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Effect of MannitolInduced on Drought Tolerant in some Sugarcane (*Saccharumofficinarum* L.) Genotypes

[1]Wafaa E.Gard, [2]Ahmed E. Khaled, [1]Nabawya S.A. Ghura and[2]Nader R. Abdelsalam

[1]*Department of Breeding and Genetics, Agriculture Research Station, Sabahia, Sugar Crops Research Institute, Agriculture Research Centre, Alexandria, Egypt.*
[2]*Agricutural Botany Department, Faculty of Agric. Saba Basha, Alexandria University.*

ABSTRACT

The present research work has been carried out to study the response of three genotypes of sugarcane(*Saccharumofficinarum* L.) for callus induction**,**day to callus initiation,embryogenic callus production and their droughttolerance*in vitro*.The threegenotypes responded well to callus induction with about 70 - 86%. The highest frequency 86±3.16 was recorded to GT 54-9. Although the two genotypes GT 54-9, G 84–47 had the same day to callus initiation in average 10 days, GT 54-9 was the highest one compared with others in mean 14±2.0 and LSD=2.38. While, the highest day to callus initiation was 14 d for ph 8013. The results showed the high embryogenic callus percentages ~ 80%. While, no significant difference was observed between GT 54-9 and G 84–47 ($L.S.D._{0.05}$) which gave the best response. Callus growth decreased under stresses among all sugarcane genotypes. Mannitol induced osmotic stress seemed to be more harmful to ph 8013 callus RGR than GT 54-9.

Key words:Genotypes**,**sugarcane**,**drought, tolerance

Introduction

Sugarcane (*Saccharumofficinarum* L.) is an important sugar crop for produced the sweetener and sugar. Commercial production of sugar from sugarcane began in India and China approximately 2500 years ago and spread to Western Europe in the eighteenth century (James, 2004). Today, sugar production, sugarcane are used as raw materials for fuel production, chemicals, bio-fertilizers, paper and pulp (Almazan*et al.*, 1998 and Arruda, 2011).

Abiotic stresses especially salinity and drought limit crop productivity (Akhtar *et al*. 2003). Sugarcane is extensively cultivated in irrigated lands worldwide. Being a typical glycophyte, itexhibits stunted or no growth under salinity, with its yieldfalling to more than 50% as compared to its true potential (Wiedenfeld 2008) due to alterations in water relations, ionic and metabolic perturbations, and tissue damage (Patade*et al.* 2011).In tolerant plants, there are many defense mechanisms such as osmoregulation, ion homeostasis, antioxidant and hormonal systems, helping plants to stay alive and development prior to their reproductive stages (Hasegawa *et al*., 2000; Wang *et al*., 2003; Reddy *et al*., 2004; Sairam&Tyagi, 2004; Mahajan &Tuteja, 2005; Ashraf, 2010).

Excess of Na^+ and Cl^- ions may lead to conformational changes in protein structures, while the osmotic stress leads to turgor loss and cell volume change (Chinnusamy and Zhu, 2003). However, the precise mechanisms underlying these effects are not fully understood because the resistance to salt stress is a multi-genic trait (Parida and Das, 2005). To achieve salt tolerance, plant cells evolve several biochemical and physiological pathways. These processes are thought to operate additively to ensure plants and cells survival and they include the exclusion of Na^+ ions and their compartmentation into vacuoles as well as the accumulation of compatible solutes such as proline, glycinebetaine and polyols (Hasegawa *et al.* 2000; Chinnusamy and Zhu,2003 andParida and Das 2005).

In vitro tissue culture constitutes an important tool to study the physiological and biochemical mechanisms that operate in response to stress conditions at the cellular level (Lerner 1985). Furthermore, the plant tissue culture allows the control of stress homogeneity and the characterization of cell behaviour under stress conditions, independently of regulatory systems that take place at the whole plant level (Lutts*et al.* 2004).In the present research, we studied the responses of Na^+ and K^+accumulation in sugarcane embryogenicwhich treated with different levels of mannitol as drought indicator.

Materials and Methods

A- Plant material and culture conditions:

ThreeSugarcane genotypesi.e. ph 8013, GT 54-9 and G 84 - 47were selected and tested in the present research. Stem sections containing two lateral buds were planted in plastic pots containing soil in greenhouse conditions until reaching ~6 months. Calli were induced from leaf segments of theyoungest leaves. Stem sections were surface sterilized with 75%(v/v) ethanol. Then, immersed in mercuricchloride HgCl2 0.03% (w/v)

Corresponding Author: Wafaa E. Gard, Department of Breeding and Genetics, Agriculture Research Station, Sabahia, Sugar Crops Research Institute, Agriculture Research Centre, Alexandria, Egypt

for 30 min followed bythree rinses with sterile distilled water for 10 min each(Tomader*et al*. 2007). Explants were aseptically inoculated onto MS medium (Murashige and Skoog 1962)supplemented with 3mg^{l-} 2,4-D and pH=5.8. Callus induction was performed in thedark at 25 ± 2_ºC in a growth chamber.

