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GENETICAL VARIATION OF CALLUS PERFORMANCES OF MAIZE GENOTYPES PRODUCED FROM TISSUE CULTURE UNDER DIFFERENT LEVELS OF SALINITY.

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ABSTRACT

Four maize hybrids (122, 168, 173 and pioneer) were investigated to determine their genotypic effects on callogenesis response to 2,4-D (2,4 dichlorophynoxyacetic acid) and *in vitro* salt tolerance using NaCl. A callus was initiated from mature embryos cultured on three MS media supplemented with 0, 2 and 4mg/l of 2,4-D where the level 0 is the control. The produced calli from the medium which contains 2 mg/L 2,4-D for each genotype were then exposed to four salinity levels (i.e. 0, 1500, 3000 and 6000 ppm) of NaCl, respectively to evaluate some physiological aspects of the produced calli from the genotypes. The results of ANOVA revealed that the mean squares of genotypes at all levels of 2,4-D were significantly indicated the presence of differences between them for all *in vitro* traits. Furthermore, the mean squares of levels and genotype × levels interaction were highly significant for all traits. This indicated that these genotypes gave different responses at different 2,4-D levels. The genetic variation was high and positive with respect to the *in vitro* traits at each 2,4-D level. This indicated that these traits are mainly controlled by genetic factors. This finding was emphasized by the heritability values, which were more than 80% for all studied traits at each 2,4-D level. In relation to callus response to salt stress, the results indicated that the presence of significant differences between these genotypes for ion content in callus cells (K^+%, Na^+%, Na^+/K^+ and Cl^-%) with respect to the four salinity levels except for K^+% at S_1,S_3 levels, Na^+% at levels S_1, S_2, S_3, Na^+/k^+ at levels S_0, S_1, S_3 and Cl^-% at levels S_2, S_3. Regarding to, the organic solutes accumulation (sugar and proline mg/g f.wt), the magnitudes of the mean squares for genotypes were significant at four salinity levels except for proline mg/g f.wt at S_3 level. For membrane permeability, relative growth rate and water content, the magnitudes of the mean squares for genotypes were significant at four salinity levels except for water content at levels S_0, S_3.The results revealed that the genetic variation was high and positive for ion content in callus cells (K^+%, Na^+%, Na^+/K^+ and Cl^-%), organic solutes accumulation (sugars and proline), membrane permeability and relative growth rate as well as water content with respect to the four levels of salinity. This finding is emphasized by the heritability values, which were more than 80% for most of studied traits.

Keywords: *Zea mays*- mature embryo culture- 2,4-D effect – Salinity –heritability.

INTRODUCTION

Maize (*Zea Mays* L.) is a widely grown cereal crop in the world. It is also the most important fodder crop among cereals in industrialized countries and many developing countries. It is used as a raw material for manufacture of large number of industrial products like corn starch and starch-based products, and in fermentation and distillation industries. Therefore, it has been extensively investigated with respect to plant regeneration from *in vitro* culture. Embryos are also the initiation of *Zea mays* tissue culture for either callus culture or DNA delivery techniques Danson *et al.,* 2006; El-Itriby *et al.,*

2003; Shohael, 2003. Immature embryos are seasonally available and have strictly limited suitable duration of culture (Odour *et al.*, 2006). In contrast, mature embryos are readily available throughout the year in large quantities. The callus is a rapidly proliferating undifferentiated mass of cells, which would be obtained by culturing explants on nutrient media containing specific growth hormones (Naqvi *et al*., 2002). Auxins and cytokinins are the main growth regulators in plants involved in the regulation of cell division and differentiation. Auxins promote, in combination with cytokinins, the growth of calli, cell suspensions and organs. They mainly also regulate the morphogenic processes. At the cellular level, auxins control basic processes such as cell division and cell elongation. Since they are capable of initiating cell division they are involved in the formation of somatic embryos. 2,4-dichlorophenoxyacetic acid (2,4-D) is a synthetic auxin and is the most commonly used growth regulator in cereal tissue culture (He *et al*., 1986; Mikami & Kinoshita, 1988; Ozawa & Komamine, 1989; Bregitzer *et al*., 1989; Naqvi *et al*., 2002).

