CHAPTER FOUR
RESULTS AND DISCUSSION

4.1. Callus Production of Sugarcane

4.1.1.Callus Induction

Stem sections of sugarcane 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 were induced to develop callus at all genotypes. The results clearly indicated that the degree of callus proliferation varied from 70 - 86% (Figure 2).

Analysis of variance indicated high significant difference between the three genotypes with LSD=3.88 in relation to percentage of callus induction. The highest frequency was (86±3.16) recorded to GT 54-9 compared to the other two genotypes (70±1.87, 80±20) in respect, as shown in (Table 4).

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 3).

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.

Table (4) 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}
$LSD_{0.05}$	2.3816				3.8815	

*Means within columns followed by the same letter are not significantly different from each other, L.S.D. test.

Figure (2) Different callus induction in three sugarcane genotypes GT 54-9, G 84–47 and ph 8013.

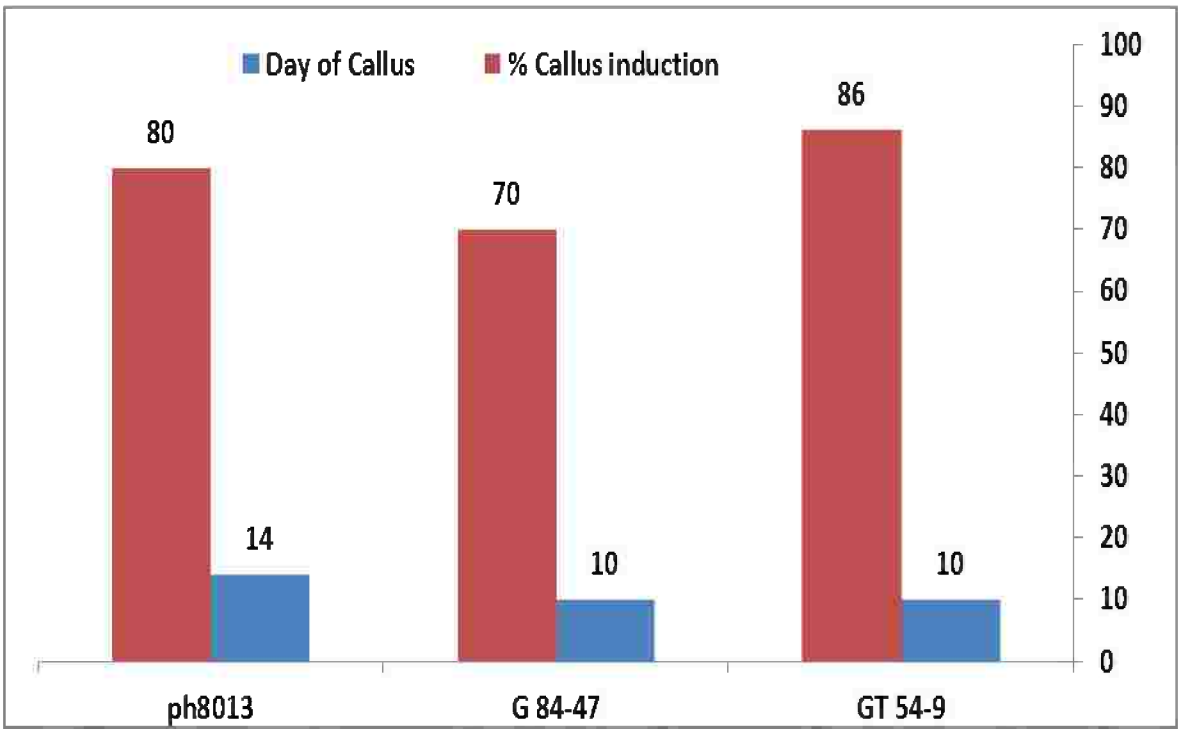

Figure (3) Callus induction and day of callus initiation in three sugarcane genotypes.

4.1.2. Embryogenic Callus Production

Distinction between embryogenic and non-embryogenic callus was carried out on the basis of callus external aspect (Figure, 4). 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 embryogenic calli 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 5 showed the high embryogenic callus percentages 80%. While no significant difference was observed between GT 54-9 and G 84 – 47 in meaning 80 ± 3.16 and 78.8 ± 2.17 with ($L.S.D._{0.05}$) = 3.67 which gave the best response compared to the other genotype, (Table. 5).

The results indicated that the different among GT 54-9 and ph 8013 was 10% when compare the percentage 80% for GT 54-9 and 70.2% for ph 8013 in relation to embryogenic callus (see Figure. 5).

Table (5) 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.

Figure (4) Embryogenic and non-embryogenic callus induced from leaf explant of sugarcane genotypes.

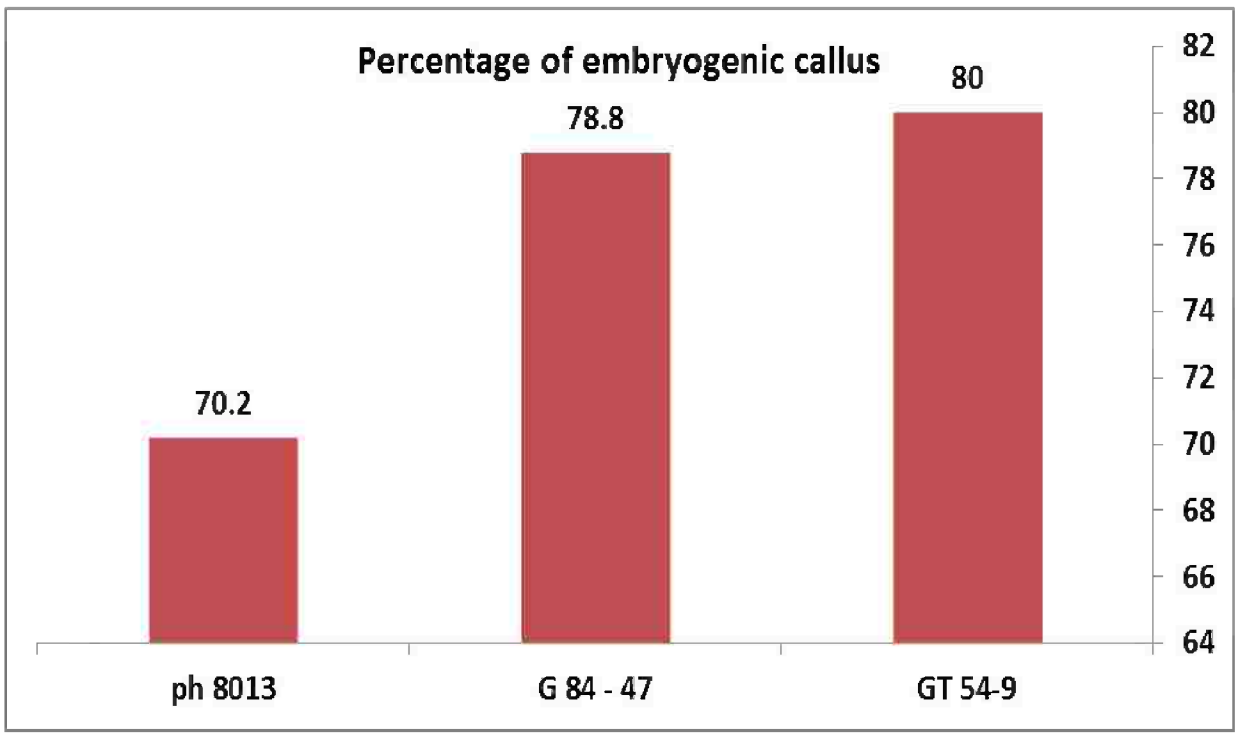

Figure (5) percentage of embryogenic callus in three sugarcane genotypes

4.2. *In Vitro* Drought Tolerance

Three genotypes of sugarcane were used to estimate the degree of drought tolerance *in vitro.*

4.2.1. Effect of Drought on Callus

Drought stress was studied on the three genotypes of sugarcane which were grown as callus cultures under different degrees of osmotic stress induced by adding mannitol at concentrations of 0, 100, 200 and 300mM. Callus relative growth rate, percentage of water content, ions determination and proline estimation were studied after 4 weeks.

4.2.1.1. Relative Growth Rate (RGR) and water content (WC)

Callus cultures initiated from leaf explants on MS medium supplemented with 3mg/L of 2,4-D mannitol was added to the medium at the final concentration of (0, 100, 200 and 300 mM). Initial callus fresh weight was recorded and after four weeks final callus fresh weight was recorded to estimate RGR. The obtained data is illustrated in Table (6, 7) and Figure (6, 7).

The results showed that the maximum RGR were obtained for GT 54-9 (2.22± 0.15) while the lowest mean was 0.73 ± 0.20 for ph8013 (Table 6). On the other hand our results showed that control callus gained the highest RGR in mean 2.83 ± 0.011 for GT 54-9 followed by mannitol treated callus (Table 7).

Among the treated samples, callus treated with 100mM mannitol showed the highest RGR in mean 2.56 ± 0.022 for GT 54-9 while, with 300mM callus recorded the lowest RGR by mean 0.09 ± 0.002 for ph8013 (Figure 7). The results clearly indicated that, by increasing the mannitol concentration, the RGR of callus decreased.

Data in Table (7) showed that significant effect between the three genotypes and treatments. The result showed that the maximum callus RGR in control and treated genotypes were decreased by increasing the concentrations of mannitol.

Comparison between means of genotypes as shown in Figure (6 and 7) indicated that genotypes differ significantly in callus RGR. Genotype GT 54 -9

gave the highest RGR after all treatment. While ph 8013 recorded the lowest after all treatment.

The obtained results clearly showed that by increasing mannitol concentration, the callus relative growth rate was decreased as showed in Figures (8, 9 and 10).

Concerning to percentage of water content, callus fresh weight and dry weight were recorded after four weeks of treatment. The obtained data is illustrated in Table (6,7) and Figure (11,12). Results showed that the highest %WC was 77.952 ± 2.36 for GT 54 -9 while, the lowest was 76.12 ± 3.38 for ph8013.

Comparison between the means of genotypes as shown in Table (6) results indicated that genotypes showed no significant variations in callus water content between GT 54 -9 and G 84-47.

Genotype GT 54 -9 recorded the highest water content after all treatment except with 100 mM mannitol concentration. While, ph8013 recorded the lowest % WC value in mean 54.45 ± 0.796 after treatment with 100mM mannitol as shown in Figure (12).

In general, callus water content was decreased with increasing mannitol concentrations. Among the 300mM mannitol concentration reduced %WC for all genotypes more than 100mM mannitol concentration as shown in Figure (13).

Results came in agreement with **Errabii *et al.* (2007)** who placed callus of sugarcane on MS medium supplemented or not with the stress factors. Iso-osmotic concentrations of NaCl (50,100 and 150 mM) and mannitol (100, 200 and 300 mM). The author reported that NaCl and mannitol-induced stress decreased RGR among all the cvs. However, a highly significant difference was recorded among the effects of each kind of stress. Mannitol-induced osmotic stress seemed to be more harmful to CP59-73 and NCo310 callus RGR than NaCl-induced stress.

Also, **Patade *et al.* (2008)** reported that callus RGR of sugarcane decreased progressively with increasing levels stress. Also, **Patade *et al.* (2014)** reported that salt treatment reduced growth rate significantly as compared to the control in sugarcane. This results agreement with **(Mohamed *et al.* 2000; Watanabe *et al.* 2000)** in other species

Our results agreement with **Patade *et al.* (2012)** reported that using iso-osmotic concentrations of PEG and salt, the mechanisms used by plants to avoid or tolerate the respective stress conditions were studied at cellular level. Sugarcane callus tissues showed a more severe reduction in growth on media containing salt or PEG at iso-osmotic levels, indicating sensitivity of the actively growing and undifferentiated tissues to these stresses. Growth reduction was primarily seen to occur due to water loss, since callus fresh weight decreased but the dry weight increased, especially in response to PEG stress.

The current research was in the line with **Errabii *et al.* (2007)** who reported that decrease of callus water content in sugarcane was observed under both NaCl and mannitol induced stress. However, a significant difference was recorded among the effects triggered by each kind of stress. The highest reduction was noticed under mannitol-induced osmotic stress at all the experimented doses and it reached to about 45, 48 and 42% of the control in the presence of the highest mannitol concentration. Thus, the water content followed the same tendency as the callus RGR among the cvs.

Data in the previous tables and figures recommended that mannitol induced stress decreased considerably either RGR or WC values among all genotypes. The highest RGR and WC decrease were noticed under mannitol-induced osmotic stress, which indicated that RGR inhibition is due to the reduction of water availability and the loss of turgor, as reported by **Errabii *et al.* (2007)** and in other species by **(Mohamed *et al.* 2000 and Watanabe *et al.* 2000**)

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Table (6) Means of relative growth rate (RGR) and Percentage water content with standard error between sugarcane genotypes.

Characters / Genotypes	Relative Growth Rate (RGR)	Percentage water content (% WC)
GT 54 -9	$2.22^{a} \pm 0.15$	$77.952^{a} \pm 2.36$
G 84 - 47	$1.57^{b} \pm 0.13$	$77.38^{a} \pm 2.57$
Ph 8013	$0.73^{c} \pm 0.20$	$76.12^{b} \pm 3.38$
L.S.D $_{0.05}$	0.0167	1.0306

*Means within columns followed by the same letter are not significantly different from each other, L.S.D. test.

Table (7) Means of callus relative growth rate (RGR) and water content (% WC) derived from leaf explants of sugarcane after treated with different concentrations mannitol.

Variety	Mannitol (mM)	RGR	% WC
GT 54 -9	Zero	$2.83^{a} \pm 0.011$	$92.50^{a} \pm 0.572$
	100	$2.56^{b} \pm 0.022$	$84.33^{c} \pm 0.125$
	200	$2.49^{c} \pm 0.014$	$71.59^{d} \pm 0.695$
	300	$1.01^{g} \pm 0.005$	$63.41^{f} \pm 0.895$
G 84 - 47	Zero	$2.49^{c} \pm 0.011$	$92.46^{a} \pm 0.718$
	100	$1.75^{e} \pm 0.007$	$85.77^{c} \pm 1.139$
	200	$1.11^{f} \pm 0.004$	$68.31^{e} \pm 0.679$
	300	$0.93^{h} \pm 0.011$	$62.96^{f} \pm 1.346$
Ph 8013	Zero	$2.39^{d} \pm 0.014$	$93.13^{a} \pm 0.387$
	100	$0.24^{i} \pm 0.007$	$90.18^{b} \pm 0.633$
	200	$0.19^{j} \pm 0.015$	$66.72^{e} \pm 0.569$
	300	$0.09^{k} \pm 0.002$	$54.45^{g} \pm 0.796$
L.S.D $_{0.05}$		0.0334	2.0612

*Means within columns followed by the same letter are not significantly different from each other, L.S.D. test.