B- Callus relative growth rate determination:

After 10 weeks, 30-35 calli were individually weighed and placed on MS medium supplemented or not with the stress factors. Iso-osmotic concentrations mannitol (100, 200 and 300 mM) were applied. After 4 weeks of the exposure to stress conditions, the calli were weighed and characterized for ion concentrations.

Callus relative growth rate (RGR) was determined on a fresh weight (FW) basis according to the formula: RGR = [(FWf – FWi)/FWi], where FWf and FWi are the final and initial FW of the calli(Vikas*et al* 2014).

Results and Discussion

A- Callus induction:

Stem sections ofsugarcane genotypes(GT 54-9, G 84 – 47 and ph 8013) were used as starting materials on MS containing 3 mg^{l-} 2,4-D for callus induction. The explants wereinduced to develop callusat all genotypes. The results clearly indicated that the degree ofcallus proliferation varied from 70-86% (Figure. 1).Analysis of variance indicated high significant difference between three genotypes with LSD=3.88 in relation to percentage of callus induction. The highest frequency (86±3.16) was recorded to GT 54-9 compared to the other two genotypes(70±1.87, 80±20) in respect, as shown in (Table. 1).Although the two genotypes GT 54-9, G 84 – 47 had the same day to callus initiation in average 10 days, GT 54-9 was the highest one compared with others in mean 14±2.0 and LSD=2.38. While the highest day to callus initiation was 14 d for ph 8013 and showed the second value in callus induction (Figure. 2). These results is agreement with those Burner (1992) and Badawy *et al*. (2008) which worked on sugarcane and found that callus induction capacity dependent on the genotype. Also, in rice Van Sint Jan *et al.* (1990) detect the same results.

GT 54-9 G 84-47 ph 8013

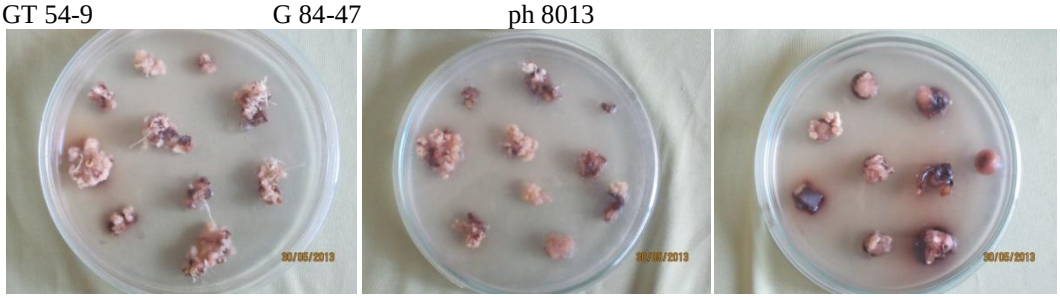

Fig. 1: Different callus induction in three sugarcane genotypes GT 54-9, G 84 – 47 and ph 8013.

Table 1: Callus induction percentage and day to callus initiation of three sugarcane genotypes.

	Day to callus initiation			% of callus induction		
Genotypes	Min.	Max.	Means±SD	Min.	Max.	Means±SD
GT 54-9	8	11	10 ± 1.22^{a}	82	90	86 ± 3.16^{c}
G 84 - 47	8	12	10 ± 1.58^{a}	68	73	70 ± 1.87^{a}
ph 8013	11	16	14 ± 2.00^{b}	78	83	80 ± 2.00^{b}

*L.S.D.$_{0.05}$ (Day to callus initiation) =2.3816 * L.S.D.$_{0.05}$ (% ofcallus initiation) =3.8815, * Means within columns followed by the same letter are not significantly different from each other, L.S.D. test.

B- Embryogeniccalli production:

Distinction between embryogenic and non-embryogenic callus was carried out on the basis of callus external aspect (Figure, 3). In the present experiments, in addition to these two previous types, results showed an intermediary type with a nonembryogenic tissue covered by an embryogenic tissue. This type of callus had been already observed for sugarcane as reported by Guiderdoni, (1986). For embryogeniccalli percentage determination, we classified the intermediary type as embryogenic because, in further subcultures, the embryogenic tissue grows faster than the nonembryogenic tissue. The results in Figure (4) showed the high embryogenic callus percentages ~ 80%. While no significant difference was observed between GT 54-9 and G 84 – 47 (L.S.D.$_{0.05}$) which gave the best response compared to the other genotype, (Table. 2).