Salinity is the main abiotic stress that has been addressed by *in vitro* selection (Parvaiz and Satyawati, 2008). Studies at cellular level provide better knowledge to understand the mechanism of salt tolerance, since they lack the differentiation and structural integrity of higher plants and require relatively little space and lower time for the selection, as well as controlled environment to research the adaptive mechanisms of plants living in saline environment. The selection of tolerant genotypes *in vitro* opens up the possibility of accelerating the improvement process, but there are some restrictions such as the difficulty of regenerating plants from the selected material (McCoy, 1987a; Lutts *et al*., 1999), and the occasional lack of correlation between tolerance *in vitro* and the whole plant *in vivo* (McCoy, 1987b). Salinity tolerance is a polygenic and complex quantitative trait with additive gene effects and its perfect evaluation depend on using authentic markers. These markers are used at callus level. First is the assessment of variation of free proline accumulation in tissues, the more proline accumulation, the salt tolerant cultivar it is. Second is assessment of Na^+, K^+ accumulation in tissue and ratio of them with increase in salinity. The less increase in Na^+/K^+ ratio observed, the more tolerant it is. The third marker is relative growth (RG). RG is a reliable index in choice of cell line. Evaluation of RG in different salt levels represents value of a cultivar salt tolerance. A variety would hold its RG in favorable level together with increasing in medium salinity; the more tolerant it is.

The objectives of this research were to study the response of different genotypes to levels of 2,4-D (2,4-dichlorophynoxyacetic acid) and their interactions on mature embryo induction using four *Zea mays* cultivars and to identify the favorable cultivar as salt tolerant. In addition, the partitioning of the sum squares from analyses variance and estimating both the genotypic and the phenotypic variances would make it possible to estimate heritability percentages for studied traits.

MATERIALS AND METHODS

Plant material

Four single hybrids of maize i.e. 122, 168, 173 and Pioneer were used. All these hybrids were supplied by Field Crops Research Institute, Agriculture Research Center, Sakha, Kafer El-sheikh, Egypt.

Embryo culture

The seeds were surface sterilized under sterile conditions by immersing them for one minute in 75% ethanol followed by immersion in 0.1% mercuric chloride solution with 2 drops of Tween 20 as a wetting agent for 20 minutes. Then, it was rinsed three times with sterile double distilled water. The seeds were moistened by soaking in sterilized distilled water containing 2 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) for 72h. The swollen mature embryos were removed from seeds with a scalpel and radicles were then separated from plumules on scutellar nodes. The plumule section (2–5 mm) was longitudinally sliced into halves and then plated cut-side down on induction media under sterile conditions. The induction medium used in this study was MS medium, which recommended by Murashige and Skoog (1962) containing 3% sucrose and supplemented with three different concentrations of 2,4-dichlorophenoxy acetic acid (2,4-D) as the following :

A: MS medium with 0 mg/l 2,4-D as a control,
B: MS medium with 2 mg/l 2,4-D.
C: MS medium with 4 mg/l 2,4-D.

The pH was adjusted to 5.8 before autoclaving at $121C^0$ for 20 minutes. The cultures were incubated in darkness at $25C^o \pm 2C^o$ for three weeks. Then, the total number of responding embryo (which gave one or more calli), the total number of calli (number of calli) and the fresh weight of calli were recorded.