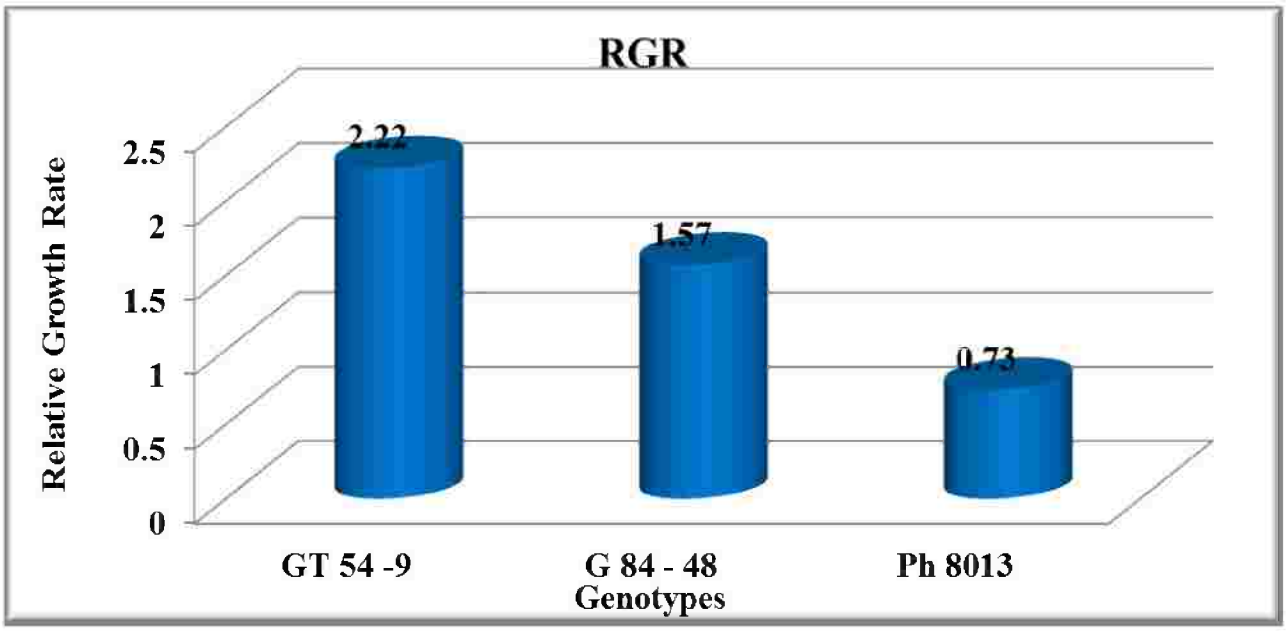


Figure (6) Difference in relative growth rate for all genotypes.

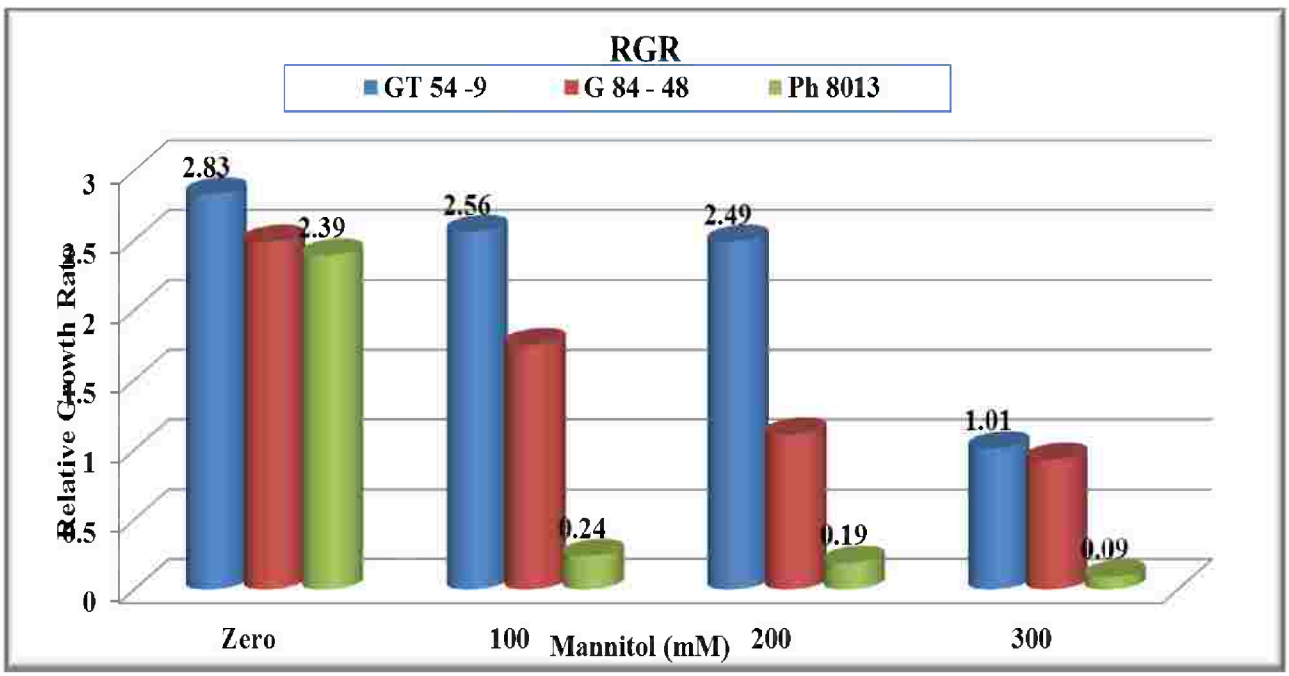


Figure (7) Means of callus relative growth rate derived from leaf explants of sugarcane after treated with different concentrations of mannitol.

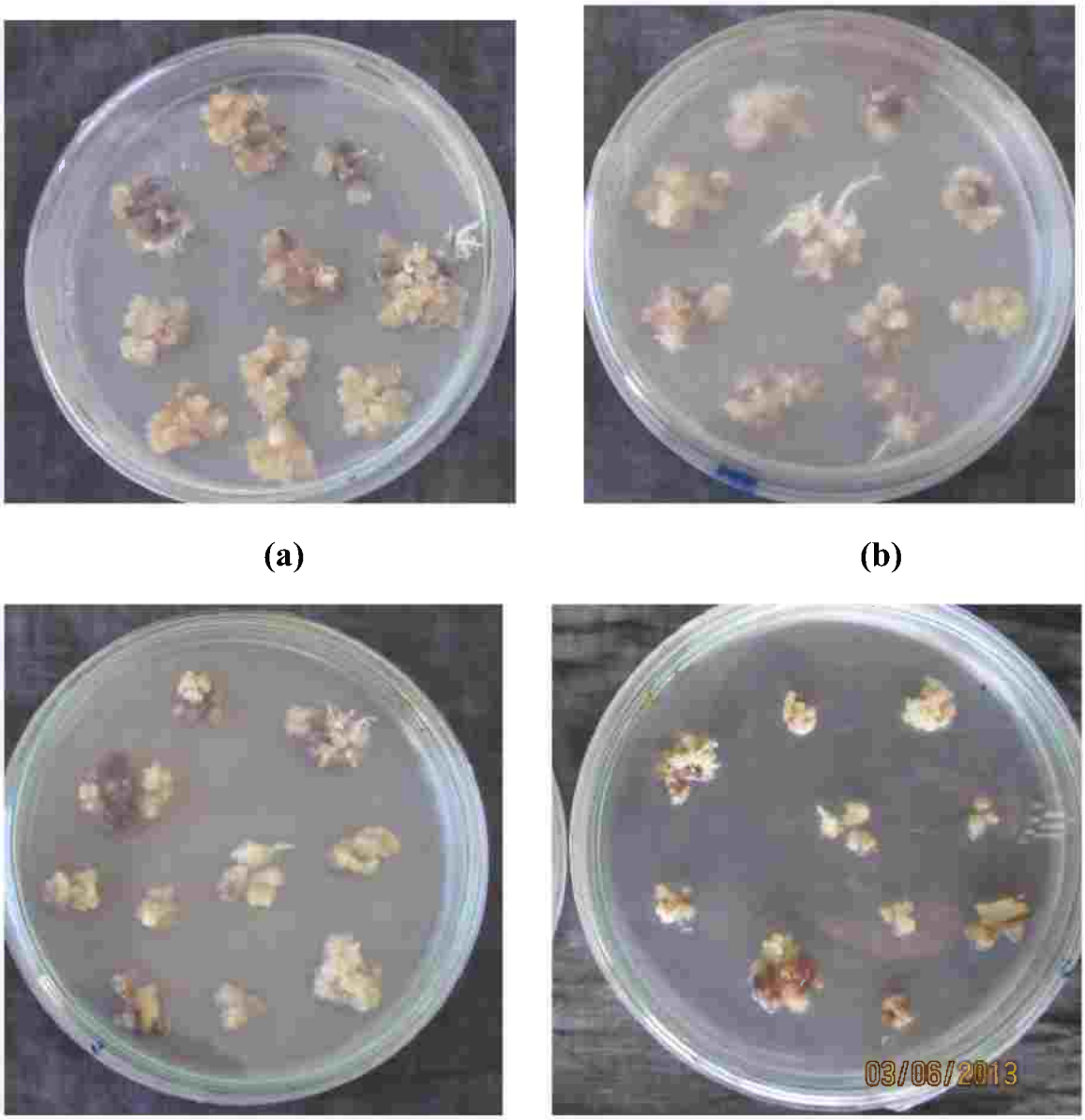


Figure (8) Effect of different concentrations of mannitol (a) 0, (b) 100, (c) 200 and (d) 300 mM on callus relative growth rate of GT 54-9 genotype.

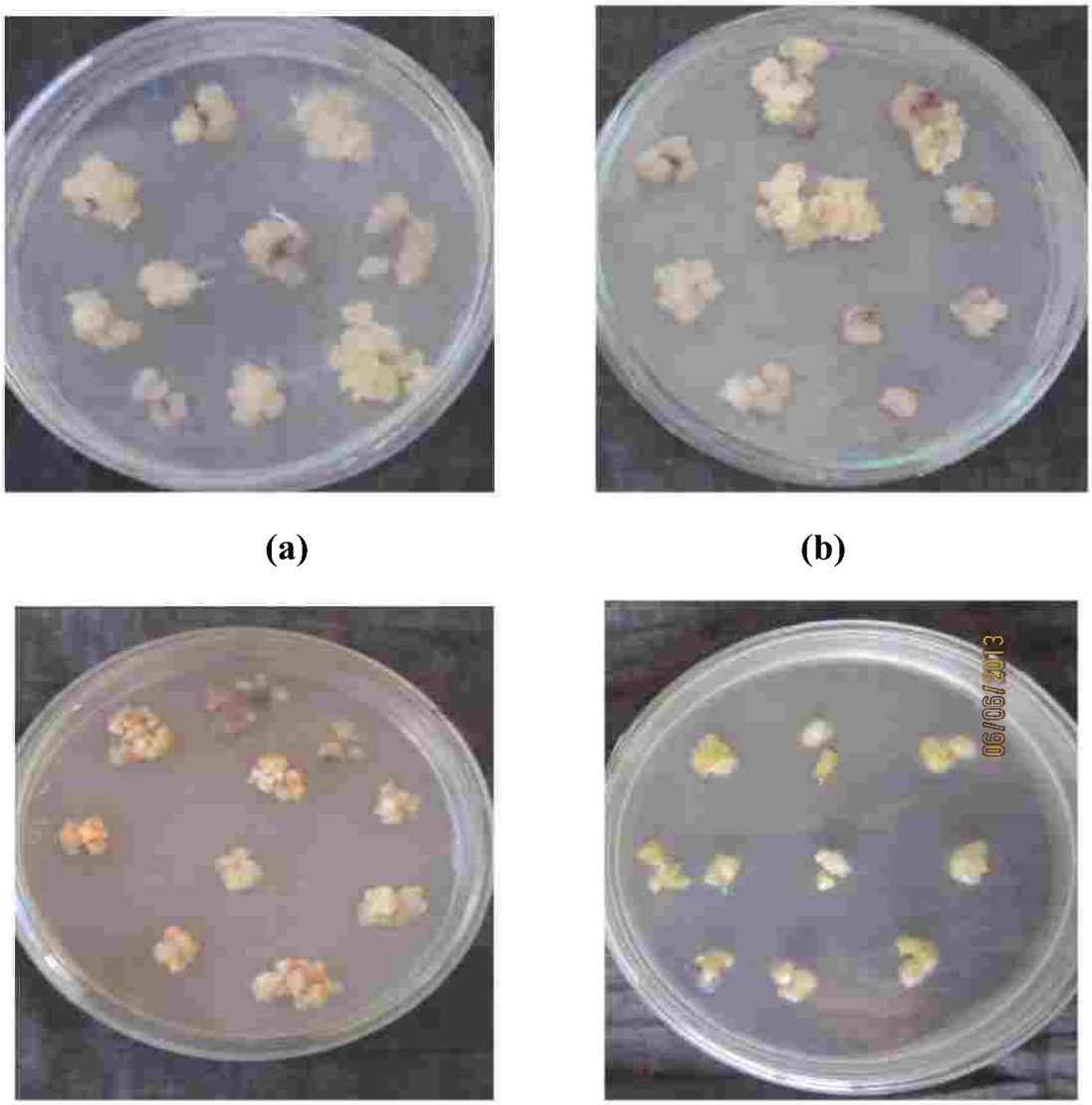
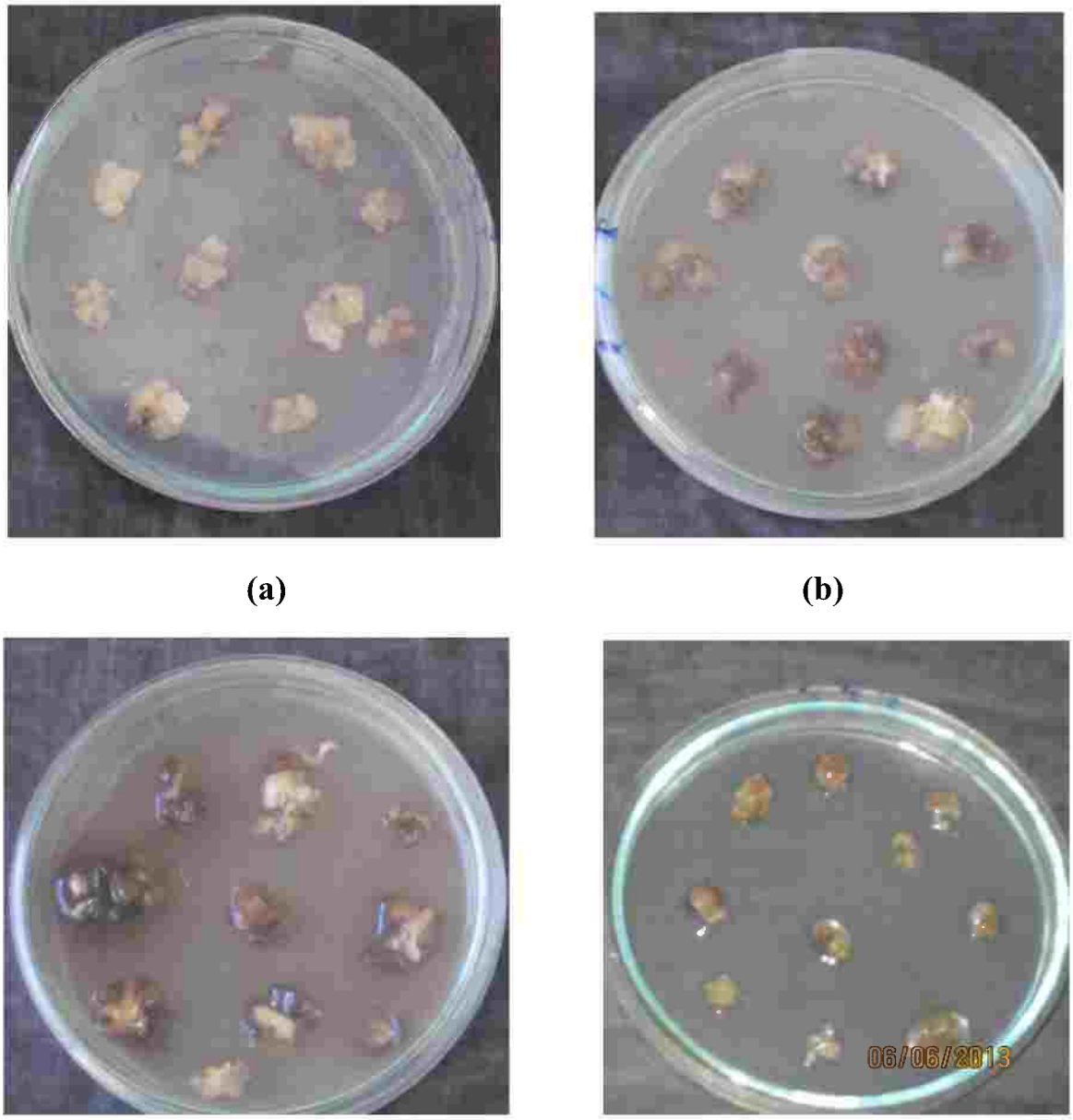


Figure (9) Effect of different concentrations of mannitol (a) 0, (b) 100, (c) 200 and (d) 300 mM on callus relative growth rate of G84-47 genotype.



