Annals of Plant Sciences
ISSN: 2287-688X
Original Research Article

Effect of Salt (NaCl) Stress on Callus Growth in Sunflower (*Helianthus Annuus* L.) Genotypes

Anuradha Chetukuri
Department of Genetics and Plant Breeding, College of Agriculture, Acharya NG Ranga Agricultural University, Rajendranagar, Hyderabad- 500030, India.

Received for publication: July 21, 2013; **Accepted**: August 17, 2013.

Abstract: Responses of sunflower tissues to NaCl stress were studied in control and salt-stressed conditions. This study was aimed to induce callus from four sunflower (*Helianthus annuus* L.) genotypes, namely APSH-11, KBSH-1, PKVSH-27 and Morden and to evaluate the response of callus for salt stress. Callus was initiated from cotyledonary leaf explants of four sunflower genotypes both under salt stress and normal conditions using MS media supplemented with 1.0mg l^{-1} NAA in combination with 0.5 mg l^{-1} BAP. Callus initiation was delayed and relative callus growth decreased as the salinity level increased. Earlier callus initiation and maximum callus growth was observed in PKVSH-27 and least in Morden. This may be attributed to the genetic architecture of the genotypes. Among the salt concentration significant differences in callus growth was observed at salt (NaCl) concentration /E, C. level of 1.3 dS/m.

Keywords*:* Sunflower, Salt stress, Callus.

Introduction

Sunflower (*Helianthus annuus* L.) is an important oil-yielding crop of the world. Its oil content ranges from 46% to 52%. The oil is of high quality and edible and has anti-cholesterol properties. It is appropriately classified as moderately salt tolerant (Francois, 1996).

Salinity is considered as the main constraint to plant production and is regarded as one of the major factors for desertification that limits crop growth. According to Mass and Hoffman (1977), nearly one third of the world irrigated lands are salt-affected. Salt stress is a complex abiotic stress in which both ionic and osmotic components are involved (Munns, 1993). Although many studies have tried to determine whether plant damage is primarily caused by the osmotic effect or due to specific ion toxicity, this point is still controversial (Nagy and Galiba, 1995; Almansouri *et al.,* 1999). In addition, the mismanagement of irrigation projects elevated the problems. Sunflower grows well in soils with salinity less than 6 ds/m. increased salinity above this level requires high energy for tolerating salinity stress and to grow in high salinity stress environment. High salinity is decreasing the quantity and quality of crops per unit area. Research findings indicated the importance of using tissue culture in parallel with classical methods by growing cells on media of high salinity and select salinity tolerant cells capable of regenerate plants that tolerate salinity stress Basu *et al.,* (1997).

Salt affected soils occur extensively in humid climates particularly in coastal areas (Hussain and Rehman, 1993). Plants exposed to saline conditions encounter basic problems like reduction in water potential of surrounding environment, limiting water availability and nutrient ions, interfering with physiological and biochemical processes etc. Although information is available on relative salt tolerance of many crops (Maas and Hoffman, 1977), much less is known about intraspecific variation for salt tolerance. Though conventional breeding methods showed some success in improving crop species to salt stress, they are time consuming, tedious and costly. Use of *in vitro* techniques was found to be efficient for screening, isolating and characterization of desirable genotypes for salt tolerance. Since much work has been not attempted on *in vitro* screening of sunflower under salt stress, the present investigation was undertaken to screen and evaluate four selected sunflower genotypes with an objective to study the effect of salt stress on callus initiation by using cotyledonary leaf as explants.

***Corresponding Author:**
Anuradha Chetukuri,
Department of Genetics and Plant Breeding, College of Agriculture,
Acharya NG Ranga Agricultural University,
Rajendranagar, Hyderabad- 500030, India
Email : anu.gene@gmail.com

Materials and Methods

The experiment was conducted at Acharya N G Ranga Agricultural University, Rajendranagar, Hyderabad. The materials used in the study and methodologies followed are presented as follows. There are two factors

Factor A-Treatments	Factor B-Genotypes
T1 (0.0 dS/m)	Sunflower genotypes-4
T2 (0.4 dS/m)	KBSH-1
T3 (0.9 dS/m)	PKVSH-27
T4 (1.3 dS/m)	APSH-11
T5 (1.7 dS/m)	MORDEN

Total number of tubes were = 4 (Genotypes) × 4 (treatments) x 10 (Replication) = 160