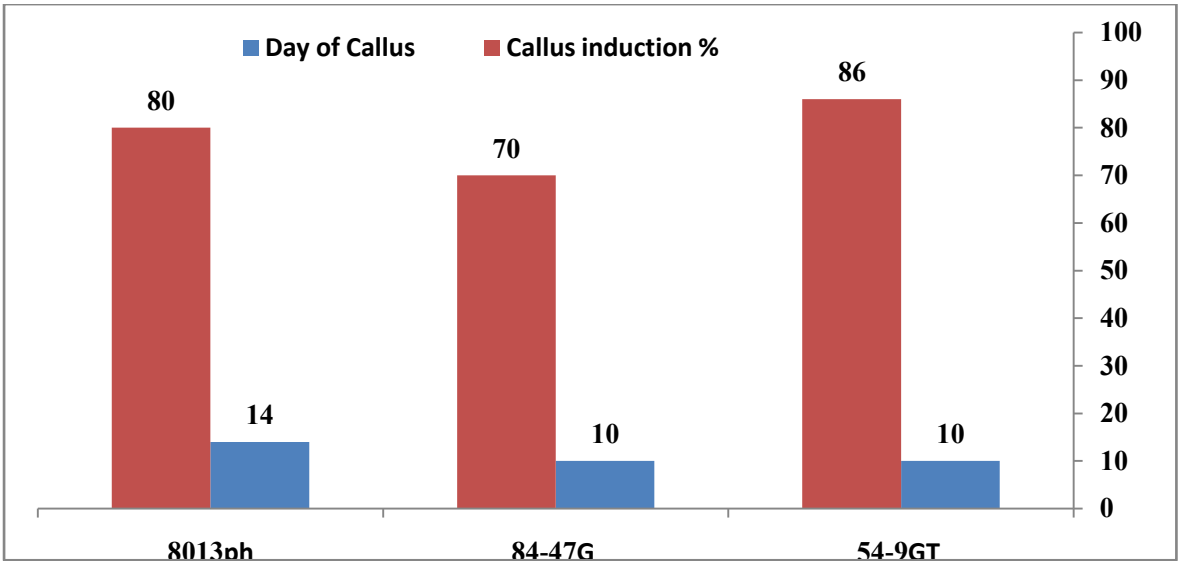

Fig. 2: Callus induction and day of callus initiation in three sugarcane genotypes.

Non-embryogenicEmbryogenic

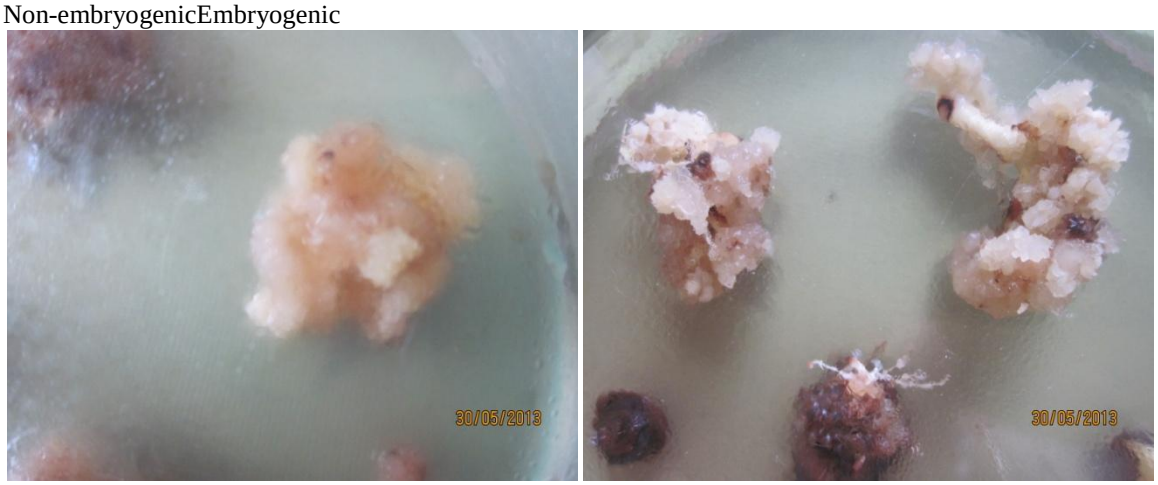

Fig. 3: Embryogenic& non embryogenic callus induced from leaf explant of sugarcane genotypes.