(a) **(b)**

(c) **(d)**

Figure (10) Effect of different concentrations of mannitol (a) 0, (b) 100, (c) 200 and (d) 300 mM on callus relative growth rate of ph8013 genotype

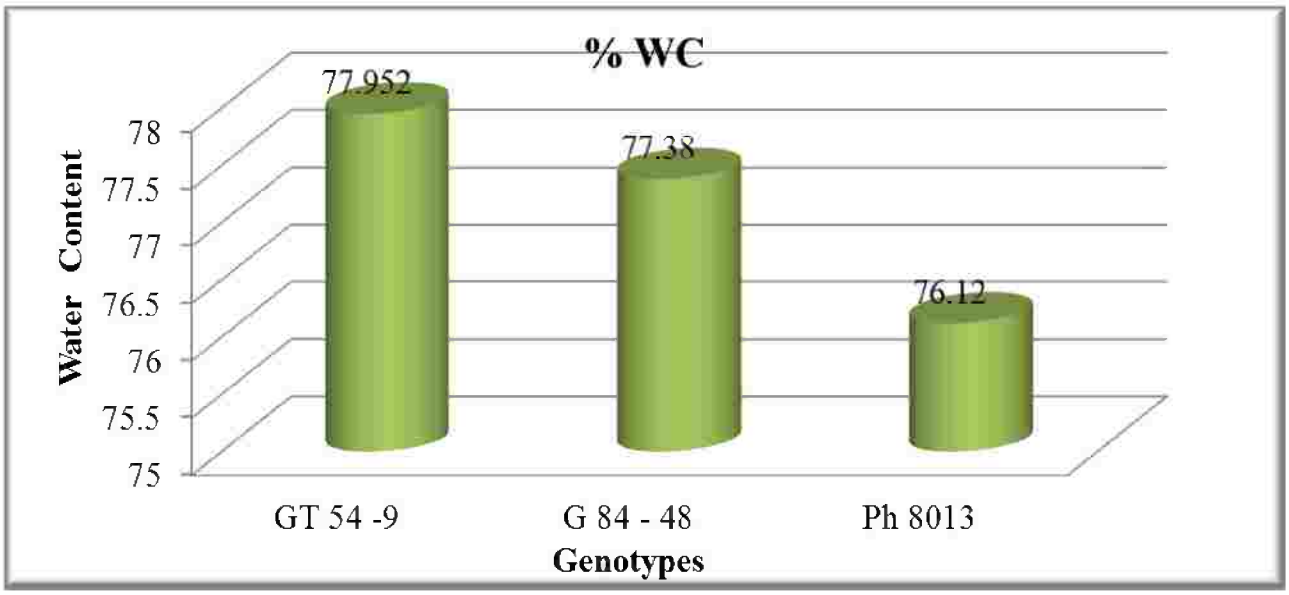


Figure (11) Percentage of water content for sugarcane genotypes.

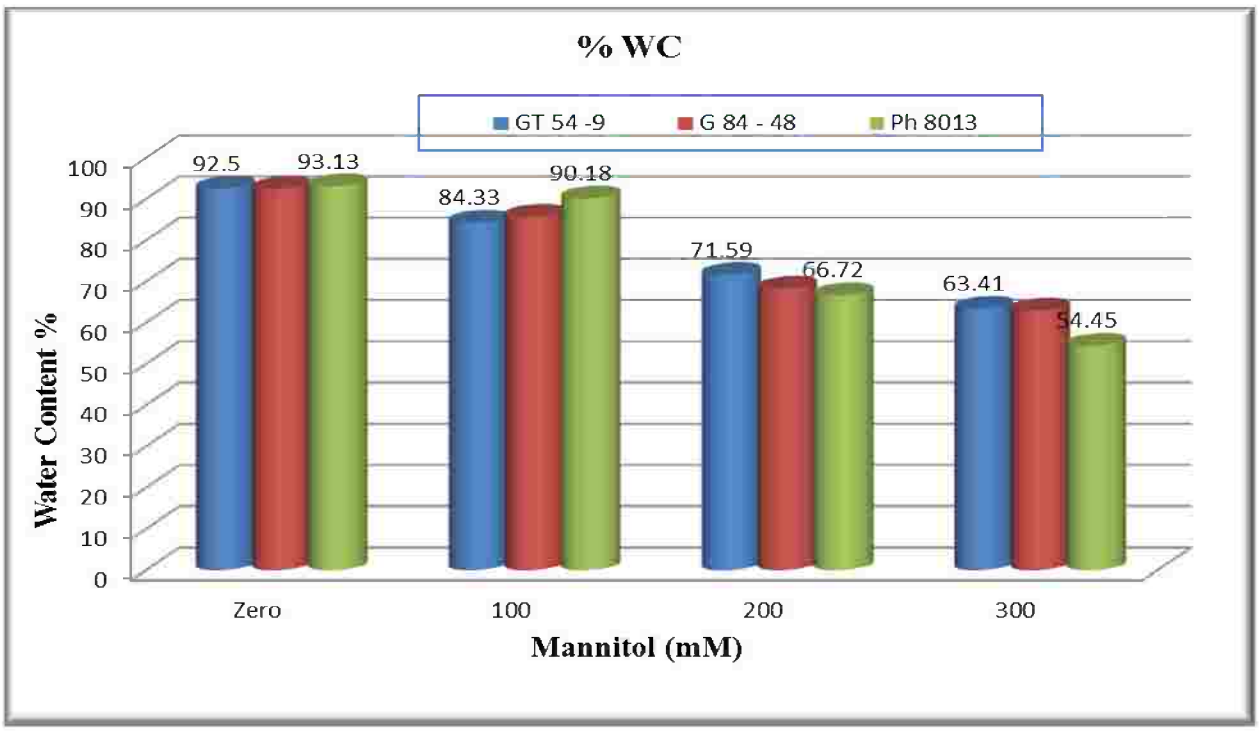


Figure (12) Percentage of callus water content of sugarcane after treated with different concentrations of mannitol.

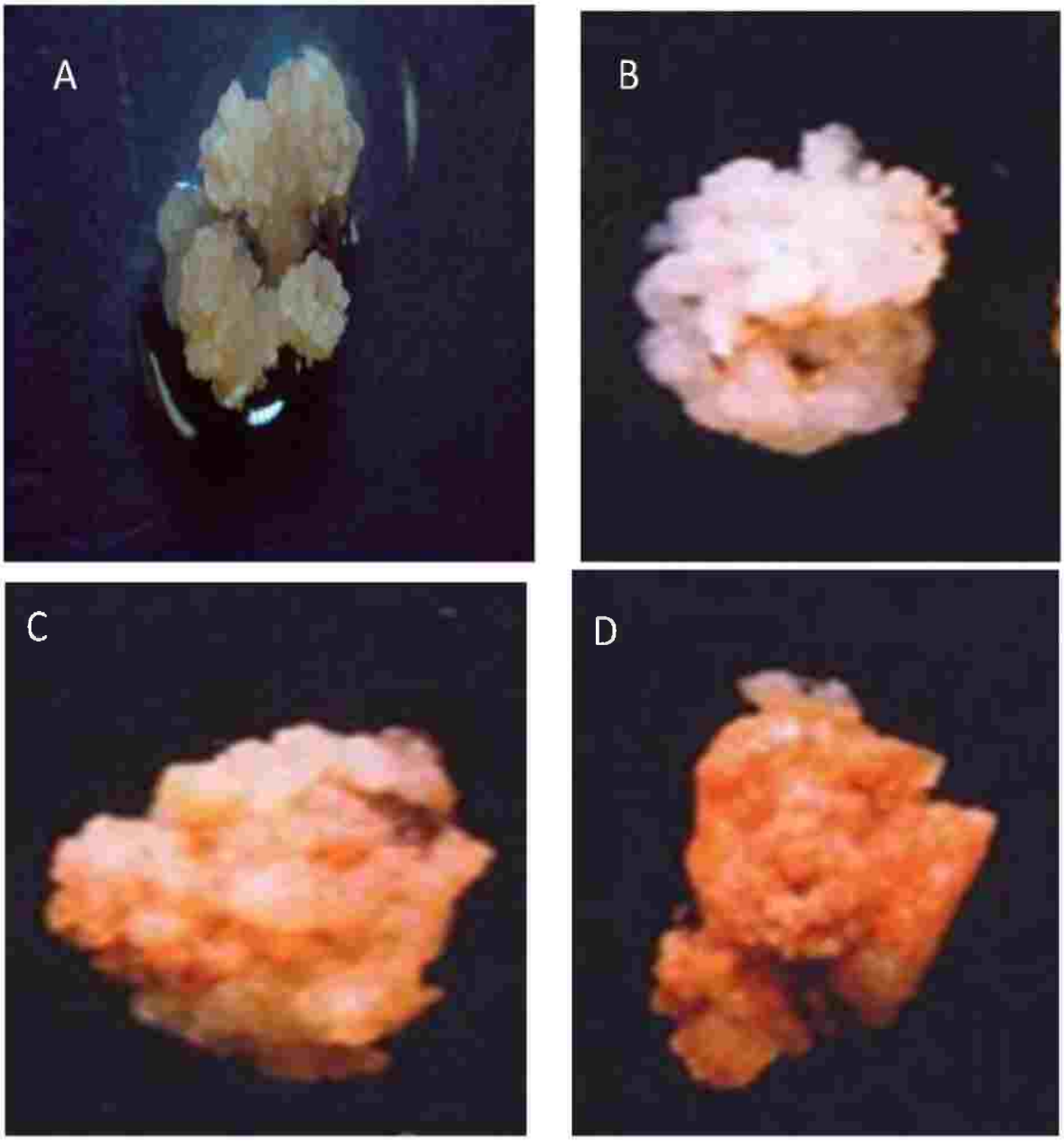


Figure (13) Effect of different concentrations of mannitol (a) 0, (b) 100, (c) 200 and (d) 300 mM on precentage of callus water content.

4.2.1.2. K^+ and Na^+ Ions Determination

Analysis of variance in Table 8 showed high significant different among the three genotypes for Na^+ and K^+ ions. The highest Na^+ value recorded for G84-47 was 0.89 ± 0.03mg/gm (FW) while, the lowest value was 0.74 ± 0.05 for ph8013.

On the other hand, the maximum K^+ were obtained for ph8013 that was 1.81 ± 0.09 mg/ gm. (FW) while, the minimum was 1.39 ± 0.03 mg/ gm. (FW) for GT 54-9 (Table 8).

The present experiment proved inverse relationship between mannitol concentration and Na^+, K^+ content. Results showed with increasing of mannitol concentrations the Na^+ is increasing, while K^+ is decreased in Parallel as shown in Table (9).

The results in Table 9 showed that there are no significant variation between the GT 54-9 and G 84–47 for Na^+ content (1.09 ± 0.031and 1.07 ± 0.033) under 300 mM of mannitol in respect. While, there are high significant interaction with the third genotype Ph 8013 in mean 0.99 ± 0.009. The same trend of K^+ content among the previous two genotype GT 54-9 and G 84–47 recorded the lowest concentrations for K^+ (1.19 ± 0.004 and 1.21 ± 0.018). While, Ph 8013 recorded the highest K^+ value in means 1.34 ± 0.043 as shown in Figure (15). In general, K^+ increased with increasing of mannitol concentrations.

Data in Figure 14 showed the different in Na^+ and K^+ content for the three sugarcane genotypes. From the above results we can calculated that ph 8013had the highest value of K^+ comparing with other genotypes.

Data in Figure 15 showed the variation in K^+ content for the three sugarcane genotypes under different mannitol concentration. It noticed that with increasing of mannitol concentration there are decrease in K^+ content

Our results are agreement with **Errabii *et al.* (2007)** reported that ion concentration was drastically affected after exposure to NaCl and mannitol. The authors reported that, salt stress induced an increase in Na^+ and Cl^- accumulation and a decrease in K^+ and Ca_2^+ concentrations. Under mannitol induced osmotic stress, K^+ and Ca_2^+ concentrations decreased significantly while Na^+ and Cl^- concentrations remained unchanged accumulation of Na^+ and Cl^- but a decrease in K^+ and Ca_2^+under stress in sugarcane calli taken together. Also, noticed in the absence of stress, Na^+ concentration differed

significantly among the cvs., and was lower in salt-sensitive than in salt-resistant cv.

The results in a line with **Patade *et al.* (2008)** suggested that accumulation of ions (Na^+ and K^+) and osmolytes (proline and glycine betaine) may have an important role in osmotic adjustment in sugarcane cells under stress.

A moderate increase of Na^+ and Cl^- within callus tissue might avoid water loss and ensure an economic way to adjust osmotically **(Dutta Gupta *et al.* 1995; Chinnusamy and Zhu, 2003; Benlloch- Gonza` lez *et al.* 2005).** However, when the ability of the cells to compartmentalize the ions into the vacuole is exceeded, ions build up in the cytoplasm and lead to severe ion imbalances and to conformational changes in the plasma membrane electrical potential **(Chinnusamy and Zhu, 2003; Sairam and Tygai, 2004).**

Under induced stress, the increase in Na^+ concentration and the subsequent decrease in K^+ concentration observed among sugarcane cvs. were previously reported in several other species **(Basu *et al.* 2002; Benlloch-Gonza` lez *et al.* 2005)**. This could be due to the fact that some species were able to substitute K^+ by Na^+ to ensure the osmotic adjustment **(Rus *et al.* 1999).**

Table (8) K^+ and Na^+ ions content between the three sugarcane genotypes.

Characters / Genotypes	K^+ mg/gm (FW)	Na^+ mg/gm (FW)
GT 54 -9	$1.39^{c} \pm 0.03$	$0.81^{b} \pm 0.05$
G 84 - 47	$1.55^{b} \pm 0.07$	$0.89^{a} \pm 0.03$
Ph 8013	$1.81^{a} \pm 0.09$	$0.74^{c} \pm 0.05$
L.S.D $_{0.05}$	0.0498	0.0258

*Means within columns followed by the same letter are not significantly different from each other, L.S.D. test.

Table (9) Means of K^+ and Na^+ in callus in sugarcane after treated with different concentrations mannitol.

Genotypes	Mannitol (mM)	K^+ mg/gm (FW)	Na^+ mg/gm (FW)
GT 54 -9	Zero	$1.61^{d} \pm 0.023$	$0.56^{e} \pm 0.016$
	100	$1.41^{fg} \pm 0.029$	$0.58^{e} \pm 0.011$
	200	$1.37^{g} \pm 0.019$	$0.99^{b} \pm 0.006$
	300	$1.19^{h} \pm 0.004$	$1.09^{a} \pm 0.031$
G 84 - 47	Zero	$2.07^{b} \pm 0.045$	$0.69^{d} \pm 0.035$
	100	$1.47^{ef} \pm 0.042$	$0.83^{c} \pm 0.008$
	200	$1.43^{fg} \pm 0.052$	$0.99^{b} \pm 0.001$
	300	$1.21^{h} \pm 0.018$	$1.07^{a} \pm 0.033$
Ph 8013	Zero	$2.57^{a} \pm 0.019$	$0.41^{f} \pm 0.011$
	100	$1.75^{c} \pm 0.048$	$0.56^{e} \pm 0.018$
	200	$1.57^{de} \pm 0.043$	$0.99^{b} \pm 0.005$
	300	$1.34^{g} \pm 0.043$	$0.99^{b} \pm 0.009$
L.S.D $_{0.05}$		0.0995	0.0516

*Means within columns followed by the same letter are not significantly different from each other, L.S.D. test.

