Journal of Crop Nutrition Science
ISSN: 2423-7353 (Print) 2538-2470 (Online)
Vol. 5, No. 1, 2019
http://JCNS.iauahvaz.ac.ir **OPEN ACCESS**

The Induction of Tolerance to Salinity Stress of Commercial Sugarcane Cultivar CP72-1312 by in Tissue Culture Condition

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RESEARCH ARTICLE

ARTICLE INFO.
Received Date: *5 Jan. 2019*
Received in revised form: *9 Feb. 2019*
Accepted Date: *15 Mar. 2019*
Available online: *30 Mar. 2019*

To Cite This Article:
Zahra Khodarahmpour, Seyed Ehsan Emam. The Induction of Tolerance to Salinity Stress of Commercial Sugarcane Cultivar CP72-1312 by in Tissue Culture Condition. ***J. Crop. Nutr. Sci.,*** 5(1): 79-85, 2019.

ABSTRACT

BACKGROUND: In order to investigate the induction of salinity tolerance in CP72-1312 commercial cultivar in tissue culture condition, an experiment was carried out in the tissue culture laboratory of Karoun Agro-Industry Company in 2017 year.

OBJECTIVES: At this study we evaluated tolerance of CP73-1312 sugarcane commercial cultivar to salt stress in tissue culture.

METHODS and RESULT: Six callus induction treatments in Murashig and Skoog based medium were studied in a CRD with 4 replications. MS based medium supplemented with 2,4-D in 3 $mg.l^{-1}$ was the best treatment for callus induction (93.3%). The effect of different levels of salinity (0.2, 0.4, 0.6, 0.8 and 1 percent) on callus tolerance was studied in a CRD with 4 replications. In the eighth weeks, the callus value reduction in compare to control was obtained 23, 51, 66, 75 and 81 percent, respectively. Five regeneration treatments in modified MS were performed as factorial experiment in a CRD with 4 replications. The highest regeneration rate was 1 $mg.l^{-1}$ BAP and 0.02 $mg.l^{-1}$ KIN. This treatment was used for indirect regeneration under salinity stress condition. The mean comparisons showed that the highest regeneration rate was observed in the control treatment and the lowest regeneration was observed in salinity treatment 0.8 and 1 percent. Five root treatments in half-strength MS medium were studied in a CRD with 4 replications with IBA. The highest rooting rates were assigned to 0.01 and 0.02 $mg.l^{-1}$ IBA treatments. The effect of the best level of IBA (0.01 $mg.l^{-1}$) on salinity stress was evaluated on rooting.

CONCLUSION: According to the results, with increasing salinity stress, rooting rate decreased.

KEYWORDS: *NaCl, Callus induction, Regeneration, Rooting, Strain.*

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1. BACKGROUND

Soil or water salinity is considered to be the major environmental factor limiting plant growth and productivity, especially in arid and semi-arid irrigated regions including Iran. Salinity limits vegetative and reproductive growth of plants by inducing severe physiological dysfunctions and causing widespread direct and indirect harmful effects, even at low salt concentrations (Altman, 2003; Munns, 2002; Al-Maskri *et al*., 2010). Salt stress has been extensively investigated since soil salinity represents a major constraint for successful production and crop yield (Munns, 2002). Sugarcane (*Saccharum officinarum* L.) is an important agro-industrial sugar crop, contributing about 70% of the world sugar production. In Iran, sugarcane is grown under irrigated systems and is seriously prone to soil salinization. This problem may be a serious problem for the production and the yield of this agricultural crop. Sugarcane growth may be suppressed due to the accumulation of toxic ions (Wahid *et al*., 2009). The complexity and polygenic nature of salinity tolerance has seriously limited the efforts to develop the tolerant crop variety through conventional breeding practices. Gandonou *et al*. (2006) studied the effects of salt stress by exposing the callus to a single level of 68 mM NaCl, and observed that physiological and biochemical indicators could play a crucial role in salt tolerance. Shomeili *et al*. (2011) studied salt tolerant variants from embryogenic calli of sugarcane cultivar CP48-103 that were cultured on a selective medium containing different levels of NaCl. A total of four plants which regenerated from the tolerant calli were selected but the best in vigor were grown in *in vitro* and hydroponic systems under salinity stress as compared to source variety. With increasing supply of NaCl in both systems, root growth was more adversely affected than shoot growth. Mohammadnejad *et al*. (2016) to evaluate induction of tolerance to salt stress CP73-21 sugarcane commercial cultivar reported that the highest callus value was obtained from treated with 3 $mg.l^{-1}$. The effect of different levels of salinity 0, 33, 66, 99 and 132 mM were investigated to tolerance of callus. After 8 weeks, the callus value reduction by 33, 66, 99 and 132 mM treatments in compare to control were obtained 31, 33, 22 and 26%, respectively.