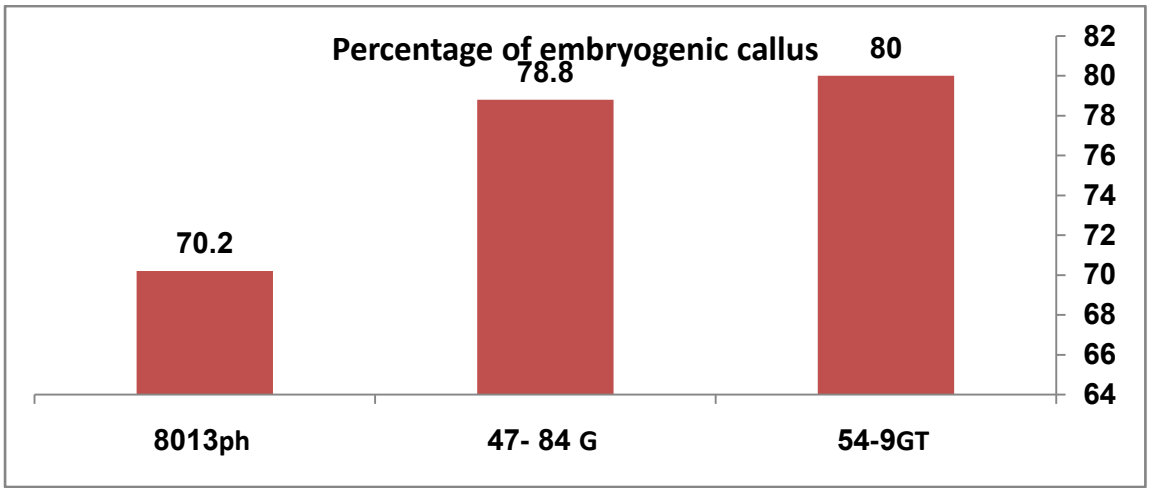

Fig. 4: Percentage of embryogenic callus in three sugarcane genotypes.

Table 2: Percentage of embryogenic callus of three sugarcane genotypes.

Genotypes	Min.	Max.	Means ± SD
GT 54-9	76	84	80± 3.16 [b]
G 84 - 47	76	81	78.8± 2.17 [b]
ph 8013	69	71	70.2± 0.84 [a]

*L.S.D.$_{0.05}$ =3.67, *Means within columns followed by the same letter are not significantly different from each other, L.S.D. test.

C- Callus relative growth rate (RGR):

Results in Table (3) proved that, mannitol-induced stress decreased Relative growth rate (RGR) among all sugarcane genotypes. Mannitol induced osmotic stress seemed to be more harmful to ph 8013 callus RGR than GT 54-9. The highest mean 2.22 was recorded to GT54-9 flowered by 1.57 and 0.73 for G84-47 and ph8013, in respect, with LSD=0.0280. These results are agreement with Basu*et al*., (2002). In contrast, callus RGR of ph 8013 decreased more under mannitol-induced osmotic stress conc. (300 mg/L) than GT 54-9 and G 84 – 47. However, a highly significant difference was recorded for each kind of genotypes (Table. 3).

However, a highly significant difference was recorded for each kind of genotypes (Table 3) . This result recorded with Alvarez *et al*., (2003). The decrease of callus water content was observed under mannitol-induced stress (Table 3). This result agreement with Tomader*et al*., (2007).

Table 3: Effect of mannitol induced stress on callus relative rate growth of sugarcane.

Genotypes	concentrations	Means ± SD	General mean
GT 54-9	control	2.83± 0.03	2.22[c]
	100 mg/L	2.56± 0.06	
	200 mg/L	2.49± 0.04	
	300 mg/L	1.01 ± 0.02	
G 84 - 47	control	2.49± 0.03	1.57[b]
	100 mg/L	1.75± 0.02	
	200 mg/L	1.11 ± 0.01	
	300 mg/L	0.93 ± 0.02	
ph 8013	control	2.39 ± 0.04	0.73[a]
	100 mg/L	0.24± 0.02	
	200 mg/L	0.19± 0.04	
	300 mg/L	0.09 ± 0.01	
L.S.D.$_{0.05}$			0.0280
Control	2.57[d]		
100 mg/L	1.52[c]		
200 mg/L	1.26[b]		
300 mg/L	0.68[a]		
L.S.D$_{0.05}$	0.0323		

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