***In vitro* salt treatments:**

The produced calli from the media which contain 2 mg/L 2,4-D for each genotype were collected in sterile petri plates and divided into 4 parts. Then, each part was transferred to MS medium supplemented with four different concentrations of NaCl as the following: S_0: MS medium with zero ppm NaCl, S_1: MS medium with 1500 ppm NaCl, S_2: MS medium with 3000 ppm NaCl and S_3: MS medium with 6000 ppm NaCl. Cultures were incubated in darkness at 26 ± 2 C°for three weeks and sub-cultured in the same media for three weeks. After six weeks the traits which were studied included: the sodium ions (Na^+), potassium ions (K^+), chloride (Cl^-) ions, Na^+/K^+ ratio, total sugars content, proline content, relative growth rate, electrolyte leakage and water content. These traits were evaluated for each genotype at all salinity treatments. Sodium, potassium and chloride contents were measured as an indicator (Chaudhary *et al*. 1996). Total sugars were determined by phenol-sulphoric acid method as described by Sadasivam and Manickam, (1996). Proline content was determined in the callus by the modified ninhydrin method of Troll and Lindsley (1955); omitting phosphoric acid to avoid interference with concentrated sugars (Magne and Larher, 1992). Relative

growth rate (RGR) was as follows: RGR= (final fresh weight – initial fresh weight) / initial fresh weight.). Electrolyte leakage (EL%) was used to assess membrane permeability according to Shalata and Neumann (2001). Water content (WC%) was calculated by following formula: WC (%) = [(FW-DW)/FW] ×100 according to (Lai and Lui,1988).

Experimental design:

The experiments were set up in a completely random design including four genotypes used under three different levels of 2,4-D and then under four levels of salinity treatments. Each treatment was replicated three times. The specific MS medium for each treatment was distributed into culture Petri dishes (12 cm inner diameter), each one contained 20 ml. One Petri dish containing 10 sterilized embryos is considered as one of experimental unit.

RESULTS AND DISCUSSION

Effect of genotypes, 2,4-D levels and their interaction:

As can be seen in Table 1, the data which were obtained from the three 2,4-D levels for the genotypes were setup in a combined analysis of variance. The magnitudes of these mean squares were highly significant for all *in vitro* traits. These findings indicated the presence of real differences among these genotypes. Therefore, the planned comparisons between these genotypes and the partitioning of the phenotypic variance to its components are valid. Many authors agreed with results of *in vitro* traits, such as Seth *et al*. (2012) reported that callus induction was significantly affected by the genotype of maize varieties, Bedada *et al.* (2012) found that callus induction frequency and formation of embryogenic callus varied significantly ($p<0.01$) depending on genotype. Furthermore, levels and genotype × levels interaction mean squares were highly significant with respect to all the studied *in vitro* traits. This indicates that these genotypes gave different responses at different 2,4-D levels. These results agreed with the results obtained by De-yi *et al*. (2011)and Bedada *et al.* (2012)

Table 1: Combined analysis of variance and mean squares of genotypes, levels and their interactions for all *in vitro* traits.

S.O.V	d.f	Responding embryo	Callus fresh weight	Number of callus
Levels (L)	2	0.191**	119.48*	2.649**
Genotypes (G)	3	0.102 **	400.45**	4.310**
G X L	6	0.048 **	39.99**	0.249**
Error	24	0.001	2.14	.008

Note: ** significant at 0.01 level of probability.

Means of four genotypes for all *in vitro* traits at the three levels of 2,4-D (A, B and C) and from the combined data over the three levels of 2,4-D are presented in Table 2.The results of means showed that the greatest mean of responding embryos, callus fresh weight and number of callus at the three studied levels of 2,4-D were observed in genotype 122 with means of 1.00,

25.74 and 3.47, respectively. On the other hand, the lowest overall for responding embryo, callus fresh weight and number of callus were observed in pioneer with means of0.45, 5.38 and 1.23,respectively.The combined data over the three 2,4-D levels could be more precise to present information concerning the behavior of these genotypes. The results revealed that there are significant differences between paired of means in most of studied traits. The results cleared that greatest percentages value for responding embryo, callus fresh weight and number of callus were observed in genotype 122 with means of 1.00, 23.57 and 2.96, respectively. Although, the genotype pioneer proved to be less efficient in all traits as compared to all other genotypes.