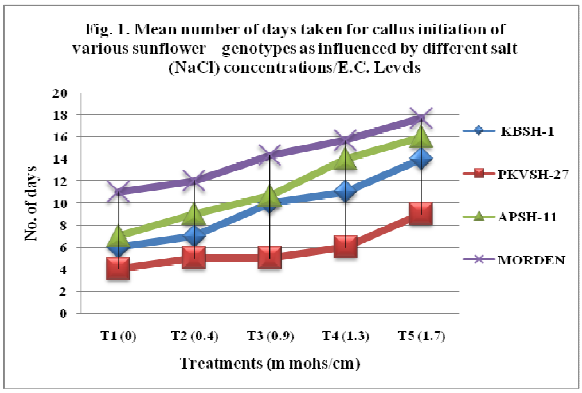
Healthy and uniform seeds of four promising genotypes of sunflower namely KBSH-1, PKVSH-27, APSH-11 and Morden were cleaned thoroughly and sterilized with 0.1% $HgCl_2$ for 15 minutes, then rinsed with sterile water and transferred onto filter paper boats in sterile culture tubes for germination and seedling growth. Seeds were cultured on MS medium and incubated on 25 ± 1°C and 1,000 Lux for 16 h/day. Cotyledonary leaf explants of 7 day old seedlings of all these genotypes were excised and inoculated on to inoculation MS media (Murashige and Skoog, 1962) sterilized by autoclaving under 1.04 kg/cm2 pressure and 121°C for 15 min which was supplemented with 1.0 mgl^{-1} Naphthalene acetic acid (NAA) in combination with 0.5 mgl^{-1} benzyl amino purine (BAP) containing five test concentrations of salt (NaCl) solutions (Table1). 30 tubes were inoculated per genotype per treatment. The parameters, time taken for callus initiation and relative callus growth by genotypes under various treatments were recorded four weeks after imposing salt stress. All experiments were carried out by using complete randomized design (CRD) with 10 replicates for each genotype and each concentration and results were analyzed statistically according to LSD in probability level of 0.05 (Franklin and Dixon, 1996).

Results and Discussion

The results indicated the presence of significant difference between the genotypes in terms of callus induction ability. The visual swelling of the cotyledonary leaf explant and creamy colour of the mass of callus was considered for ascertaining the callus initiation. Significant individual effects of genotype, treatments were observed with respect to days to callus initiation but trivial in interaction between genotypes and treatments. Callus initiation was found to be earlier in control treatment in all the selected four genotypes. As the salt (NaCl) concentration/E.C. level increased, number of days taken for callus initiation also increased. Among the genotypes, callusing was found to be initiated earlier in PKVSH-27 (4^{th} day) followed by KBSH-1, APSH-11 (6^{th} and 7^{th} day) respectively, and very late in Morden (11^{th} day) (Table1) (Fig.1). Similar results were reported by Jyotsna (1997). It can be concluded that, the callus induction is affected by many factors included the composition of the media. It also depends on the type and concentration of growth regulator, source of plant tissue cultured, and genotype (Mohammad & Roof (1981); Mohamand & Nabors (1991). Al-Jibouri *et al.,* (2003)) in their test on callus induction of sunflower and wheat confirmed that the genotype and media composition have the main role in *in vitro* callus induction.

Table.1: Mean number of days taken for callus initiation of various sunflower genotypes as influenced by different salt (NaCl) concentrations/E.C. Levels

Genotypes	Treatments [salt (NaCl) concentrations/ E.C. levels in dS/m					Mean
	T1	T2	T3	T4	T5	
	0	0.4	0.9	1.3	1.7	
KBSH-1	6	7	10	11	14	9.6
PKVSH-27	4	5	5	6	9	5.8
APSH-11	7	9	11	14	16	11.3
MORDEN	11	12	14	16	18	14.1
Mean	7.0	8.3	10.0	11.7	14.2	10.2
	Genotype (G)	Treatment (T)	Interaction (GXT)			
SED	0.39	0.44	0.88			
C.D (p=0.05)	0.80	0.89	1.78			
C.D (p=0.01)	1.07	1.19	2.39			
CV%	10.44					
S/NS	S	S	NS			



