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# *In vitro* studies on effect of different concentration of NaCl on *Jatropha curcas*

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**Abstract**

Short-term effect of different concentrations of NaCl on callus cultures of *Jatropha curcas* was investigated at different concentration of NaCl (0, 20, 40, 60, 80, 100 mM).Results showed a decrease in fresh weight of callus cultures when subjected to increasing concentration of salt in the medium. Callus morphology correspondingly changed from off-white to blackish–brown above 40mM to acutely necrotic stage at 100 mM NaCl .The callus cultures after recurrent selection (at 20mM for 20 days) were transferred to salt free optimized callus regeneration medium expressed 90.0% recovery. The callus placed in 40mM, 60mM concentration of NaCl exhibited moderate tolerance and showed 64.0% and 56.0% recovery. In 80mM concentration, callus showed moderate susceptibility and showed 6.9 % recovery of callus.

**Key words**

Necrotic, Recovery, Stress, Susceptibility, Tolerance



## Introduction

Soil salinity problem has been aggravated during the last decades as a consequence of some agricultural practices such as irrigation and poor drainage systems. Around 20% of the irrigated land in the world is affected by salinity, and it is expected that increase of salinization in agricultural fields will reduce the land available for cultivation by 30% in the next 25 years and up to 50% by the year 2050 (Rozema and Flowers ,2008). Nearly 40% of India is waste land. Wastelands are considered as a high soil salinity regions with low fertility and extremely limited water supply.

There is a good reason for developing *Jatropha curcas* as a new energy crop, as it is fit for agro-forestry and other afforestation programmes in wasteland development (Behera *et al.,* 2010; Jingura,2011) and does not compete with conventional food crops for land, water and manpower resources . Jatropha (*Jatropha curcas* L.) is a multipurpose shrub (Family Euphorbiaceae) that is native to tropical America but now thrives in many parts of the tropics and subtropics in Africa and Asia (Kumar and Sharma,2008). It grows well in lands with low rainfall harsh climatic conditions and capable of growing in marginal and poor soil conditions able to grow where most other crops cannot survive (Fairless, 2007; Divakara *et al.,* 2010, Reubens *et al.,* 2011). Their seeds contain 25 to 32% oil with yields of 1.5 tons of oil per hectare after five years of growth (Tiwari *et al.,* 2007; King *et al.,* 2009).

The study of stress / recovery response contributes to a better understanding of the plant's ability to adapt to different environments and climatic conditions. Plant tissue culture techniques can be used as an important tool to study the salt stress response of callus cultures to salinity in controlled and uniform environmental conditions (Bajji *et al.,* 1998; Queiros *et al.,* 2007),thus avoiding complications arising from physiological and structural variability of whole plant (Bajji *et al.,* 1998, Elkahoui et al.,2005) and means of rapid selection and improvement for salinity tolerance. Few reports on salt tolerance of *Jatropha curcas* has been reported so far. Although there are other sources of biodiesel, as a nonfood crop, *Jatropha curcas* ranks first among all possible crops after considering the social, economic and humanitarian aspects (Behera *et al.,* 2010; Reubens *et al.,* 2011).The use of unconventional crop improvement methods such as selection of tissue culture or the screening of existing germplasm for obtaining salt tolerant Jatropha curcas-biodiesel plant should be explored. Therefore, the effect of increasing NaCl concentration at cell levels (callus) of *Jatropha curcas* was investigated.

## Materials and Methods

Young tender vegetative shoots of 20-25cm length were excised from Experimental Orchard of the Department of Horticulture and Home Sciences Garden of CCS Haryana Agricultural University, Hisar, Haryana, India. The explants (nodal segment) were excised from young tender shoot with the help of scalpel. The excised explants were washed with detergent (Teepol) followed by washing under running tap water and finally rinsed with double distilled water. Explants were surface sterilized with Streptocycline (0.1%) for 8min + $HgCl_2$ (0.1%) for 15min followed by successive washing with sterile distilled water three times in a laminar flow. The callus induction and regeneration experiment was carried on MSC1 media supplemented with MS + IBA (0.5 mg $l^{-1}$) + BAP (1.5mg $l^{-1}$) using nodal segments. The pH of the medium was adjusted to 5.8 using 1N KOH or HCl, prior to adding agar. The culture medium was autoclaved at 1.05 kgcm$^{-2}$ pressure at 121◦C for 20 min. The cultures were maintained at 25 ± 2◦C under a 16 hr photoperiod with light intensity of 35–40µmol $m^{-2}s^{-1}$ (cool white fluorescent tubes). The well developed calli were cultured on MSC1 (MS + IBA (0.5 mg $l^{-1}$) + BAP (1.5mg $l^{-1}$) supplemented with a range of NaCl concentration (0,20,40,60,80 and 100mM) media. The calli were incubated under controlled culture conditions for 20 days. The calli were rated as tolerant, moderately susceptible and susceptible on the basis of normal growth, browning and high necrosis of callus.

The experiment was conducted in triplicate and each treatment consisted of ten replicates. The data was analyzed using ANOVA in completely randomized design. The significance level was determined at p = 0.05. Mean values of treatments were compared with Newman-Keul's multiple range.

## Results and Discussion

A significant difference was observed in callus proliferation response when subjected to different concentrations of NaCl (0-100mM). At 20mM NaCl, 10.82% decrease in fresh weight of callus was observed. By increasing the concentration of salt in the media, gradual decrease in fresh weight of callus was observed. At 40,60,80 and 100mM concentration, the corresponding decrease in fresh weight of callus was more i.e. 36.33,39.96,72.0, 84 and 97% respectively (Table 1) .

