcultured on to regeneration medium containing MS + 4mg/L BAP showed the highest regeneration frequency with a mean number of 18.3 shoots. This was used as the control to study the effect of various concentrations of NaCl on callus induction and regeneration (Table 2). The firm role of BAP as an effective congenial supplement for shoot regeneration in sorghum has been reported earlier (Abubachker and Murugesan, 1999; Nirwan and Kothari, 2004).

Effect of temperature and light on callus induction

A study on the effect of temperature on callus induction from shoot apex explants inoculated in MS media supplemented with 2.5mg/L 2,4-D + 0.25mg/L kinetin, resulted in maximum callus formation of about 98% at 25±2°C and most of the calli formed turned to be embryogenic (nearly 95%) at the end of 4 weeks. On the other hand, it was observed that callus formation was very lesser at 30±2°C (Fig.1) with considerable shoot elongation and root

formation from shoot apex explants. This may be attributed to the fact that higher temperatures favour vegetative growth and lower temperatures are essentially involved in reproductive development. In sorghum, the optimum temperature for vegetative growth is between 26ºC and 34ºC, and for reproductive growth it is between 25ºC and 28ºC (Maiti, 1996). Recent reports have indicated that an increase in temperature by 1ºC have decreased the global sorghum yield by 8.4% (David and Christopher, 2007). Calli grown at 25±2°C were relatively nodular, creamy- white in color and compact whereas calli grown at 30±2°C were translucent and friable in nature. The effect of light (16/8 h photoperiod) on callus induction studied against the dark incubation cycle revealed that the frequency of callus induction was higher (96%) when callus was induced in total darkness rather than in 16/8 h photoperiod (Table 1). It was observed that explants grown under dark incubation produced more compact embryogenic calli, while the explants incubated in light conditions showed distinct shoot and root organogenesis. Hence, maintaining the cultures in dark is an essential parameter for retardation of shoot growth in the callus induction phase (Maheswari et al., 2006).

Table 1. Effect of temperature and light on callus induction

Callus induction medium (CIM)	**Percentage of callus formation** (Mean± SD)			
	Incubation Temperature		Dark incubation	Light incubation
	25±2°C	30±2°C		
MS + 2,4-D (2.5mg/L) + kinetin (0.25mg/L)	*98±0.2*	65±0.8	*96±0.1*	30±0.7

Each treatment included 5 flasks, each containing 4 shoot apex explants (20 explants per treatment). The optimum response is shown in italics.