Table 2: Mean performance of genotypes for the studied *in vitro* traits at each level of 2,4-D and the combined data over the three levels (A, B and C).

Genotype		Responding embryo	Callus fresh weight	Number of callus
122	A	1.00^{A}	25.74^{A}	3.47^{A}
	B	0.99^{A}	23.00^{A}	3.02^{A}
	C	1.00^{A}	21.98^{A}	2.39^{A}
	Com.	1.00^{A}	23.57^{A}	2.96^{A}
168	A	0.90^{B}	24.62^{A}	2.27^{B}
	B	0.99^{A}	17.87^{B}	1.89^{B}
	C	0.99^{A}	11.41^{B}	1.18^{B}
	Com	0.95^{B}	17.97^{B}	1.76^{B}
173	A	0.63^{C}	13.94^{B}	2.23^{B}
	B	0.97^{A}	15.64^{CB}	1.87^{B}
	C	0.96^{A}	8.77^{C}	1.11^{B}
	Com	0.86^{C}	12.78^{C}	1.73^{B}
Pioneer	A	0.45^{D}	5.39^{C}	1.23^{D}
	B	0.90^{B}	13.20^{C}	1.82^{B}
	C	0.92^{B}	5.69^{D}	1.00^{B}
	Com	0.76^{D}	8.09^{D}	1.37^{C}

Note: Means followed by the same letter in the same column are not significantly different at the 0.05 level of probability.

A: M.S medium with 0.0 mg/l 2,4-D B: M.S medium with 2.0 mg/l 2,4-D C: M.S medium with 4.0 mg/l 2,4-D

The genetic variation and heritability in broad (H_b%) sense were estimated within each 2,4-D levels and from the data combined over three levels of 2,4-D for all *in vitro* studied traits and the obtained results are presented in Table 3. The results revealed that the magnitude of genetic variation was positive for all *in vitro* traits at the three levels of 2,4-D.The results showed that genetic variation was high for all traits with respect to the three levels of 2,4-D concentration (0.0 mg, 2 mg and 4 mg/l). These results were confirmed by the values of heritability, which ranged from 84.62 to 99.79 % for responding embryos at level C and number of callus at level B, respectively. Moreover, the results also showed that heritability in broad sense (H_b%) was high (more than 99%) for all traits in the absence of 2,4-D. Thus, it could be more precise to estimate these parameters from the data

combined over three levels of 2,4-D concentrations. The results revealed that the genetic variations were high and positive for most of studied traits. This finding is emphasized by the heritability values, which were more than 80% for all studied traits except for responding embryos. In addition the values of genetic by levels interaction variations were positive in all studied *in vitro* traits.

Table 3: Estimates of relative magnitudes of different genetic parameters for *in vitro* traits at the three levels of 2,4-D (A, B and C) and from the combined data over the three levels of 2,4-D .

Genetic parameters	Responding embryo				Callus fresh weight				Number of callus			
	A	B	C	Com.	A	B	C	Com.	A	B	C	Com.
$\sigma^2 g$	0.0622	0.0018	0.0011	0.006	91.839	16.769	49.479	40.05	0.837	0.339	0.419	0.451
$\sigma^2 gL$	-	-	-	0.015	-	-	-	12.65	-	-	-	0.081
$\sigma^2 e$	0.0008	0.0003	0.0005	0.001	2.383	2.033	1.749	2.06	0.007	0.002	0.012	0.007
$\sigma^2 ph$	0.0625	0.0019	0.0013	0.011	92.634	17.446	50.062	44.95	0.839	0.341	0.423	0.481
$H_{b\%}$	99.52	94.74	84.62	54.55	99.14	96.12	98.83	89.10	99.73	99.79	99.03	93.76

A: M.S medium with 0.0 mg/l 2,4-D
B: M.S medium with 2.0 mg/l 2,4-D
C: M.S medium with 4.0 mg/l 2,4-D

Genotypic (r_g) and phenotypic (r_{ph}) correlation among different pairs of the studied traits were studied from the data combined over the three levels of 2,4-D and the obtained results are shown in Table 4. The results revealed that both phenotypic (above) and genotypic (below) correlation coefficient values were close with respect to most of studied traits. A positive and significant correlation was found between responding embryos and callus weight (0.98). This trait responding embryos is genetically correlated with callus weight and the coefficient value was 0.98. Owing to these results, responding embryos could be used as indicator trait for selecting the genotypes which are suitable for mature embryos culture purpose in maize.