2. OBJECTIVES

At this study we evaluated tolerance of CP73-1312 sugarcane commercial cultivar to salt stress in tissue culture.

3. MATERIALS AND METHODS

3.1. Lab and Treatments Information

This study was carried out in the Biotechnology-Tissue Culture Laboratory, Department of Sugarcane Research Center, Karun Agro-industrial Co., Iran in 2017 year.

3.2. Lab Management

The early materials of this study included 40 stem cuttings of sugarcane commercial variety CP72-1312. After transferring the samples to the laboratory, samples were washed washing

with running water for an hour. After washing, the samples were transferred under the hood, the samples were disinfected with 70% ethanol and 20% sodium hypochlorite for 120 seconds. Six treatments callus were investigated.

3.3. Measured Traits

Callusing treatments included 1, 1.5, 2, 2.5 and 3 mg.l^{-1} 2, 4-D in a CRD with 4 replications. Cultures were kept in darkness at 25 ± 1°C. After 3 weeks, number of explants that induced callus was noted and data was analyzed statistically using ANOVA technique. After 2 month, sodium chloride was used for to determine the salinity tolerance of viable callus. For this purpose, 5 treatments were prepared 0, 0.2, 0.4, 0.6, 0.8 and 1 percent. The calli were transferred to the test tubes containing the medium of regeneration. Each replication was a rack containing ten test tubes in which one callus was placed. Modified MS including 60 g.l^{-1} sucrose and 500 mg.l^{-1} casein hydrolyzate was chosen as the based medium for treatments. Treatments included 1 and 2 mg l^{-1} of BAP + 0.01 and 0.02 mg.l^{-1} Kinetin. Cultures were incubated in a growth cabinet at 25 ± 1°C under 16 h photoperiod. After 6 weeks, the number of regenerated calli was noted. The data so obtained was analyzed statistically with using ANOVA technique. After of determination the best regeneration treatment, sodium chloride was used for determine the salinity tolerance of regenerated plantlet under salinity. For this purpose, 5 treatments were prepared 0, 0.2, 0.4, 0.6, 0.8 and 1 percent. The shoots were transferred to the rooting medium. Five treatments were studied in a CRD with four replications (each replication was a rack containing ten test tubes in which one shoot was placed). Half-strength MS medium was used as a based medium for root formation. The treatments included 0.01, 0.02, 0.03, 0.04 and 0.05 mg l^{-1} IBA. After of determination the best rooting treatment, sodium chloride was used for determine the salinity tolerance of rooted plantlet under salinity. For this purpose, 5 treatments were prepared 0, 0.2, 0.4, 0.6, 0.8 and 1 percent.

3.4. Statistical Analysis

Analysis of variance and Duncan mean comparison were performed by using SAS statistical software (Ver.8) and graphs were drawn by using MS. Excel software (Ver.2010).

4. RESULT AND DISCUSSION

Table 1 shows the results of variance analysis for all experiments after data transformation. Significant differences ($p<0.01$) were observed among all treatments for all experiments.

Callus induction: Based on the results of analysis of variance, it was founded that callus induction treatments had significant effects on explants at 1%. 2,4-D. Treatments led to a callus from explants and the highest callus (93.3 percent) was obtained by 3 mg.l^{-1} treatment and it was best treatment for induction of callus (Fig. 1). These results are in agreement with results obtained previously in CP73-21 cultivar (Mohammadnejad et al., 2016), while Sadat *et al*. (2013) in NCO310 cultivar medium supplemented with 2,4-D in 3 mg.l^{-1}

along with BAP in 0.3 $mg.l^{-1}$ was best treatment for callus induction.

Tolerance of callus to salinity: According to ANOVA, it was determined that treatments had significant effect on tolerance of callus to salinity, so, callus value decreased by increasing of salinity stress and this reduction was obtained 23, 51, 66, 75 and 81% by application of 0.2, 0.4, 0.6, 0.8 and 1 percent NaCl treatments, respectively (Fig. 2).