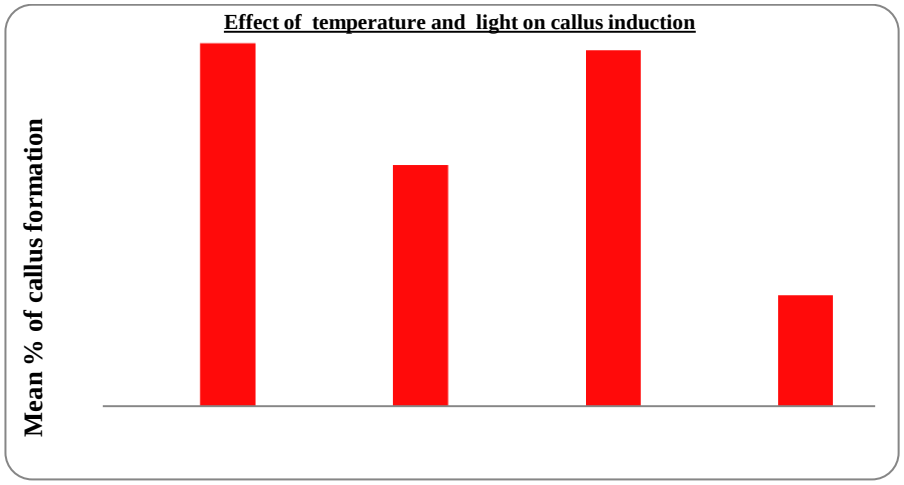

Fig. 1. Effect of temperature and light on callus induction

Effect of NaCl on callus induction and regeneration

Different strategies have been employed for the development of NaCl-tolerant crops, one strategy being the use of *in vitro* selection procedures. Sorghum is relatively salt tolerant (Igartua *et al*., 1995). The effect of different concentrations of NaCl ranging from 20-100mM was tested on callus induction and regeneration response of sorghum, grown in CIM and RM respectively. It was observed that the percentage of callus formation and regeneration showed a drastic decrease with increasing concentrations of NaCl (Table 2, Fig. 2). Moreover, the morphological degradation was prominent with necrosis, when the negligible calli formed in CIM containing 100mM NaCl were transferred to RM containing 100mM NaCl (Fig. 4e). The shoot regeneration was completely inhibited at a higher concentration of NaCl (100mM). Excess salt interferes with several physiological and biochemical processes, resulting in problems such as ion imbalance, mineral deficiency, osmotic stress, ion toxicity and oxidative stress; these conditions ultimately interact with several cellular components, including DNA, proteins, lipids, and pigments in plants (Zhu, 2002), impeding the growth and development of a vast majority of crops (Manoj *et al.*, 2011).

Table 2. Effect of NaCl on callus induction and regeneration

Concentration of NaCl (mM) *	**CIM = MS + 2,4-D (2.5mg/L) + kinetin (0.25mg/L)**		**RM = MS + BAP (4.0mg/L)**	
	% of callus frequency (Mean± SD)	Nature of callus	Percentage of regeneration (Mean± SD)	No. of regenerated shoots (Mean± SD)
0	*97±0.2a*	creamy white, compact	*89±0.4a*	*18.3±0.5a*
20	85±0.8b	yellow, compact	64±0.1b	10.2±0.8b
40	72±0.4c	yellow, friable	53±0.4c	9.4±0.4c
60	68±0.5d	dark yellow, friable	31±0.2d	4.7±0.1d
80	34±0.2e	brown, necrotic	15±0.8e	2.2±0.2e
100	28±0.1f	brown, necrotic	0.0f	0.0f

Different levels indicate significantly different values at a probability level of P=0.05 based on Fisher's LSD test. The optimum response is shown in italics.

*20 explants per treatment, CIM- callus induction medium, RM- regeneration medium

A large number of plant species accumulate proline in response to salinity stress and this accumulation may be involved in the defense mechanisms against salinity stress (Premachandra *et al.*, 1992). The role of proline in cell osmotic adjustment, membrane stabilization and detoxification of injurious ions in plants exposed to salt stress is widely reported (Hare *et al.*, 1999; Kavikishor *et al.*, 2005; Ashraf and Foolad, 2007). In addition, proline accumulation

protects plants against free radical induced damage by quenching of singlet oxygen (Hossein and Fatemeh, 2012). Thus, endogenous proline acts as a critical component for stress protection in plants. With this perspective, the current study was conducted with a view that exogenous proline added to cultured plant cells would further improve the stress tolerance mechanism in plants.

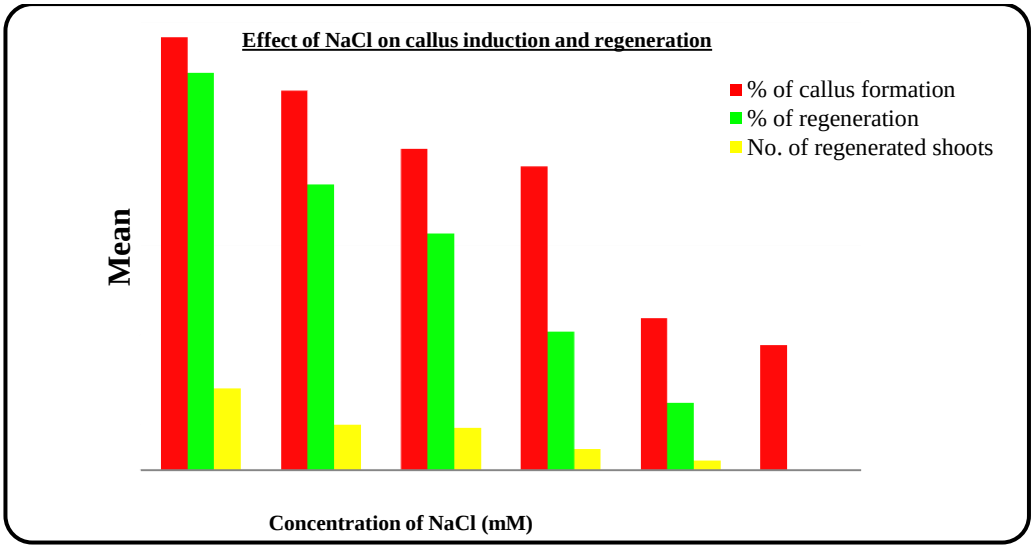

Fig. 2. Effect of NaCl on callus induction and regeneration

Effect of exogenous proline on callus induction and regeneration under induced salt stress