Farhatullah *et al.* (2002) and Queiros *et al.* (2007) had also reported similar results at higher salt levels in potato, Elkahoui *et al.* (2005) in *Catharanthus roseus*, Cherian and Reddy (2003) in *Suaeda nudiflora*, Niknam *et al.* (2006) in *Trigonella speies* and Shibli *et al.* (2007) in *Lycopersicon species*. This reduction of callus growth at high salinity stress was also linked to lesser absorption of water, ionic imbalance and induction of metabolic disturbance and oxidative stress (Hasegawa *et al.,* 2000; Niknam *et al.,* 2006; Errabii *et al.,* 2007). Sodium accumulation in tissues under salinity stress is generally considered as a major factor behind the adverse effect of salinity on nutrient uptake and growth (Shibli *et al.,* 2001). This is apparently achieved by several mechanisms that may include regulation of $K^+$, $Na^+$ and $Cl^-$ uptake across the plasma membrane and /or compartmentalization of $Na^+$ and $Cl^-$ in the vacuole (Parida and Das, 2005; Shibli *et al.,* 2007). The increase in $Na^+$ content of cells was accompanied by a decrease in $K^+$ accumulation and differences in $Na^+/K^+$ ratio under saline conditions (Cherian and Reddy 2003). Potassium takes part in many enzymatic activities in plant cell and maintaining cystosolic $Na^+/K^+$ ratio is a key requirement for growth under high saline conditions (Apse *et al.,* 1999).

The callus cultures after recurrent selection (20 days) were transferred to salt–free optimized callus regeneration

**Table 1 :** Effect of different concentrations of NaCl on callus proliferation response in *Jatropha curcas*

| $MSC_1$+ NaCl (mM) | Fresh weight of callus at the time of salt tolerance | Fresh weight of callus after 20 days of salt treatment(g*) | Increase/Decrease in fresh weight (g)** | Increase/Decrease in fresh weight (%**) | Rating of callus placed on NaCl conc.for 20 days*** |
|---|---|---|---|---|---|
| Control | 7.12± 0.22 | 7.54 ±0.19 | +0.42 ±0.03 | +5.89 | +++ |
| 20 | 7.02±0.31 | 6.26±0.24 | - 0.76 ±0.07 | - 10.82 | +++ |
| 40 | 6.22±0.18 | 3.96±0.14 | - 2.26±0.17 | - 36.33 | ++ |
| 60 | 6.08±0.29 | 3.65±0.12 | - 2.43±0.17 | - 39.96 | ++ |
| 80 | 7.00±0.16 | 1.96±0.10 | -5.04±0.06 | -72.00 | + |
| 100 | 6.79±0.14 | 1.02±0.05 | - 5.77±0.09 | -84.97 | —— |

*Result is mean ± S.E. from 30 replicate cultures; **The + and – signs represent increase or decrease in fresh weight in comparison with the initial weight in the respective treatment; ***Callus response: +++ = Tolerant (Normal growth), ++ = Moderately tolerant ((Very less browning of callus); + = Moderately susceptible (Browning of callus); —— = Susceptible (Highly necrotic callus); $MSC_1$ : Optimized callus induction medium (MS+ IBA(0.5mg $l^{-1}$+BAP (1.5mg $l^{-1}$)

medium. The recovery percentage decreased with increase in NaCl concentration. It was observed that regeneration potential of callus cultures without salt was better as compared to NaCl treated (40 to 80mM) ones. Shoot formation via organogenesis was observed in callus regeneration medium. The callus placed in 40mM and 60mM concentration of NaCl exhibited moderate tolerance and showed 64.0% and 56.0% recovery. In 80mM concentration callus showed moderate susceptibility and showed 6.9 % recovery of callus while at 100mM concentration of NaCl highly necrotic callus was observed.

Regeneration of selected salt-tolerant callus cultures on salt free regeneration medium is well documented in literature (Jaiswal and Singh, 2001).This decrease in growth of callus cultures at higher salt concentration in *Jatropha curcas* is considered a common phenomenon (Niu *et al.,* 2012; Kumar *et al.,* 2008). It had been previously observed by many workers that the presence of salt in the medium generally reduced or even completely inhibited the plant regeneration (Lutts *et al.,* 1999). These results also corroborate previous studies of El-Enany (1997) and Hassanein, (2004) on regeneration of tomato under salt stress in which they obtained relatively lesser regeneration response in salt-treated calluses. This phenomenon is justified partly due to the loss of regeneration potential during the long periods required for selection or the presence of high concentrations of NaCl in the regeneration medium (Shankhdhar *et al.,* 2000). Reduction in the growth is a common phenomenon in cultured cells grown on medium supplemented with NaCl ( Shankdhar *et al.,* 2000) and it has been interpreted that a certain amount of the total energy available for tissue metabolism is channeled to resist the stress . Under stress conditions, one of the strategies that higher plants in general have probably adopted is to slow down their growth and metabolism (Zhu, 2001). One other possibility is to better utilize and manage the available resources under nutritional imbalance, osmotic and metabolic disturbances. This reduction in growth not only helps the plants to save energy for defense purpose but also limits the risk of heritable damage (May *et al.,* 1998). Change in callus morphology (brownish to black) at higher salt concentrations may directly be linked to cell death at higher salt concentrations.

Our results provide guidelines for the selection of salt tolerant *Jatropha curcas* plantlets and this information is relevant and important to breeders and plant physiologists interested in improving salt tolerance of *Jatropha curcas*.

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