Table 1. Results of ANOVA for experiments on CP72-1312 cultivar

Experiment	S.O.V	df	MS	CV (%)
Callus induction	2, 4-D	5	21.7**	7
	Error of experimental	18	0.2	
Salinity	NaCl	5	13.3**	5
	Error of experimental	18	0.06	
Regeneration	BAP	1	676**	7.5
	KIN	1	1482	
	BAP×KIN	2	81**	
	Error of experimental	12	17.7	
Regeneration under salinity	Salinity	5	17.2**	6
	Error of experimental	18	0.07	
Root formation	IBA	4	1250**	7.3
	Error of experimental	10	12.9	
Root formation under salinity	Salinity	5	14.4**	4.6
	Error of experimental	12	0.06	

**: Significant at 0.01 probability level.

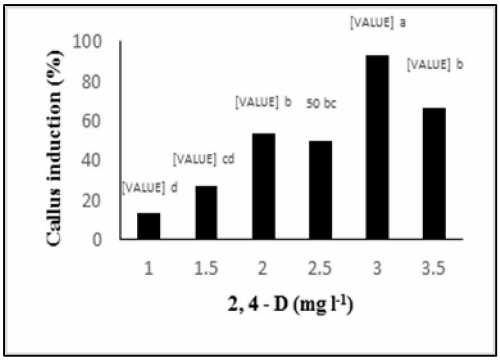

Fig. 1. Mean of comparisons (Duncan test in 0.01 probability level) of 2, 4-D treatments on callus induction.

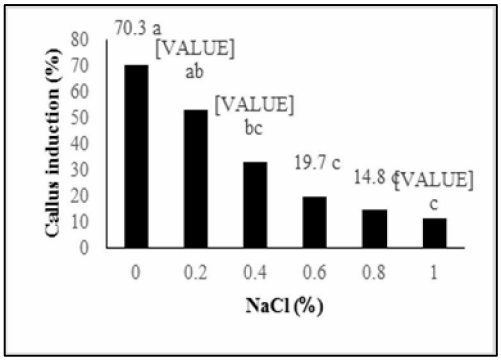

Fig. 2. Mean of comparisons (Duncan test in 0.01 probability level) of effect of salinity treatments on callus induction.

Along 8-week study of percentage of callus showed average yield decreased. This reduction was observed in all treatments including control and stress.

Mohammadnejad *et al*. (2016) in CP73-21 cultivar reported that callus value reduction by 33, 66, 99 and 132 mM treatments of NaCl in compare to con-

trol were obtained 31, 33, 22 and 26%, respectively.

Indirect regeneration: Between treatments, highest regenerations were obtained by application of 1 BAP + 0.02 KIN $mg.l^{-1}$ (Fig.3).

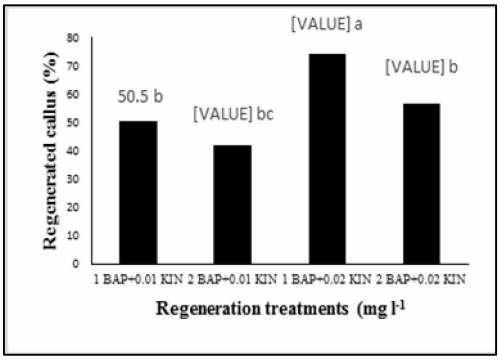

Fig. 3. Mean of comparisons (Duncan test in 0.01 probability level) of regeneration treatments.

These results confirm the results reported in CP73-21 (Mohammadnejad *et al*., 2016), CP69-1062 (Rahimi, 2016) and NCO310 (Sadat *et al*., 2013).

Indirect regeneration at salinity stress: According to previews results, 1BAP + 0.02 KIN was selected as best treatment for indirect regeneration at salinity stress. Means comparisons showed that the highest regeneration were observed in control and after that 0.2 percent. On the other hand a little bit regeneration was observed in 0.8 and 1 percent (Fig. 4). The success of in vitro culture depends mainly on the growth conditions of the source material (Caswell *et al*., 2000, Delporte *et al*., 2001), medium composition and culture conditions (Saharan *et al*., 2004) and on the genotypes of donor plants.

Root formation: Duncan test (Fig. 5) indicated that treatment IBA in 0.01 and 0.02 $mg.l^{-1}$ is the best for root formation of plantlets with average of 72.3 and 66.7 % rooted plantles. Use of 0.01 $mg.l^{-1}$ IBA for root formation has been reported previously in different varieties of sugarcane (Sadat *et al*., 2012; Mohammadnejad *et al*., 2016).

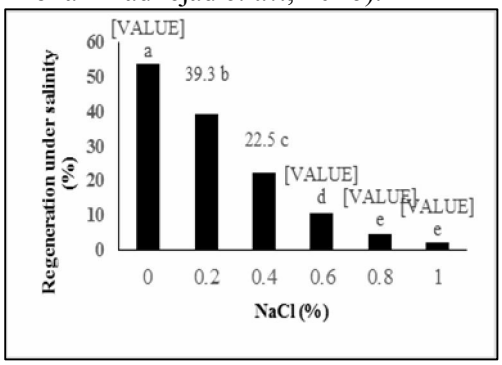

Fig. 4. Mean of comparisons (Duncan test in 0.01 probability level) of salinity treatments on indirect regeneration.