The effect of exogenous proline on callus induction and regeneration response under saline culture conditions was studied by adding L-proline at five different concentrations, each added to CIM and RM containing 100mM NaCl respectively. The results indicated that addition of proline at a concentration of 1000mg/L was efficient in ameliorating the stress imposed by 100mM NaCl to a significant extent. The calli was observed to be white and semi-friable with 88% (mean) callus frequency (Fig. 4c). The regeneration which was earlier inhibited by the presence of 100mM NaCl in RM, was indeed counteracted by the addition of proline (1000mg/L) yielding a mean number of 11.5 shoots (Fig. 3). However, increased addition of proline at concentrations above 1000mg/L decreased the callus formation and regeneration, thereby signifying 1000mg/L of proline to be the optimal concentration for alleviating the adverse effects of

salt stress (Table 3). These results indicated that exogenous proline at an optimal concentration can be used by the cultured cells for mitigating the suppressive effects of imposed salt stress and thereby improve the mechanisms of salt tolerance in such cells. Earlier reports had also indicated that the effectiveness of proline on the growth of salt stressed plants depends on the type of plant species, growth stage and concentration of osmoprotectant and mode of application (Amzallag, 2002). There are also findings stating that exogenous proline improved the protein content and enhanced the photosynthetic pigments in rice under salt stress (Deivanai *et al.*, 2011).

Table 3. Effect of exogenous proline on callus induction and regeneration under induced salt stress

Concentration of L-Proline (mg/L)	**Concentration of NaCl (mM)**	**CIM = MS + 2,4-D (2.5mg/L) + kinetin (0.25mg/L)**		**RM =MS + BAP (4.0mg/L)**	
		% of callus frequency (Mean± SD)	Nature of callus	Percentage of regeneration (Mean± SD)	No. of regenerated shoots (Mean± SD)
0	100	28±0.1f	Brown, necrotic	0.0f	0.0e
200	100	52±0.4e	White, friable	45±0.4e	3.6±0.1d
500	100	64±0.1c	Creamy, friable	60±0.2b	7.8±0.9b
1000	100	*88±0.8a*	white, semi-friable	*82±0.3a*	*11.5±0.7a*
2000	100	70±0.6b	Yellow, friable	56±0.6c	7.3±0.4b
3000	100	58±0.5d	Yellow, friable	50±0.1d	4.2±0.6c

Means followed by the same letter are not significantly different at a probability level of P=0.05 based on Fisher's LSD test. The optimum response is shown in italics.
Each treatment included 20 shoot apex explants. CIM- callus induction medium, RM- regeneration medium.

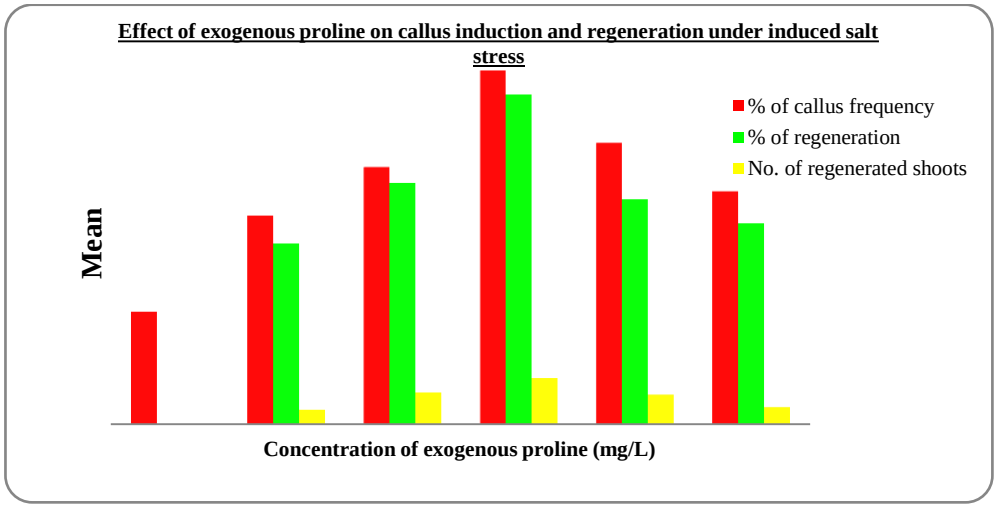

Fig. 3. Effect of exogenous proline on callus induction and regeneration under induced salt stress