The callus growth decreased with the increased salt concentration of media. Relative callus growth also significantly differed among genotypes, treatments as well as their interaction (Table 2). The control treatment recorded maximum callus growth in all the genotypes and decreased significantly as the salt (NaCl) concentration increased. Genotype PKVSH-27 recorded maximum relative callus growth of 4.48 and was significantly superior over other genotypes, followed by KBSH-1 (3.14) and APSH-11 (2.35). Morden recorded the least value of 0.94 (Table2). Irrespective of treatments, the genotype PKVSH - 27 gave mean 2.9 and differed significant from the other genotypes APSH-11 and Morden which gave mean growth of 1.4 and 0.5 respectively. Also it is noticed the presence of significant interaction among genotypes and the concentration levels of salinity (treatments). The mean growth of callus was 4.48 for genotype PKVSH-27 under salt (NaCl) concentration T1 and differed significantly from most of the interactions, whereas the lowest callus mean weight was 0.20 for the genotype, Morden at salt concentration T5 (1.7 dS/m) (Fig.2.)

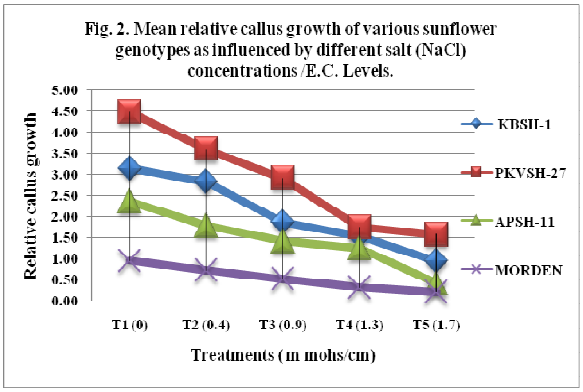


Table.2: Mean relative callus growth of various sunflower genotypes as influenced by different salt (NaCl) concentrations /E.C. Levels.

Genotypes	Treatments [salt (NaCl) concentrations/ E.C. levels in dS/m					Mean
	T1	T2	T3	T4	T5	
	0	0.4	0.9	1.3	1.7	
KBSH-1	3.14	2.83	1.86	1.53	0.94	2.1
PKVSH-27	4.48	3.60	2.91	1.75	1.57	2.9
APSH-11	2.35	1.77	1.41	1.24	0.40	1.4
MORDEN	0.94	0.72	0.51	0.31	0.20	0.5
Mean	2.7	2.2	1.7	1.2	0.8	1.7
	Genotype (G)	Treatment (T)	Interaction (GXT)			
SED	0.07	0.08	0.16			
C.D (p=0.05)	0.14	0.16	0.31			
C.D (p=0.01)	0.19	0.21	0.42			
CV%	11.87					
S/NS	S	S	S			

These results are in conformity with the findings of Prakash *et al*., 1993. Carceller and Ambrogio (1994) developed callus in sunflower with and without salt stress and reported that salinity significantly decreased callus growth. Salinity would lower availability of water and nutrients which have negative effect on growth and division of callus cells. Similar results were obtained by Al-Jibouri *et al.,* (2008) in their study on four cultivars of bread wheat. Alhabaidy *et al.,* (1993) found results in accordance with above, in callus growth of two genotypes of Soyabeans with the increased salinity of media.

A significant difference in callus growth was brought about at salt (NaCl) concentration/E.C. level of 1.3 dS/m using cotyledonary leaf as explant. Therefore cotyledonary leaf explant and salt (NaCl) concentration/E.C. level of 1.3 dS/m could be helpful for large scale screening of sunflower genotypes for salt tolerance. The genotypes, which exhibited earlier callusing and high callus growth, could be considered salt

tolerant. These findings are the results of preliminary evaluation in laboratory and these genotypes could be further evaluated in field conditions for confirmation and further studies.

Conclusion

Using of *in vitro* screening and tissue culture is a successful technique for evaluation of genotypes for salt tolerance and recommendations can be given on the salt-tolerant genotypes for cultivation in salt-affected lands in India. From the findings of the present study it might be concluded that, among the genotypes, PKVSH-27 was found to be salt tolerant with regard to induction and relative growth of the callus. It was observed that PKVSH-27 genotype cultured in salt (NaCl) solution treated MS media gave the best callus which can be utilized for further salt resistance analysis in future. However, more research needs to be undertaken on the mechanism of salt tolerance at the biochemical and molecular level.

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Source of support: Nil
Conflict of interest: None Declared