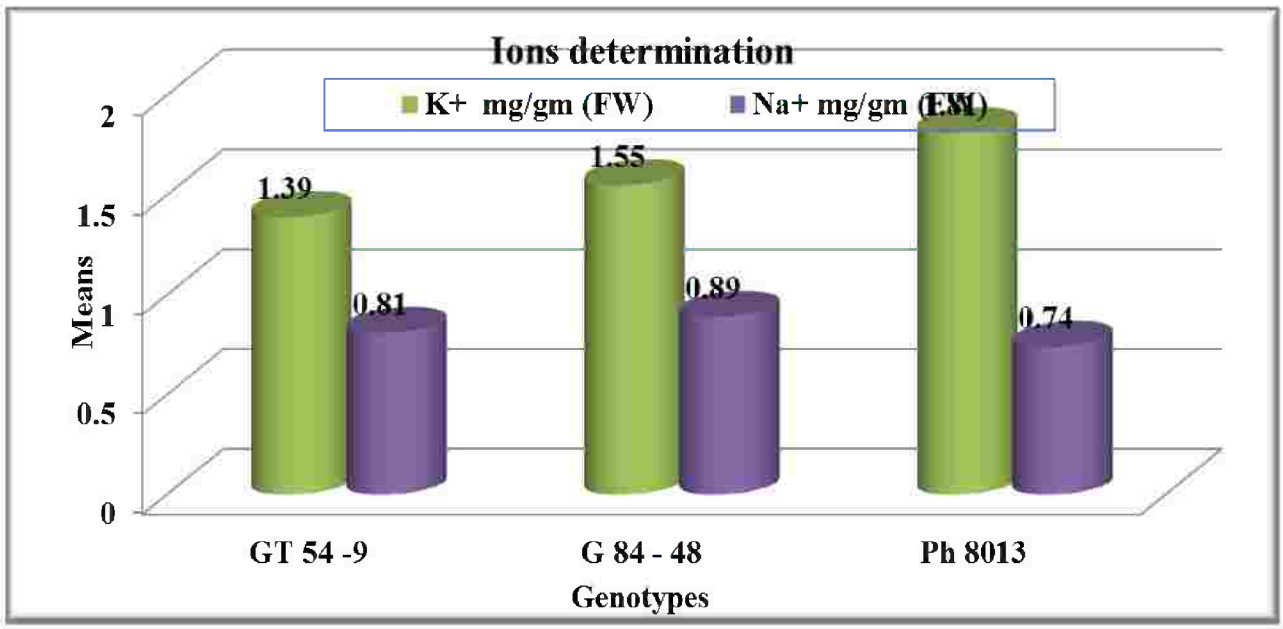


Figure (14) K^+ and Na^+ ions determination for sugarcane genotypes.

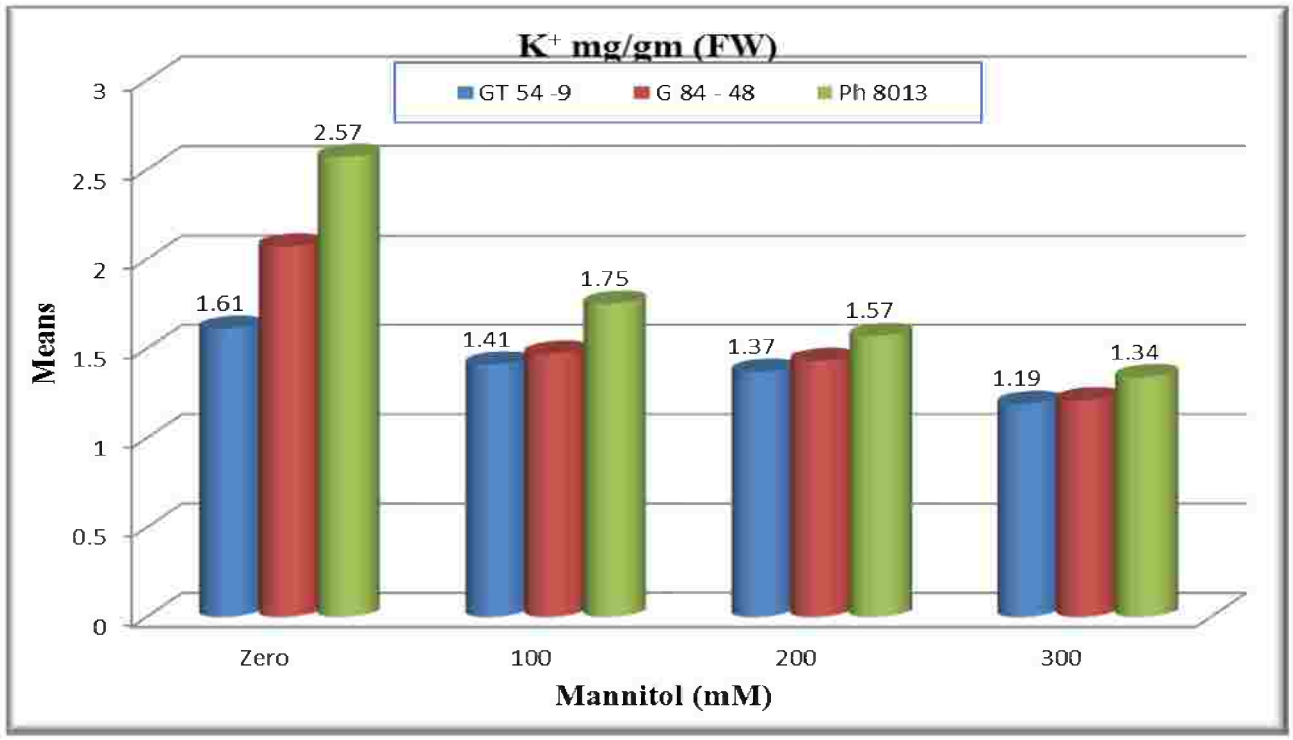


Figure (15) Means of K^+ in callus derived from leaf explants of sugarcane after treated with different concentrations of mannitol.

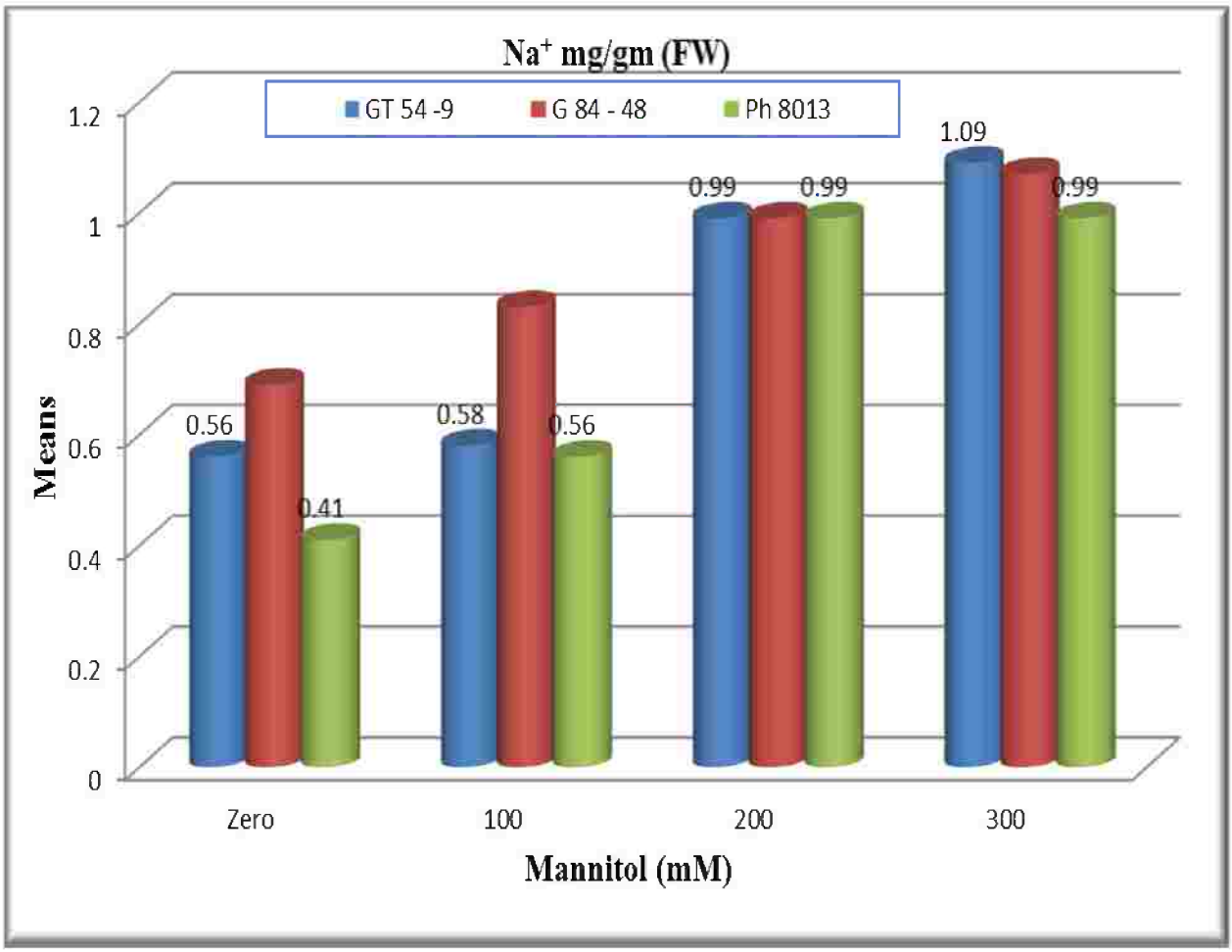


Figure (16) Means of Na^+ in callus derived from leaf explants of sugarcane after treated with different concentrations of mannitol.

4.2.1.3. Proline Determination

Proline content was determined in present study as indicator for drought tolerant in the sugarcane genotypes.

Results showed that the proline content was increased by increasing concentration of mannitol. The regression coeffiancy was done to determine the relationship between the two variables. Proline was considered as the dependent one (Y) while the drought concentration was determined as the independent variable (X) for the genotypes as shown in Figures (17,18 and19) and Tables (10, 11 and 12).

Results showed that increasing or decreasing in proline was due to the change in mannitol concentration. The results in Table (10) showed if mannitol increased by 100mM, proline will be increased by 36.333 ± 0.667mM for GT 54-9.

Whereas in ph 8013 callus, if mannitol increased by 100mM, proline will be increased by 24.667 ± 1.453 mM (Tables, 10 and 12).

The decrease or increase of proline induction due to iso-osmotic stress (drought) indicated the suitability or tolerance of the species to afford abiotic stress, so that, when the proline production is increased, this indicates that the genotype can afford drought stress and it is tolerant to iso-osmotic stress. Again this indicates that GT 54-9 is more tolerant to drought stress than ph 8013 as shown in Figures (17 and 19) and Tables (10 and 12).

The disturbances in plant metabolism induced by abiotic treatments affect generally the various metabolic pools of iso-osmotic stressed plants. These changes in the contents of the various metabolites under drought treatments may indicate an enhancement or retardation in the synthesis, accumulation or consumption of these cellular metabolites.

Proline, which frequently accumulates in stressed cells than any other free amino acid, was always correlated with the stress to which the plant cell is subjected. However, the values of these contents varied according to the degree of stress, the plant species was tested and organ analyzed.

These results are in accordance with those obtained by some other authors, i.e. The accumulation of proline is frequently reported for most of the plant cells and tissues exposed to stress **(Nayyar and Walia, 2004; Errabii *et al.* 2007).**

Also, **Balibrea *et al.* (1999)** mentioned that a change in proline content has been correlated with its capacity to tolerate and adapt to salinity conditions.

Gandonou *et al.* (2006) reported a higher accumulation of proline in salt-tolerant than in non-selected calli of sugarcane. Further studies are needed to investigate the possible involvement of other osmolytes in osmotic adjustments and to determine the significance of proline accumulation in NaCl-treated sugarcane callus cultures.

Our results in the same trend with those **Cano *et al.* (1996); Garcia *et al.* (1997); Tonon *et al.* (2004)** revealed that under both stresses, the stress-resistant cv. accumulated proline at lesser extent than the stress-sensitive one. This finding let us suggest that proline accumulation among sugarcane cvs. is merely a symptom of injury rather than a stress resistance trait. Identical statements were reported in sev- eral other species.

In contrast, the assumption that proline is a stress resistance marker has been widely adopted **(Alvarez *et al.* 2003; Ehsanpour and Fatahian, 2003).** As well, proline can serve as an organic nitrogen reserve ready to be used after stress relief to sustain both amino acid and protein synthesis **(Sairam and Tygai, 2004).** Generally, it can be seen that the increase in mannitol concentration as iso-osmotic stress in sugarcane parallel with the accumulation of proline.

Table (10) Regression between mannitol concentrations (X-factor) and the proline (Y-factor) in GT 54-9 callus.

Genotype (X)	Treatment	Mean ± standard Error (Y)
GT 54-9	0	15.333 ± 0.882
	100	38.333 ± 1.209
	200	72.333 ± 3.180
	300	112.00 ± 1.732

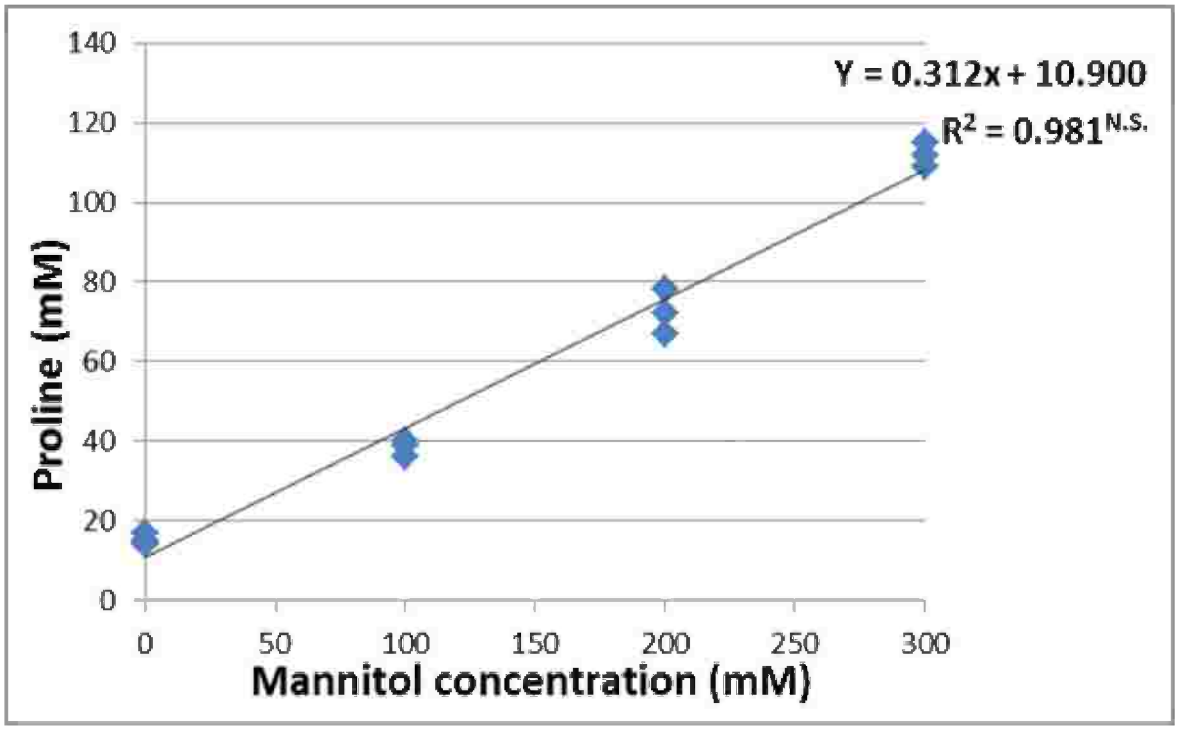


Figure (17) Regression between mannitol concentrations (X-factor) and the proline (Y-factor) in GT 54-9 callus.

Table (11) Regression between mannitol concentrations (X-factor) and the proline (Y-factor) in G 84-47 callus.