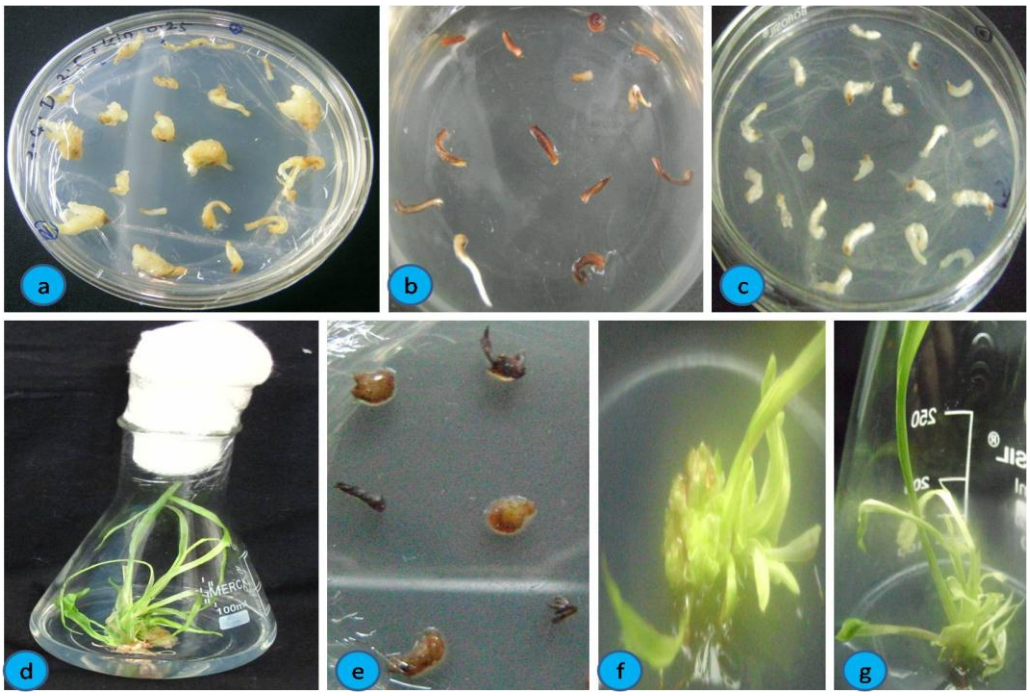

Fig. 4. Callus induction and shoot regeneration of *Sorghum bicolor* M35-1. a) Callus induction in MS supplemented with 2.5mg/L of 2,4-D and 0.25mg/L of kinetin (callus induction medium). b) Effect of 100mM NaCl on callus induction in callus induction medium. c) Effect of 1000mg/L proline on callus induction in callus induction medium containing 100mM NaCl. d) Shoot regeneration in MS supplemented with 4mg/L of BAP (regeneration medium). e) Effect of 100mM NaCl on shoot regeneration in regeneration medium. f) and g) Effect of 1000mg/L proline on shoot regeneration in regeneration medium containing 100mM NaCl.

Conclusion

The study indicated that NaCl addition in the callus induction medium and regeneration medium at varying concentrations imparted a stress on the *in vitro* regeneration of sorghum, which was indicated by the gradual decrease in callus induction and regeneration frequency with the increasing concentrations of NaCl from 20-100mM. Further, the exogenous application of proline at a concentration of 1000mg/L was effective in alleviating the adverse effects of induced salt stress. The current study could provide a platform for improving the sustainability and agricultural productivity of sorghum crop in saline soils.

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References

Abubachker, M.N. and Murugesan, N.P. (1999). *In vitro* organogenesis of various explants of *Sorghum bicolor* (L.) Moench (sweet sorghum). Plant tissue culture 9:173-176.

Allakhverdiev, S.I., Sakamoto, A., Nishiyama, Y., Inaba, M. and Murata, N. (2000). Ionic and osmotic effects of NaCl induced inactivation of photosystems I and II in *Synechococcus sp*. Journal of Plant Physiology 123:1047-1056.