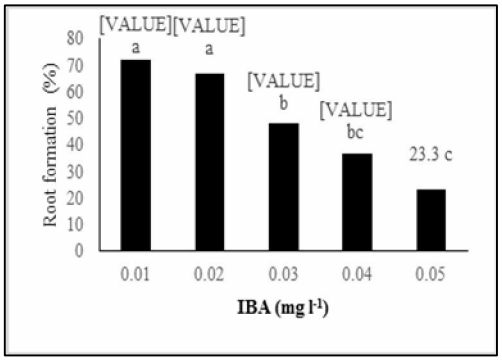

Fig. 5. Mean of comparisons (Duncan test in 0.01 probability level) of root formation treatments.

Root formation at salinity stress: According to previews results, 0.01 IBA was selected as best treatment for root formation at salinity stresses. Means comparisons showed highest root formation was observed in control and after that 0.2%. So a little bit root formation was in 0.8 and 1 % (Fig. 6).

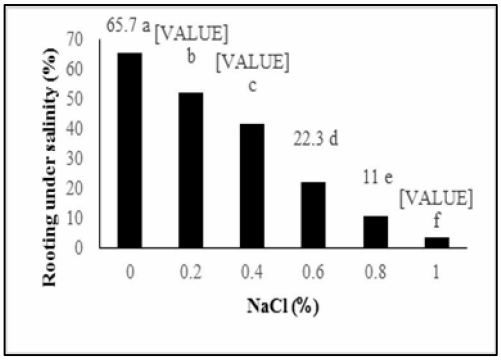

Fig. 6. Mean of comparisons (Duncan test in 0.01 probability level) of root formation under salinity stress

Totally, the highest callus value (67.5%) was obtained from treated with 3 mg l^{-1}. After 8 weeks, the callus value reduction by 0.2, 0.4, 0.6, 0.8 and 1 percent treatments in compare to control were obtained 23, 51, 66, 75 and 81%, respectively. The effect of treatments was significant at 1% on indirect regeneration. In between treatments the highest of mean was related to 1 BAP + 0.02 KIN $mg.l^{-1}$ treatments, also the effect of salinity on regeneration was significant at 1%. The highest regeneration value was obtained in control and 0.2 percent treatments.

5. CONCLUSION

According the results of present investigation indicate a procedure and feasibility of *in vitro* regenerated plantlet production and selection *in vitro* by adding NaCl in the medium for study salinity tolerant plantlets. However, for confirmation of the results, the growth performance of selected plantlets in field is need to be evaluated.

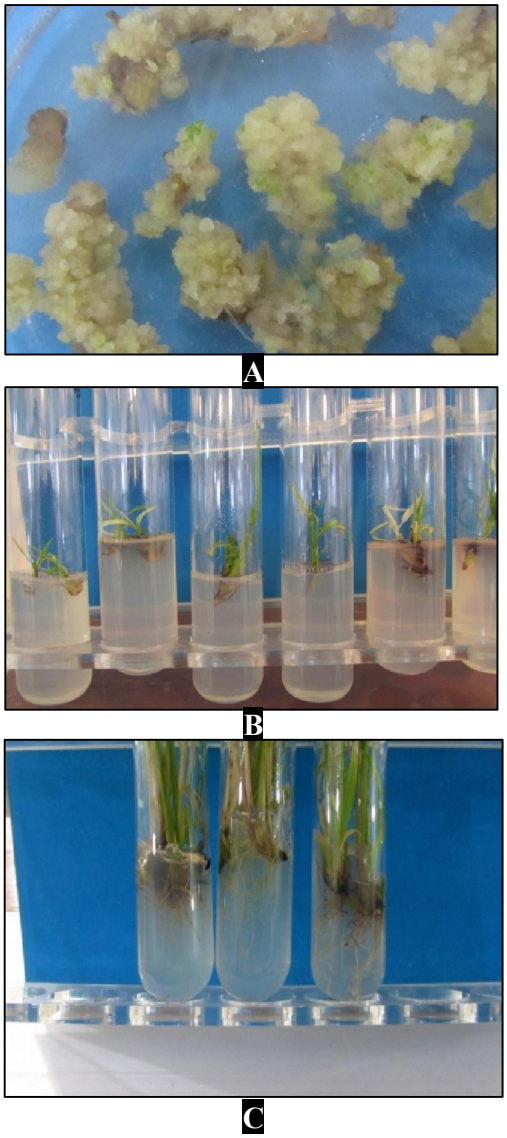

Fig. 7. Callus induction (A), regeneration (B) and root formation (C) in cultivar CP72-1312.

ACKNOWLEDGEMENT

The authors thank all colleagues and other participants, who took part in the study.

FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

CONFLICT OF INTEREST: Authors declared no conflict of interest.

FUNDING/SUPPORT: This work was supported by Islamic Azad University, Shoushtar Branch, Iran.

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