Table 4: Phenotypic (above) and genotypic (below) correlations among pairs of *in vitro* traits over the three levels of 2,4-D.

	Responding embryo	Callus fresh weight
Responding embryo	---	
Callus fresh weight	0.98* 0.98*	---
Number of callus	0.79 0.80	0.90 0.90

*** Significant at 0.05 of probability.**

Effect of genotype, salinity levels and their interaction:

The data which were obtained from the four salinity levels for varieties were set up in a combined analysis of variance and the obtained results are presented in Table 5. Significance testes on the mean squares of genotype were significant for all studied traits except for Na^+/k^+ ratio. These findings indicated the presence of real differences among these genotypes. Therefore, the planned comparisons between these genotypes and the partitioning of the phenotypic variance to its components are valid.

T5

Furthermore, levels and genotype × levels interaction mean squares were highly significant with respect to all the studied traits except for a few cases such as levels for K^+% content and genotype × levels for Na^+ %, water content and sugars accumulation. This indicates that these genotypes gave different responses at different salinity levels.

Effect of salt stress levels on Na^+, K^+, Na^+/K^+ ratio, Cl^-, sugars, proline, electrolyte leakage (EL), relative growth rate (RGR) and water content (W.C) in the callus of different studied genotypes are shown in Table 6. The results showed the level of sodium (Na^+) content was significantly increased in the salt-stressed cells. The results cleared that greatest percentages value for Na^+ was observed in genotype 173 at levels S_0, S_1 and S_2 (0.51%, 0.97% and 1.27%, respectively), whereas the genotype 168 showed greatest percentages at level S_3 (2.02%). Significantly less Na^+ was accumulated in the callus of 122 and pioneer than the other two genotypes. Also, potassium (K^+) content did not change regularly in all genotypes. But generally K^+ trend tend to decrease with increasing stress levels, this was evident when medium salt concentration was raised to 6000 ppm. Therefore, Na^+/K^+ ratio was increased in all genotypes with increasing salt stress level. In a similar trend, the Cl^- content in the callus was increased in parallel to increasing salt stress level. These results are agreed with Summart *et al.* (2010) who found that rice cells accumulated high level of Na^+ during stress, whereas the accumulation of K^+ was decreased. High level of Na^+ inside the cells inhibited the K^+ uptake resulted in increased level of the Na^+/K^+ ratio.

The overall mean of Na^+ levels was differed significantly ($P<0.05$) among genotypes. Both genotypes 122 and pioneer were significantly lower than genotypes 168 and 173. The lowest significantly ($P<0.05$) level of potassium (K^+) was in genotype 122. Also, K^+ level significantly higher in genotypes 168 and 173. The difference between genotype 173 and pioneer was not significant. Otherwise, the lowest significantly ($P<0.05$) ratio of Na^+/K^+ was in genotype pioneer which was 0.73, whereas the other genotypes were not significantly differed. The mean of chloride (Cl^-) concentration was similar in genotypes 122, 168 and 173, which were significantly ($P<0.05$) lower than those in pioneer.

Data clearly revealed that sugars content were generally increased with increasing salt stress levels, and this increase was most pronounced with increasing medium salt concentration from 3000 to 6000 ppm. Sugars content were significantly higher in hybrid 173 and hybrid 122 (10.21, 9.69 mg/g f.wt, respectively) than other genotypes and there was not noticed significant difference between them in most cases and the lowest content was observed in hybrid 168 (3.59 mg/g f.wt). Our results disagreed with those reported in tomato Perez-Alfocea *et al.* (1994), they found that soluble sugars content increased significantly in calli issued from salt-sensitive species while a significant decrease (50%) was observed in calli issued from the salt-tolerant species indicating that soluble sugars were not implied in salt tolerance in these species.