Amzallag, G.N. (2002). The adaptive potential of plant development: evidence from the response to salinity. In: Lauchli, A., Luttage, U. (Eds.), Salinity: Environment-Plant-Molecules, Kluwer, The Netherlands, pp. 291- 312.

Anthony, J.H. (1986). The maximum familywise error rate of Fisher's least significant difference test. Journal of the American Statistical Association 81:1000-1004.

Ashraf, M., Athar, H.R., Harris, P.J.C. and Kwon, T.R. (2008). Some prospective strategies for improving crop salt tolerance. Advances in Agronomy 97:45–110.

Ashraf, M. and Foolad, M.R. (2007). Roles of glycinebetaine and proline in improving plant abiotic stress tolerance. *Environmental* and Experimental Botany 59:206-216.

Carvajal, M., Cerda, A. and Martinez, V. (2000). Modification of the response of saline stressed tomato plants by the correction of cation disorders. Plant Growth Regulation 30:37–47.

David, B.L. and Christopher, B.F. (2007). Global scale climate–crop yield relationships and the impacts of recent Warming. Environmental Research Letters 2:014002.

Deivanai, S., Xavier, R., Vinod, V., Timalata, K. and Lim, O.F. (2011). Role of exogenous proline in ameliorating salt stress at early stage in two rice cultivars. Journal of Stress Physiology & Biochemistry 7(4):157-174.

Fisher, R.A. (1935). The design of experiments. University of Adelaide.

Hare, P.D., Cress, W.A. and Staden, J.V. (1999). Proline biosynthesis and degradation: a model system for elucidating stress-related signal transduction. Journal of Experimental Botany 50:413-434.

Hoque, M.A., Okuma, E., Banu, M.N.A., Nakamura, Y., Shimoishi, Y. and Murat, Y. (2007). Exogenous proline mitigates the detrimental effects of salt stress more than exogenous betaine by increasing antioxidant enzyme activities. Journal of Plant Physiology 164:553-561.

Hossein, S. and Fatemeh, A.S. (2012). Salinity induced effects on growth parameters, chemical and biochemical characteristics of two forage sorghum (*Sorghum bicolor* L.) cultivars. Asian Journal of Plant Sciences 11:19-27.

Igartua, E., Gracia, M.P. and Lasa, J.M. (1994). Characterization and genetic control of germination-emergence responses of grain sorghum to salinity. Euphytica 76:185-193.

Igartua, E., Gracia, M.P. and Lasa, J.M. (1995). Field responses of grain sorghum to a salinity gradient. Field Crops Research 42:15-25.

Karley, A., Leigh, R. and Sanders, D. (2000). Differential ion accumulation and ion fluxes in the mesophyll and epidermis of barley. Journal of Plant Physiology 122:835-844.

Kavikishor, P.B., Sangam, S., Amrutha, R.N., Laxmi, P.S., Naidu, K.R., Rao, K.R.S.S., Rao, S., Reddy, K.J., Theriappan, P. and Sreenivasulu, N. (2005). Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Current Science 88:424-438.

Khalid, N., Aqsa, T., Iqra, Khalid, H. and Abdul, M. (2010). Induction of salt tolerance in two cultivars of sorghum (*Sorghum bicolor* L.) by exogenous application of proline at seedling stage. World Applied Sciences Journal 10(1):93-99.

Koca, M., Bor, M., Ozdemir, F. and Turkan, I. (2007). The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. Environmental Experimental Botany 60:344-351.

Kresovich, S., Casa, A.M., Mitchell, S.E., Hamblin, M.T., Sun, H., Bowers, J.E., Paterson, A.H. and Aquadro, C.F. (2005). Diversity and selection in sorghum: simultaneous analyses using simple sequence repeats. Theoretical and Applied Genetics 111(1):23-30.

Kusuma, K.P. (2012). *In vitro* response of *Sorghum bicolor* to salinity. Journal of Biotechnology and Biomaterials 2: pp. 6.

Liming, Z., Shujun, L. and Songquan, S. (2010). Optimization of callus induction and plant regeneration from germinating seeds of sweet sorghum (*Sorghum bicolor* Moench). African Journal of Biotechnology 9(16):2367-2374.

Lone, M.I., Kueh, J.S., Wyn, R.J. and Bright, S.J. (1987). Influence of proline and glycinebetaine on salt tolerance of cultured barley embryos. Journal of Experimental Botany 38:479-490.