Genotype (X)	Treatment	Mean ± standard Error (Y)
G84-47	0	11.000 ± 0.577
	100	36.333 ± 0.667
	200	65.667 ± 2.333
	300	104.33 ± 3.180

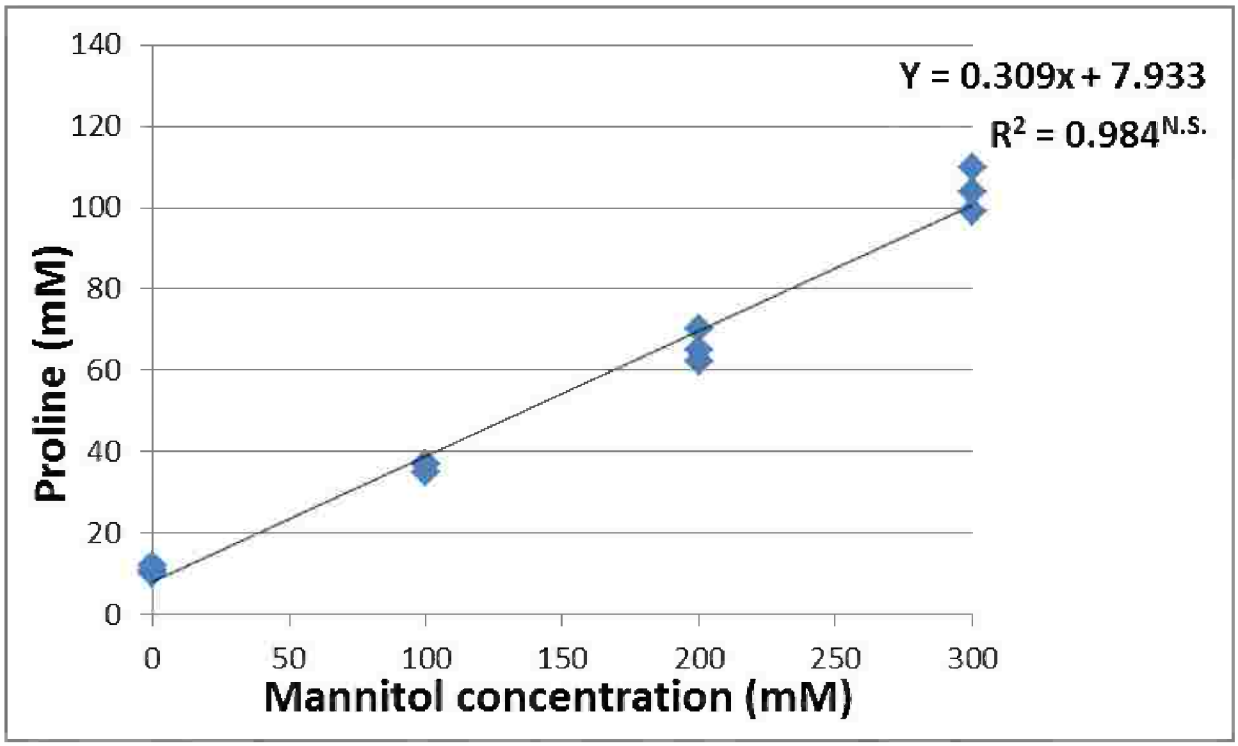


Figure (18) Regression between mannitol concentrations (X-factor) and the proline (Y-factor) in G 84-47 callus.

Table (12) Regression between mannitol concentrations (X-factor) and the proline (Y-factor) in ph 8013 callus.

Genotype (X)	Treatment	Mean ± standard Error (Y)
Ph8013	0	11.333 ± 0.882
	100	24.667 ± 1.453
	200	60.333 ± 2.728
	300	86.000 ± 3.000

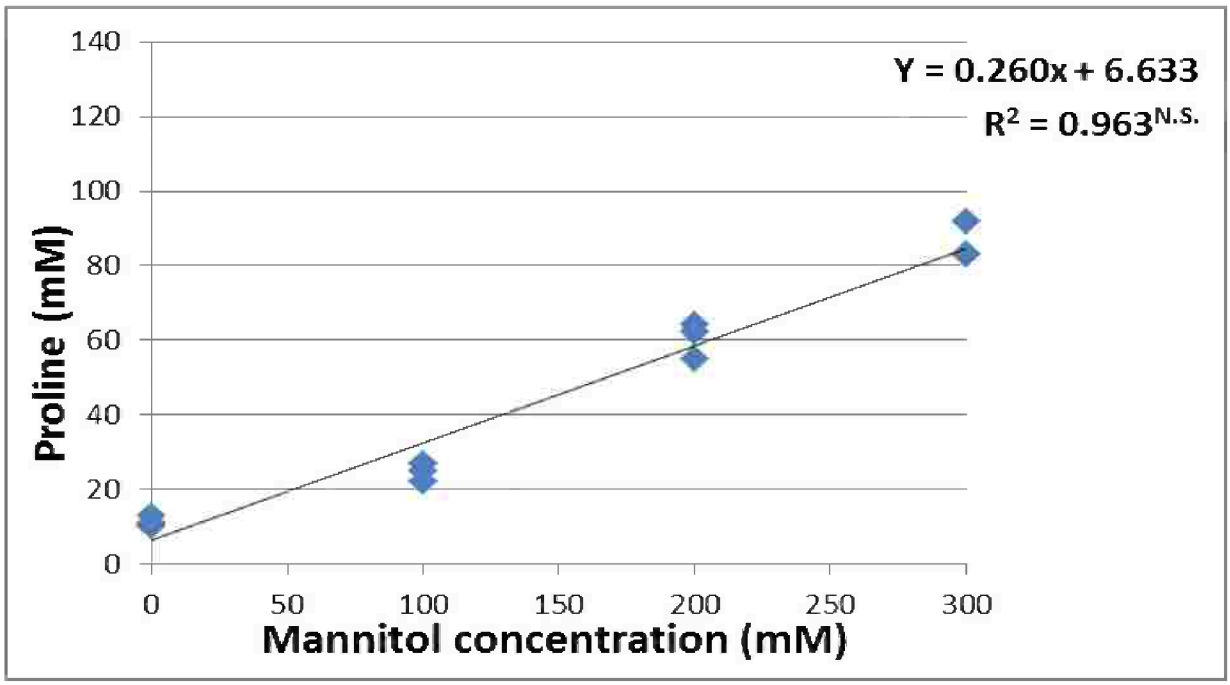


Figure (19) Regression between mannitol concentrations (X-factor) and the proline (Y-factor) in ph 8013 callus.

4.2.2. Effect of Drought on Morphological Characters in Regeneration

4.2.2.1 Shoot and Root Formation

The results in Table 13 indicated that there are not significant different among GT 54-9 and G 84–47 in shoot and root formation. Almost 6 shoot comparing with the other genotype Ph 8013 had the lowest shoot in mean 4.67 ± 0.59 (Figure 20).

In the other hand, Ph 8013 showed the highest value of day of root formation in mean 11.25 ± 0.46, comparing with the other genotypes GT 54 -9 and G 84–47 recorded the means 7.58 ± 0.51 and 9.25 ± 0.49, in respect.

While with number of root the GT 54-9 showed the highest root in mean 11.75 ± 0.51foewared by G 84-47 and Ph 8013 in means 10.33 ± 0.45 and 9.58 ± 0.41, respectively (Table 13 and Figure 20)

Analysis of variance in Table 14 indicated that GT 54-9 showed the highest values for shoot formation comparing with other two genotypes and ph 8013 showed the lowest drought tolerant under different concentration of mannitol (Figures 22 and 23)

Analysis of variance in Table 14 indicated that ph 8013 showed the highest values for day of root formation comparing with other two genotypes GT 54-9 and G 84-47 for control, 100, 200 and 300 mM Mannitol. Results in Table 14 and Figure 24 of GT 54-9 showed the lowest values for day of root formation under different concentration of mannitol

Analysis of variance in Table 14 indicated that GT54-9 showed the highest values for number of root formation comparing with other two genotypes G 84-47 and ph 8013 for control, 100, 200 and 300 mM Mannitol.

Results in Table 14 and Figures 25, 26and 27 of ph 8013 showed the lowest values for number of root formation under different concentration of mannitol.

Nieves *et al.* (2008) reported that somatic embryogenesis is known as an important pathway for plant regeneration and a useful model system for the investigation of molecular, biochemical and morphological events that take place during the early development of plants.

Our results in the same line with **Watt *et al.* (2009)** They reported early work on *in vitro* regeneration of sugarcane, different results have been obtained for the genetic stability of *in vitro*-derived plantlets. These range from little or no evidence of somaclonal variation to high levels regard- less of morphogenesis path. Further, where phenotypic variations were detected, they were found to be transitory as the variants reverted to the original parental phenotype in the first ratoon crop.

Our results agreement with **Haq *et al.* (2011)** who reported after somatic embryo induction, calluses were sub-cultured on plant regeneration medium. Their results indicated that the maximum plant regeneration was observed in control (MS4) plant regeneration cultures of each cultivar. The authors reported that plant regeneration efficiency was decreased with the increase in NaCl stress. Also, no plant regeneration was observed in MS4c in all cultivars. Similarly, plant regeneration was not observed on MS4b cultures of Thatta-10 and SPHS-19, while 2.21±0.17 plantlets callus was regenerated in CPF-237 sugarcane cultivar.

The same trend with **Rao and FTZ, (2013)** who mention that a system for *in vitro* selection of drought tolerant callus lines in sugarcane was developed. Their results showed that, number of shoots per calli was higher (98±0.15) in non-selected callus when compared to selected calli (36±0.18). Whereas plantlets obtained from selected calli showed 86 % rooting within 10 days of transfer, each plantlet showing 10–12 roots.

Other studies such as **Koch *et al.* (2012)** observed a significant reduction in plantlet of sugarcane production compared with the untreated control at 32.2 to 96.6 mM EMS, with no plantlet development from callus exposed to 96.6 mM EMS. The results indicated that, a reduction in callus proliferation, embryo induction, germination, and plantlet. The reduction in plant yield may have been due to the decrease in viable embryogenic callus production. Significant differences in plantlet development were observed between the control and treated cultures for total number of plants

Table (13) Shoot, root and number of shoot formation for the three sugarcane genotypes under the study.

Characters / Genotypes	Number of shoot formation	Number of days for root formation	Number of root
GT 54 -9	$6.33^{a} \pm 0.55$	$7.58^{c} \pm 0.51$	$11.75^{a} \pm 0.51$
G 84 - 47	$6.25^{a} \pm 0.49$	$9.25^{b} \pm 0.49$	$10.33^{b} \pm 0.45$
Ph 8013	$4.67^{b} \pm 0.59$	$11.25^{a} \pm 0.46$	$9.58^{c} \pm 0.41$
L.S.D $_{0.05}$	0.4574	1.2933	1.1849

*Means within columns followed by the same letter are not significantly different from each other, L.S.D. test.

Table (14) Interaction between shoot, root formation and number day for root formation for the three sugarcane genotypes under the study.

Variety	Mannitol) (mM	No. SF	No. DRF	No. R
GT 54 -9	Zero	$9.33^{a} \pm 0.422$	$07.33^{de} \pm 0.913$	$14.00^{a} \pm 1.316$
	100	$8.00^{c} \pm 0.365$	$07.00^{e} \pm 0.966$	$12.00^{ab} \pm 0.730$
	200	$4.67^{e} \pm 0.558$	$08.00^{cde} \pm 1.320$	$10.67^{cb} \pm 0.557$
	300	$3.33^{f} \pm 0.421$	$08.00^{cde} \pm 1.095$	$10.33^{cb} \pm 0.760$
G 84 - 47	Zero	$9.00^{ab} \pm 0.356$	$09.00^{cde} \pm 1.095$	$11.33^{b} \pm 0.919$
	100	$7.67^{c} \pm 0.422$	$09.00^{cde} \pm 1.460$	$11.33^{b} \pm 0.760$
	200	$5.00^{e} \pm 0.365$	$09.33^{cdeb} \pm 0.558$	$10.33^{cb} \pm 0.558$
	300	$3.33^{f} \pm 0.211$	$09.67^{cdb} \pm 0.919$	$08.33^{c} \pm 0.919$
Ph 8013	Zero	$8.33^{bc} \pm 0.211$	$11.67^{ab} \pm 1.280$	$10.33^{cb} \pm 0.919$
	100	$6.00^{d} \pm 0.365$	$10.33^{cab} \pm 0.558$	$09.67^{cb} \pm 0.518$
	200	$3.33^{f} \pm 0.211$	$12.67^{a} \pm 0.558$	$10.00^{cb} \pm 0.356$
	300	$1.00^{g} \pm 0.365$	$10.33^{cab} \pm 0.919$	$08.33^{c} \pm 1.174$
L.S.D $_{0.05}$		0.914	2.587	2.3699

*Means within columns followed by the same letter are not significantly different from each other, L.S.D. test.

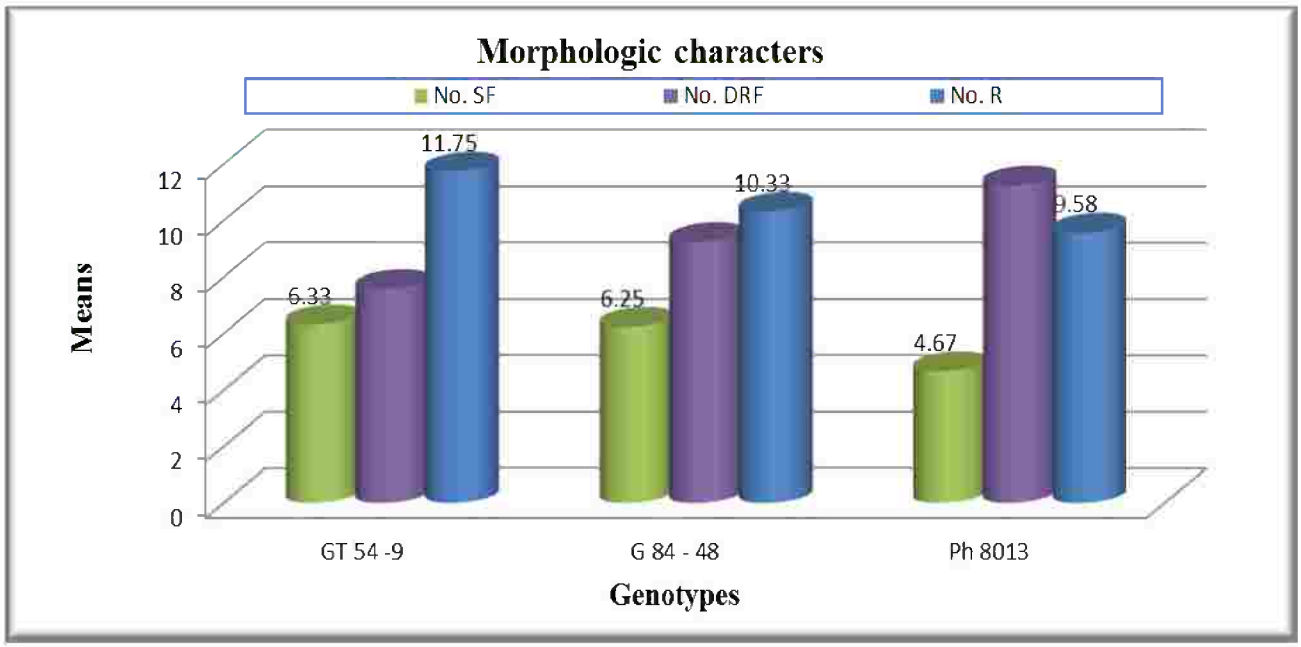


Figure (20) Morphological performance of sugarcane genotypes (No. SF: number of shoot formation; No. DRF: day numbers for root formation; NO. R: Root number).