Maheswari, M., Jyothilakshmi, N., Yadav, S.K., Varalaxmi, Y., Vijayalakshmi, A., Vanaja, M. and Venkateswarlu, B. (2006). Efficient plant regeneration from shoot apices of sorghum. Biologia Plantarum 50(4):741-744.

Maiti, R. (1996). Sorghum science. New Delhi, India: Oxford & IBH Publishing Co. Pvt. Ltd. pp. 352.

Maiti, R.K., Rosa-Ibarra, M.D. and Sandowal, N.D. (1994). Genotypic variability in glossy sorghum lines for resistance to drought, salinity and temperature-stress at seedling stage. Journal of Plant Physiology 143:241-244.

Manoj, K.R., Rajwant, K.K., Rohtas, S., Manugangola, P. and Dhawana, A.K. (2011). Developing stress tolerant plants through *in vitro* selection—an overview of the recent progress. Environmental and Experimental Botany 71:89–98.

Meloni, D.A., Marta, G.R., Martínez, C.A. and Oliva, M.A. (2004). The effects of salt stress on growth, nitrate reduction and proline and glycinebetaine accumulation in *Prosopis alba* Brazilian. Journal of Plant Physiology 16:39-46.

Munns, R. (2005). Genes and salt tolerance: bringing them together. New Phytologist 167:645–663.

Munns, R. and Tester, M. (2008). Mechanisms of salinity tolerance. Annual Review of Plant Biology 59:651-681.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiologia Plantarum 15:473-497.

Nirwan, R.S. and Kothari, S.L. (2004). High frequency shoot organogenesis in *Sorghum bicolor* (L.) Moench. Journal of Plant Biochemistry and Biotechnology 13:149-152.

Premachandra, G.S., Saneoka, H., Fujita, K. and Ogata, S. (1992). Leaf water relations, osmotic adjustment, cell membrane stability, epicuticular wax load and growth as affected by increasing water deficits in sorghum. Journal of Experimental Botany 43:1569-1576.

Sanjay, G., Khanna, V. K., Rameshwar, S. and Garg, G.K. (2006). Strategies for overcoming genotypic limitations of *in vitro* regeneration and determination of genetic components of variability of plant regeneration traits in sorghum. Plant Cell Tissue and Organ Culture 86:379–388.

Shyamala B. and Smith, R.H. (1988). Enhanced somatic embryogenesis in *Sorghum bicolor* from shoot tip culture. *In Vitro* Cellular and Developmental Biology 24:65–70.

Songul, G., Ekrem, G., Rajvinder, K., Joshua, W., Ling, M., Han-Qi, T. and Peggy, L.G. (2009). Efficient, reproducible Agrobacterium-mediated transformation of sorghum using heat treatment of immature embryos. Plant Cell Reports 28:429–444.

Sudhakar, P., Saradamani, N. and Ramana, T. (2008). Plant tissue culture studies in *Sorghum bicolor*: immature embryo explants as the source material. International Journal of Plant Production 2:1-14.

Tal, M. and Katz, A. (1980). Salt tolerance in the wild relatives of the cultivated tomato: the effect of proline on the growth of callus tissue of *Lycopersicon esculentum* and *L. peruvianum* under salt and water stress. Z Pflanzenphysiol Bd 98:283-288.

Vera-Estrella, R., Barkla, B., Bohnert, H. and Pantoja, O. (2004). Novel regulation of aquaporins during osmotic stress. Journal of Plant Physiology 135:2318-2329.

Withers, L.A. and King, P.J. (1979). Proline: a novel cryoprotectant for the freeze preservation of cultured cells of *Zea mays*. Plant Physiology 64:675-678.

Wyn, R.J, Storey, R., Leigh, R.A., Ahmad, N. and Pollard, A. (1977). A hypothesis on cytoplasmic osmoregulation. In "Regulation of Cell Membrane Activities in Plants" (E Marre, O Cifferi eds.), Elsevier, Amsterdam, pp. 121-136.

Yamaguchi, T. and Blumwald, E. (2005). Developing salt-tolerant crop plants: challenges and opportunities. Trends in Plant Science 10:615–620.

Zhu, J.K. (2002). Salt and drought stress signal transduction in plants. Annual Review of Plant Biology 53:247–273.

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