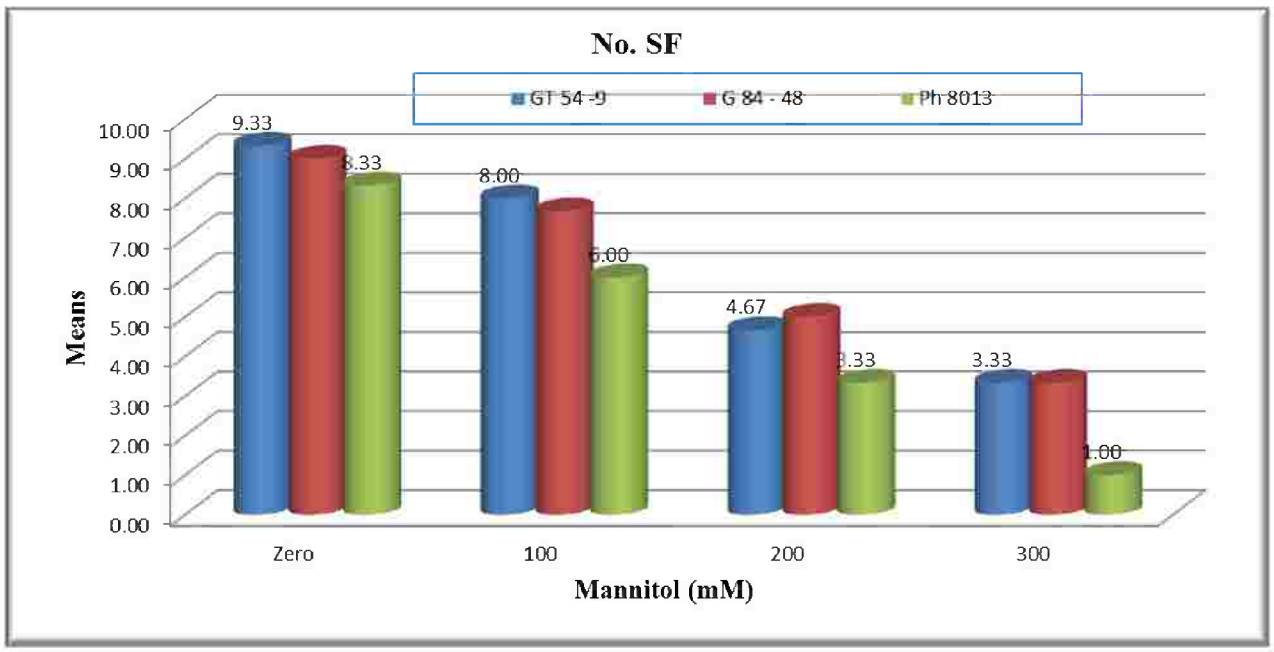


Figure (21) Interaction of shoot number formation of sugarcane genotypes (No. SF: number of shoot formation).

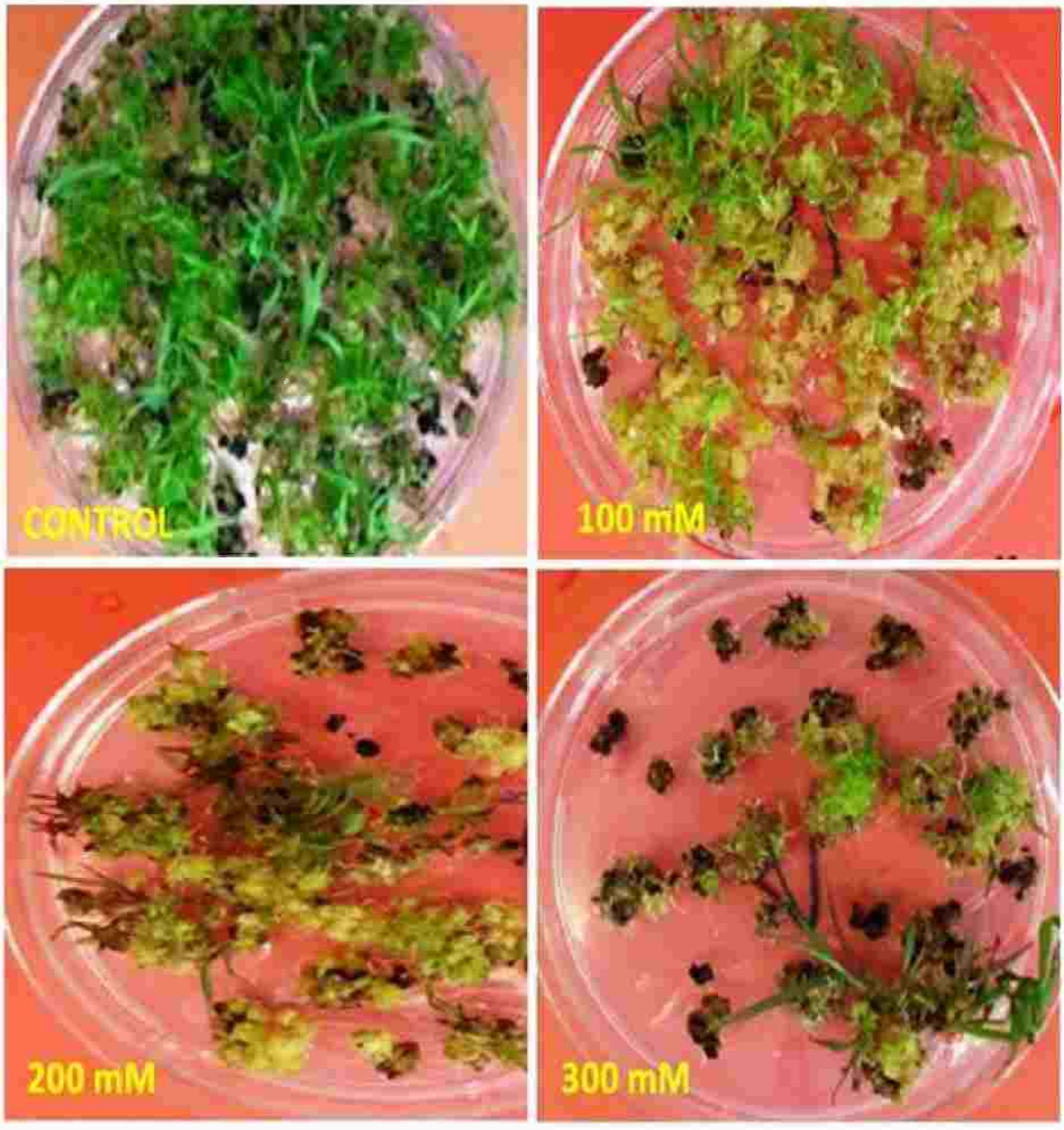


Figure (22) Regeneration of GT54-9 obtained from tolerant callus under different mannitol concentrations.

Figure (23) Regeneration of ph 8013 obtained from tolerant callus under different mannitol concentrations.

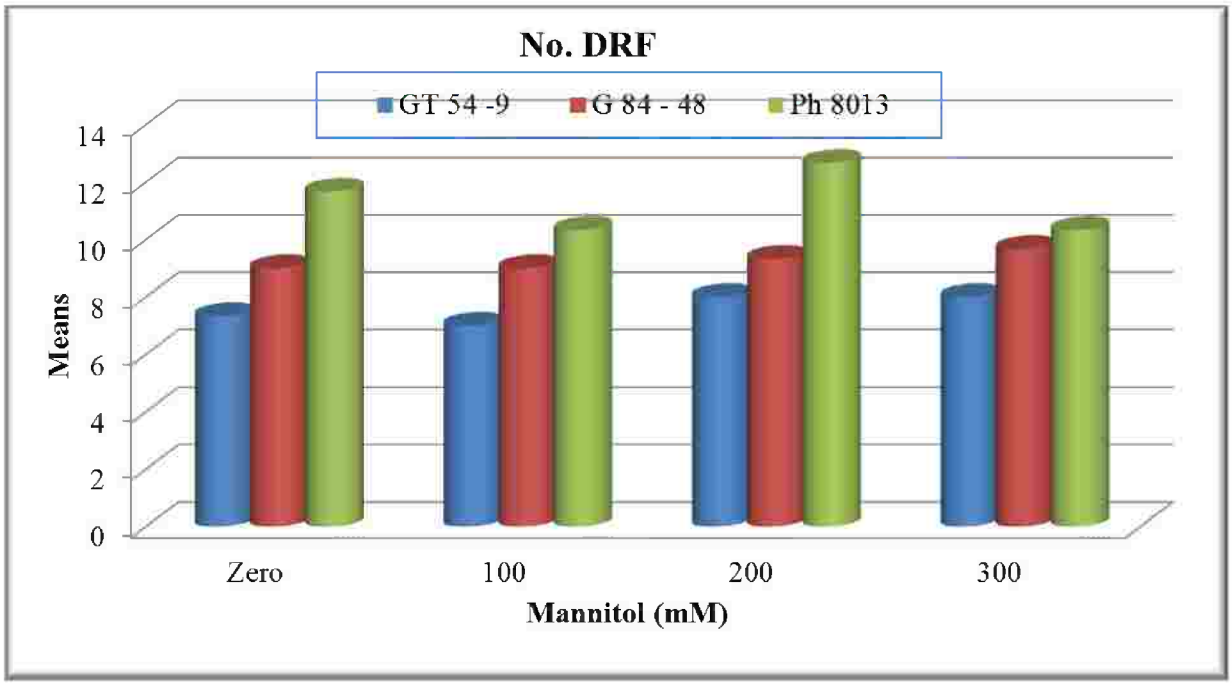


Figure (24) Day number of root formation for three sugarcane genotypes.

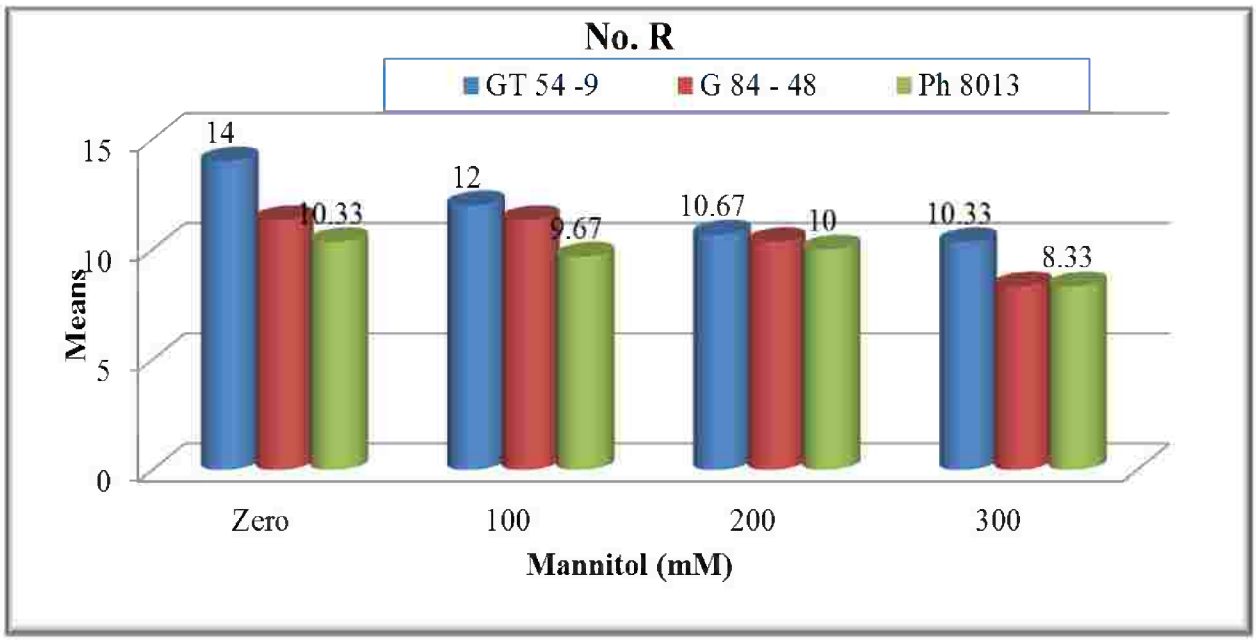


Figure (25) Root number formation for three sugarcane genotypes.

Figure (26) Difference in number of root formation for GT 54-9 under different mannitol concentration.

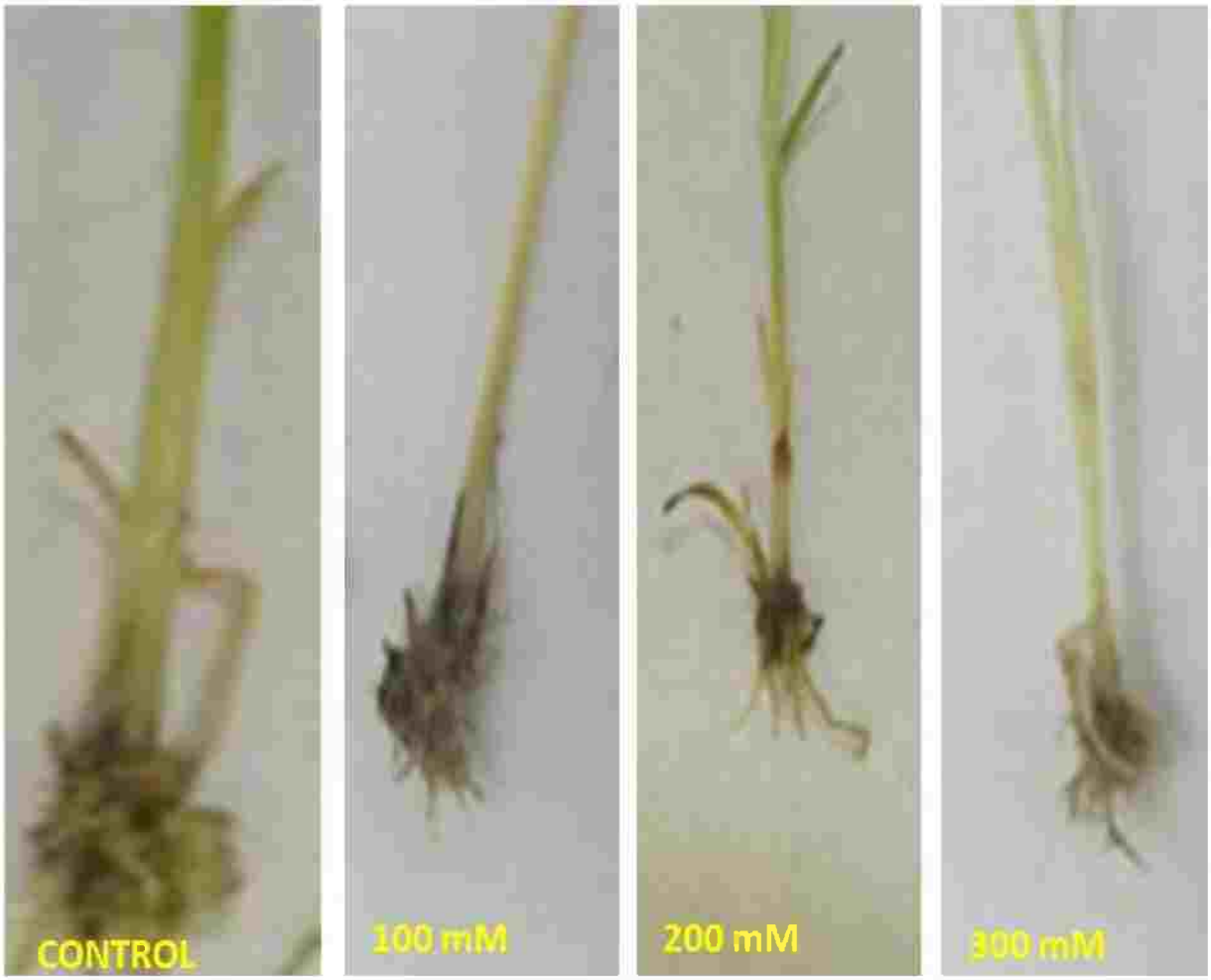


Figure (27) Difference in number of root formation for G84-47 under different mannitol concentration.

4.3. Molecular Study

4.3.1. Randomly Amplified Polymorphic DNA (RAPD) Analysis

In the present study five random primers were used to differentiate through RAPD analysis among tested samples of Sugarcane (*Saccharum officinarum* L.). The results of primer OPD-02 are illustrated in Plate (1) and Table (15). It gave a maximum of 17 amplification products at the fragment lengths ranged between 174 bp. to 1685 bp. and all tasted samples were polymorphic fragments.

The percentage of the polymorphism was 94%. Two unique (specific) fragment (283 and 1685 bp.) was exhibited for G 84-47 cultivar and GT 54-9 cultivars, in respect, with frequency 0.083.

Results showed that GT 54-9 cultivar has the highest number of fragments (31 fragments), while, the ph 8013 cultivar give the lowest number of DNA fragments (21 fragments). On the other hand, the G 84-47 cultivar give (25 fragments) including control treatment.

Polymorphism between the three sugarcane genotypes ranged from 57% to 89%.

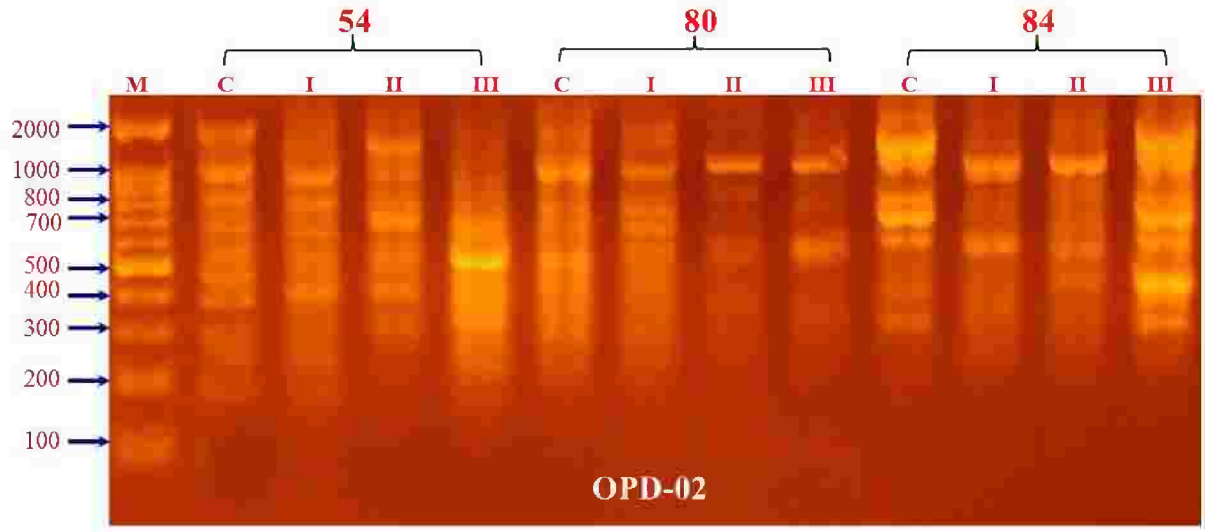


Plate (1) DNA polymorphism of the Sugarcane (*Saccharum officinarum* L.) treated with mannitol using randomly amplified polymorphic DNA with primer OPD-02. (54: GT 54-9, 80: ph 8013, 84:G 84-47, M: marker, C: control, I: 100mM, II: 200mM and III: 300mM mannitol).

Table (15) DNA polymorphism using randomly amplified polymorphic DNA with primer OPD-02 (54: GT 54-9, 80: ph 8013, 84:G 84-47, M: marker, C: control, I: 100mM, II: 200mM and III: 300mM mannitol).

Fragments Size (bp)	54				80				84				Frequency	Polymorphism
	C	I	II	III	C	I	II	III	C	I	II	III		
1685	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0.083**	**Unique**
1538	1	0	0	0	1	1	0	0	0	0	1	0	0.333	Polymorphic
1384	0	1	1	0	0	0	0	0	1	0	0	1	0.333	Polymorphic
1071	1	1	1	1	1	1	1	1	1	1	1	1	1.000	Monomorphic
934	0	0	0	1	1	0	1	0	0	0	0	0	0.250	Polymorphic
861	1	1	1	0	1	0	0	1	1	1	1	0	0.667	Polymorphic
730	0	0	1	1	0	1	0	0	1	0	1	1	0.500	Polymorphic
674	1	1	0	0	1	1	0	0	0	0	0	0	0.333	Polymorphic
600	0	0	0	0	0	1	1	1	1	1	1	1	0.583	Polymorphic
516	1	1	1	1	1	0	0	0	0	0	0	0	0.417	Polymorphic
454	1	1	0	0	0	0	0	0	0	0	0	0	0.167	Polymorphic
419	0	0	0	0	0	0	0	0	1	1	1	1	0.333	Polymorphic
373	1	1	1	0	0	0	1	1	1	0	0	0	0.500	Polymorphic
314	0	0	1	1	1	1	0	0	1	0	0	1	0.500	Polymorphic
283	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.083**	**Unique**
224	1	1	0	0	0	0	0	0	0	0	0	0	0.167	Polymorphic
174	1	1	0	0	0	0	0	0	0	0	0	0	0.167	Polymorphic
Cultivars Polymorphism														
Monomorphic fragments	1	1	1	1	1	1	1	1	1	1	1	1		
Polymorphic - Unique	8	8	6	4	6	5	3	3	7	3	5	5		
Unique	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**		
Polymorphic + Unique	9	8	6	4	6	5	3	3	8	3	5	5		
Total fragments	**10**	**9**	**7**	**5**	**7**	**6**	**4**	**4**	**9**	**4**	**6**	**6**		
Polymorphism (%)	90	89	86	80	86	83	75	75	89	75	83	83		

Primer Polymorphism	
Monomorphic fragments	1
Polymorphic (without Unique)	14
Unique fragments	2
Polymorphic (with Unique)	16
Total number of fragments	17
Polymorphism (%)	**94%**
Mean of fragments frequency	0.377

1.0 = Fragments presence 0.0 = Fragments absence

As shown in Plate (2) and Table (16), the RAPD pattern of primer OPD-03 have detected 17 fragments that ranged between 126 to 1619 bp. and the percentages of the polymorphism 100%. This primer was created out 100% of polymorphism with the DNAs of the different tasted samples. The polymorphic fragments distributed as follow: 22 fragments for GT 54-9; 21 fragments for ph 8013; 23 fragments for G 84-47. Furthermore, the results showed that six unique (specific) was exhibited for GT 54-9 cultivars and G 84-47 cultivars, in respect, with frequency 0.083. Results of this primer showed that all the three genotypes have the same fragment number (16) without the control.

From the 6 unique fragments G 84-47 had four fragments followed by GT 54-9 by two fragments.

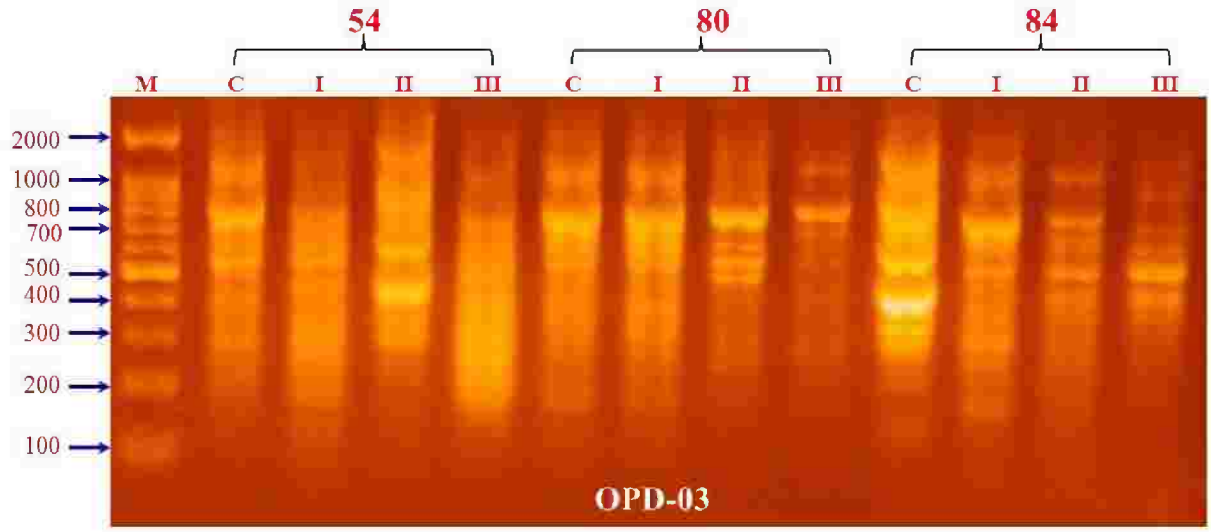


Plate (2) DNA polymorphism of the Sugarcane (*Saccharum officinarum* L.) treated with mannitol using randomly amplified polymorphic DNA with primer OPD-03. (54: GT 54-9, 80: ph 8013, 84:G 84-47, M: marker, C: control, I: 100mM, II: 200mM and III: 300mM mannitol).

Table (16) DNA polymorphism using randomly amplified polymorphic DNA with primer OPD-03(54: GT 54-9 80:ph 8013 84:G 84-47) (C: 0 I: 100mM II: 200mM III: 300mM mannitol).

Fragments Size (bp)	54				80				84				Frequency	Polymorphism
	C	I	II	III	C	I	II	III	C	I	II	III		
1619	1	0	0	1	1	0	0	0	0	0	0	0	0.250	Polymorphic
1337	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.083**	**Unique**
1132	1	0	1	1	1	1	0	1	1	1	1	1	0.833	Polymorphic
925	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0.083**	**Unique**
775	1	1	1	1	1	1	1	1	1	1	1	0	0.917	Polymorphic
694	0	0	0	0	0	0	0	1	0	0	0	1	0.167	Polymorphic
609	0	0	0	0	0	0	1	0	0	0	1	1	0.250	Polymorphic
542	1	1	1	1	1	1	1	1	1	0	0	0	0.750	Polymorphic
464	0	0	0	0	0	0	1	0	0	1	1	1	0.333	Polymorphic
383	1	1	1	1	0	1	0	1	1	0	1	1	0.750	Polymorphic
298	0	0	0	0	0	0	0	0	1	1	0	0	0.167	Polymorphic
265	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.083**	**Unique**
261	0	0	1	0	0	1	1	1	0	0	0	0	0.333	Polymorphic
199	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0.083**	**Unique**
164	0	1	0	1	1	0	0	0	0	0	0	0	0.250	Polymorphic
156	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0.083**	**Unique**
126	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0.083**	**Unique**
Cultivars Polymorphism														
Monomorphic fragments	0	0	0	0	0	0	0	0	0	0	0	0		
Polymorphic - Unique	5	4	5	6	5	5	5	6	5	4	5	5		
Unique	1	0	1	0	0	0	0	0	2	1	0	1		
Polymorphic + Unique	6	4	6	6	5	5	5	6	7	5	5	6		
Total fragments	6	4	6	6	5	5	5	6	7	5	5	6		
Polymorphism (%)	100	100	100	100	100	100	100	100	100	100	100	100		

Primer Polymorphism	
Monomorphic fragments	0
Polymorphic (without Unique)	11
Unique fragments	6
Polymorphic (with Unique)	17
Total number of fragments	17
Polymorphism (%)	**100%**
Mean of fragments frequency	0.323

1.0 = Fragments presence 0.0 = Fragments absence

Furthermore in Plate (3) and Table (17), the RAPD pattern of primer OPH-03 produced a total of 12 fragments ranged between 239 to 1157 bp. The four unique fragments were distributed as follow: Two fragments for G 84-47 at 383 and 1157 bp. and one fragment for GT 54-9 at 343 bp. and finally one fragment at 418 bp. for ph 8013. The percentages of the polymorphism ranged from 67% to 83%. This primer was created out 92% of polymorphism with the DNAs of the different tasted samples.

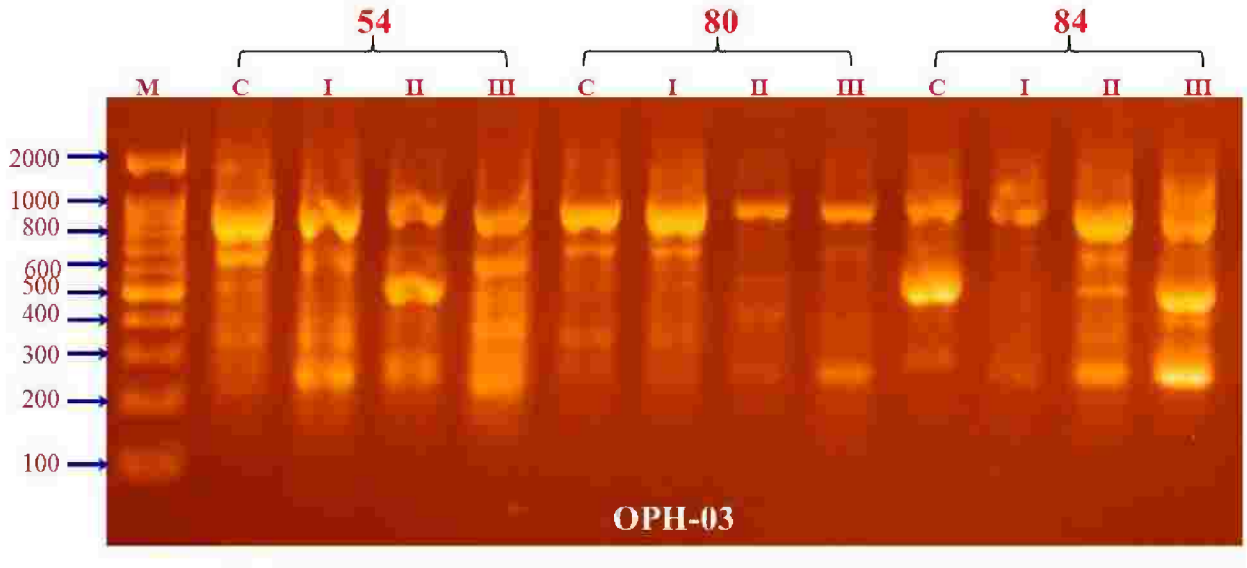


Plate (3) DNA polymorphism of the Sugarcane (*Saccharum officinarum* L.) treated with mannitol using randomly amplified polymorphic DNA with primer OPH-03 (54: GT 54-9, 80: ph 8013, 84:G 84-47, M: marker, C: control, I: 100mM, II: 200mM and III: 300mM mannitol).

Table (17) DNA polymorphism using randomly amplified polymorphic DNA with primer OPH-03(54: GT 54-9, 80: ph 8013, 84: G 84-47, M: marker, C: control, I: 100mM, II: 200mM and III: 300mM mannitol).

Fragments Size (bp)	54				80				84				Frequency	Polymorphism
	C	I	II	III	C	I	II	III	C	I	II	III		
1157	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0.083**	**Unique**
921	1	1	1	1	1	1	1	1	1	1	1	1	1.000	Monomorphic
714	0	0	0	0	1	1	0	1	0	0	0	0	0.250	Polymorphic
671	1	1	1	1	0	0	0	0	0	1	1	1	0.583	Polymorphic
614	0	0	0	0	1	1	0	0	0	0	0	0	0.167	Polymorphic
520	1	1	1	1	1	1	1	1	1	0	1	1	0.917	Polymorphic
434	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.083**	**Unique**
418	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0.083**	**Unique**
383	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0.083**	**Unique**
369	1	1	0	0	0	0	0	0	0	0	0	0	0.167	Polymorphic
324	1	1	0	1	1	1	0	1	0	0	1	1	0.667	Polymorphic
239	0	1	1	1	1	1	1	1	1	1	1	1	0.917	Polymorphic
Cultivars Polymorphism														
Monomorphic fragments	1	1	1	1	1	1	1	1	1	1	1	1		
Polymorphic - Unique	4	5	3	4	5	5	2	4	2	2	4	4		
Unique	1	0	0	0	0	0	1	0	0	0	0	2		
Polymorphic + Unique	5	5	3	4	5	5	3	4	2	2	4	6		
Total fragments	6	6	4	5	6	6	4	5	3	3	5	7		
Polymorphism (%)	83	83	75	80	83	83	75	80	67	67	80	86		

Primer Polymorphism	
Monomorphic fragments	1
Polymorphic (without Unique)	7
Unique fragments	4
Polymorphic (with Unique)	11
Total number of fragments	12
Polymorphism (%)	**92%**
Mean of fragments frequency	0.417

1.0 = Fragments presence 0.0 = Fragments absence

The results of primer OPO-01 are illustrated in Plate (4) and Table (18). It gave a maximum of 9 amplification products at the fragment lengths ranged between 285 bp. to 546 bp. and the percentages of the polymorphism ranged from 33% to 67%. The polymorphic fragments distributed as follow: 16 fragments for GT 54-9; 17 fragments for ph 8013; 22 fragments for 84. Moreover, the two unique fragments were distributed for G 84-47 at 381 and 377 bp., respectively.

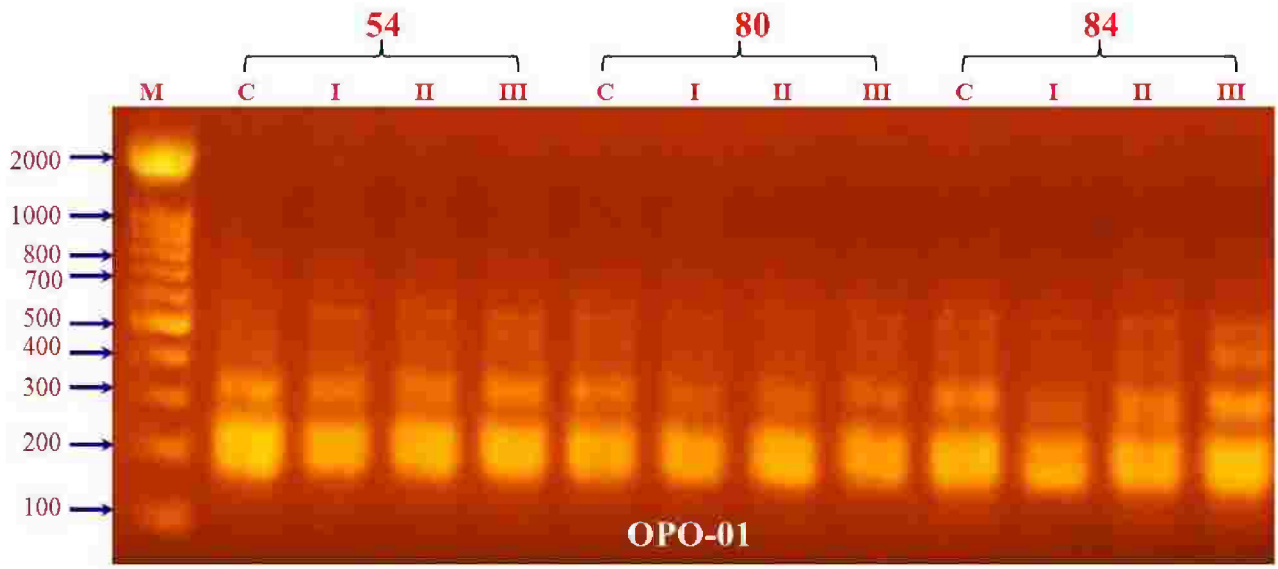


Plate (4) DNA polymorphism of the Sugarcane (*Saccharum officinarum* L.) treated with mannitol using randomly amplified polymorphic DNA with primer OPO-01. (54: GT 54-9, 80: ph 8013, 84:G 84-47, M: marker, C: control, I: 100mM, II: 200mM and III: 300mM mannitol).

Table (18) DNA polymorphism using randomly amplified polymorphic DNA with primer OPO-01(54: GT 54-9, 80: ph 8013, 84: G 84-47, M: marker, C: control, I: 100mM, II: 200mM and III: 300mM mannitol).

Fragments Size (bp)	54				80				84				Frequency	Polymorphism
	C	I	II	III	C	I	II	III	C	I	II	III		
546	0	1	1	1	1	1	1	1	1	0	0	0	0.667	Polymorphic
462	0	0	0	1	1	0	0	0	1	1	1	1	0.500	Polymorphic
381	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0.083**	**Unique**
377	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0.083**	**Unique**
312	1	1	1	1	1	1	1	1	0	0	0	0	0.667	Polymorphic
252	0	0	0	0	0	0	0	0	1	1	1	1	0.333	Polymorphic
216	1	1	1	1	1	1	1	1	1	1	1	1	1.000	Monomorphic
178	1	1	1	1	1	1	1	1	1	1	1	1	1.000	Monomorphic
285	0	0	0	0	0	0	0	0	1	0	1	1	0.250	Polymorphic
Cultivars Polymorphism														
Monomorphic fragments	2	2	2	2	2	2	2	2	2	2	2	2		
Polymorphic - Unique	1	2	2	3	3	2	2	2	4	2	3	3		
Unique	0	0	0	0	0	0	0	0	0	0	1	1		
Polymorphic + Unique	1	2	2	3	3	2	2	2	4	2	4	4		
Total fragments	3	4	4	5	5	4	4	4	6	4	6	6		
Polymorphism (%)	33	50	50	60	60	50	50	50	67	50	67	67		
Primer Polymorphism														
Monomorphic fragments	2													
Polymorphic (without Unique)	5													
Unique fragments	2													
Polymorphic (with Unique)	7													
Total number of fragments	9													
Polymorphism (%)	**78%**													
Mean of fragments frequency	0.509													

1.0 = Fragments presence 0.0 = Fragments absence

The results illustrated in Plate (5) and Table (19), the RAPD pattern of primer OPO-02 produced a total of 7 fragments ranged between 156 to 787 bp. The polymorphic fragments distributed as follow: nine fragments for GT 54-9; seven fragments for ph 8013; eight fragments for G 84-47. The percentages of the polymorphism ranged from 25% to 50%. This primer was created out 57% of polymorphism with the DNAs of the different tested samples. One unique fragment was detected for GT 54-9 at 515 bp.

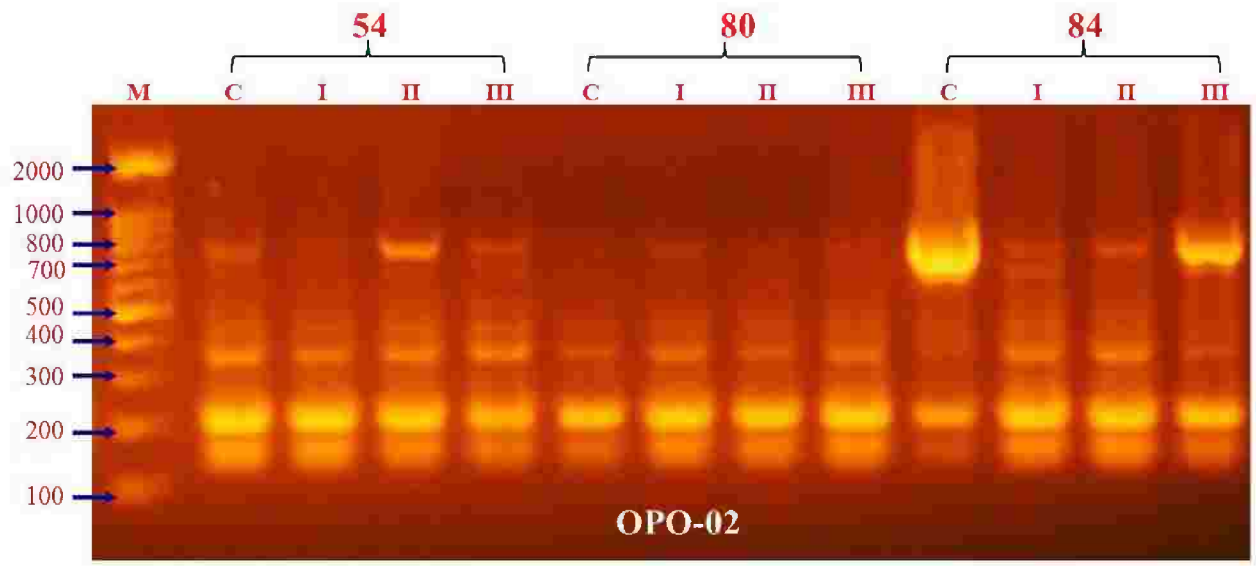


Plate (5) DNA polymorphism of the Sugarcane (*Saccharum officinarum* L.) treated with mannitol using randomly amplified polymorphic DNA with primer OPO-02 (54: GT 54-9, 80: ph 8013, 84: G 84-47, M: marker, C: control, I: 100mM, II: 200mM and III: 300mM mannitol).

Table (19) DNA polymorphism using randomly amplified polymorphic DNA with primer OPO-02 (54: GT 54-9, 80: ph 8013, 84: G 84-47, M: marker, C: control, I: 100mM, II: 200mM and III: 300mM mannitol).

Fragments Size (bp)	54				80				84				Frequency	Polymorphism
	C	I	II	III	C	I	II	III	C	I	II	III		
787	1	1	1	1	0	1	1	1	1	1	1	1	0.917	Polymorphic
669	0	0	1	1	0	0	0	0	0	1	0	1	0.333	Polymorphic
515	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.083**	**Unique**
408	0	1	1	1	1	1	1	1	0	1	1	0	0.750	Polymorphic
338	1	1	1	1	1	1	1	1	1	1	1	1	1.000	Monomorphic
202	1	1	1	1	1	1	1	1	1	1	1	1	1.000	Monomorphic
156	1	1	1	1	1	1	1	1	1	1	1	1	1.000	Monomorphic
Cultivars Polymorphism														
Monomorphic fragments	3	3	3	3	3	3	3	3	3	3	3	3		
Polymorphic - Unique	1	2	3	3	1	2	2	2	1	3	2	2		
Unique	0	0	0	1	0	0	0	0	0	0	0	0		
Polymorphic + Unique	1	2	3	4	1	2	2	2	1	3	2	2		
Total fragments	4	5	6	7	4	5	5	5	4	6	5	5		
Polymorphism (%)	25	40	50	57	25	40	40	40	25	50	40	40		

Primer Polymorphism	
Monomorphic fragments	3
Polymorphic (without Unique)	3
Unique fragments	1
Polymorphic (with Unique)	4
Total number of fragments	7
Polymorphism (%)	57%
Mean of fragments frequency	0.726

1.0 = Fragments presence 0.0 = Fragments absence

In the current study, five oligonucleotide primers (100%) used in the RAPD analysis gave unique markers. Out of the five primers, five detected for GT 54-9 and 8 for G 84-47. Data in Table 20 showed the similarity matrix of the five RABD-PCR markers.

In the present study five random primers were used to differentiate through RAPD analysis among tested samples of Sugarcane (*Saccharum officinarum* L.) name of markers OPD-02, OPD-03, OPH-03, OPO-01 and OPO-02.

Cluster analysis of the current research, divided the three sugarcane genotypes into two main groups in similarity percentage 59%. The first group includes G84-47 and GT 54-9 in similarity 65% within this group G 84-47 divided by the three concentrations 100, 200 and 300 mM mannitol by similarity 70%. While, control with the other concentrations by 68%.

On the second group, the genotypes divided into two sub groups on 65% similarity. The two sub group includes the genotypes ph-8013 and GT-54-9 (Figure 28).

The main consolation of the present study indicated that, GT-54-9 genotype considers the promising genotypes other than G84-47 and ph-80-13, in respect, in all the morphological and molecular studies.

Table (20) Similarity indices (%) among the three cultivars using five random amplified (RAPD) primers.

	C54	I54	II54	III54	C80	I80	II80	III80	C84	I84	II84	III84
C54	1.00											
I54	0.80	1.00										
II54	0.64	0.76	1.00									
III54	0.62	0.71	0.80	1.00								
C80	0.64	0.69	0.67	0.80	1.00							
I80	0.62	0.67	0.76	0.75	0.80	1.00						
II80	0.55	0.67	0.72	0.67	0.66	0.75	1.00					
III80	0.66	0.75	0.80	0.71	0.73	0.86	0.82	1.00				
C84	0.49	0.58	0.71	0.62	0.56	0.66	0.62	0.69	1.00			
I84	0.51	0.56	0.66	0.64	0.58	0.60	0.67	0.67	0.69	1.00		
II84	0.564	0.58	0.64	0.66	0.60	0.69	0.66	0.69	0.71	0.80	1.00	
III84	0.40	0.46	0.58	0.60	0.44	0.56	0.53	0.56	0.66	0.67	0.76	1.00

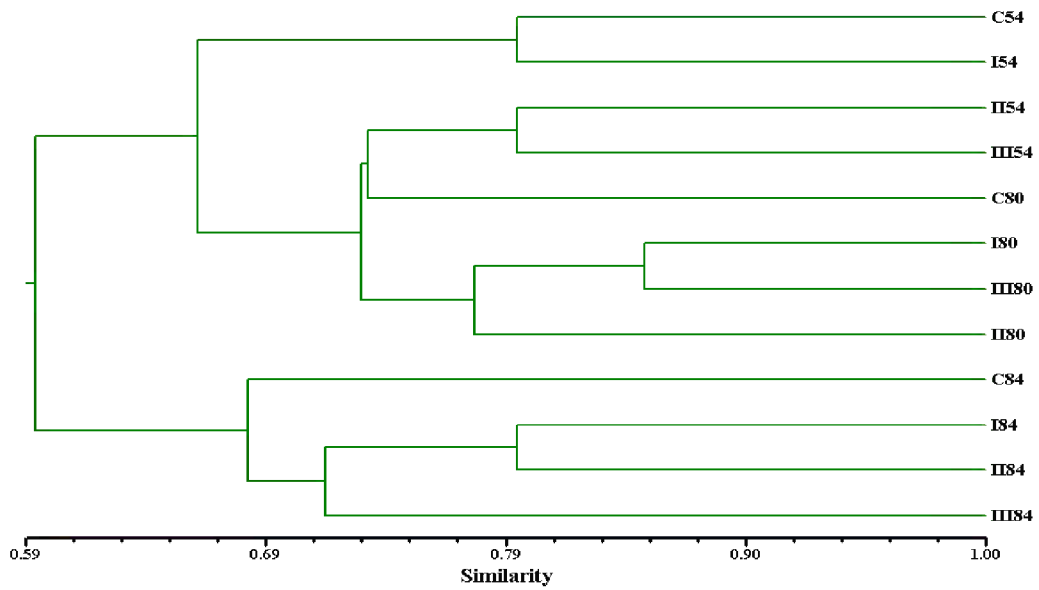


Figure (28) Dendrogram of the three cultivars and using five random amplified (RAPD) primers (54: GT 54-9, 80: ph 8013, 84:G 84-47, M: marker, C: control, I: 100mM, II: 200mM and III: 300mM mannitol).

The present work used the review of **Jones *et al.* (1997)** they, reported genetic markers represent genetic differences between individual organisms or species. Generally, all genetic markers occupy specific genomic positions within chromosomes (like genes) called 'loci' (singular 'locus'). There are three major types of genetic markers: (1) morphological (also 'classical' or 'visible') markers which themselves are phenotypic traits or characters; (2) biochemical markers, which include allelic variants of enzymes called isozymes; and (3) DNA (or molecular) markers, which reveal sites of variation in DNA.

Analysis of the changes that occur at the DNA level resulting from culture-induced somaclonal variation and mutagenic treatments are important to understand the resulting variation **(Rasheed *et al.* 2005)**.

Our results was agreement with **Khan *et al.* (2013)** who reported that drought tolerance is polygenic and complex trait interplay with the environment makes phenotypic evaluation difficult. Hence, the use of DNA markers can help breeders in improving the speed as well as reliability of the process. Gene tagging and DNA fingerprinting is particularly suitable for pyramiding of desired traits. Ten elite sugarcane clones were tested for genetic diversity through RAPD, sucrose synthase activity was determined via TRAP and drought tolerance was examined with the help of STS techniques / field trial. RAPD study revealed that genetically most similar genotypes were Thatta-10 and AEC82-223 (80.4%) and most dissimilar genotypes were AEC71- 2011 and NIA-2004 (49.8%). On the basis of dendrogram, the varieties could be divided into four clusters (A to D). Variety AEC82-223 produced a specific allele of 311bp with primer B-02.

Hoezel and Green, (1998) mentioned that RAPD results in amplification of few random segments of DNA, allowing for variation in length and number of amplified segments when the sequence of the segments is altered. RAPDs have been used widely for analysis of genetic variation **(Afiah *et al.* 2007; Ehsanpour *et al.* 2007;Ngezahayo *et al.* 2007; Cuesta *et al.* 2010)** recommended the use of RAPDs for analysis of somaclonal variation.