The results revealed that the accumulation of proline was higher under salt stress treatment than the untreated calli for all genotypes. The level of increase in the proline concentration in response to salt stress varied

between the maize varieties. In particular, hybrid 173 was observed to have the highest proline accumulation under salinity stress treatment among the genotypes and at the combined data over all four levels of salinity. While, pioneer variety had the lowest proline accumulation among the other varieties. Similar results have been reported in rice by Summart *et al*. (2010); Htwe *et al.*(2011)

In order to assess membrane permeability (M.P), electrolyte leakage was determined. Electrolyte leakage reflects the changes of cell membrane structure under salt stresses. Its relative conductivity can be used to evaluate the damage on structure and function of cell membranes under salt stresses. The results showed that salinity stress significantly increased electrolyte leakage in all genotypes. The largest increase in electrolyte leakage was observed in pioneer (59.69%), which was significantly different over 122, 173 and 186 genotypes as increasing of 6.5%, 29% and 32%, respectively. It showed the increase of damage to cell membrane and increase of electrolyte leakage of the membrane and a criterion of the damage to the plant in salinity stress conditions. The results are in agreement with Mansour *et al.*(2005)who found that NaCl increased plasma membrane permeability in maize; Kaya *et al*. (2010) who reported that membrane permeability was increased in the leaves of maize plants in the salt treatment compared to the non-stressed plants and Bayat *et al*. (2012) who determined that electrolyte leakage of calendula plant was intensively increased by salt treatment.

The relative growth rate (RGR%) were reduced with increasing levels of NaCl in all genotypes. The reduction was significantly stronger in pioneer than other genotypes with ratio (63%, 81.9% and 90.6%) fresh weight reduction relative to the control when cultured in 1500, 3000 and 6000 ppm followed by173, 122 and 168. The reduction in fresh weight of the callus as the salt stress increased in the medium may refer to the reduction in the water availability to the callus cell due to the increase of sodium chloride concentration in the medium. Reduction in callus growth in response to increasing concentrations on NaCl has been observed in *in vitro* cultures of several plants as alfalfa (Shah *et al*., 1990), sunflower (Carceller and D'Ambrogio, 1994), mustard (Gangopadhyay *et al*., 1997), orange(El-Yacoubi *et al*., 2010) and rice (Htwe *et al*., 2011) tomato (Shibli *et al.,* 2011).

Water content (WC) was decreased with increasing levels of NaCl compared to control in all genotypes. From the combined data, the largest amount of water content belonged to genotype 122 with ratio 94.37; while the lowest ratio was attributed to genotype pioneer (92.45). These results indicate that salinity also negatively affects water absorption. The results are in agreement with Cicek and Cakirlar (2002) who suggested that high osmotic pressure resulted from increasing salinity restricted plant cells water uptake; Pesqueira *et al*. (2003)who found water content evolution clearly decreased in NaCl treated plant compared to control ones and Koutoua *et al*. (2011) who found the water content varies according to genotype and type of calli.

The genetic variation and heritability in broad (H_b%) sense were estimated within each salinity level for Na^+, K^+ content, Na^+/K^+ ratio and Cl^- %

and the obtained results are presented in Table 7. The results revealed that the magnitude of genetic variation was positive for all traits at the four levels of salinity. The results showed that genetic variation was high for all traits with respect to the four levels of salinity (0 ppm, 1500 ppm, 3000 ppm and 6000 ppm) except for K^+% at level S_3(6000 ppm), for Na^+% and Cl^-at levels S_2 and S_3 and for Na^+/k^+ at levels S_1 and S_3.These results were confirmed by the values of heritability, which ranged from 98.37 to62.50% for Na^+ % at level S_0 and Cl^-% at level S_3, respectively. Moreover, the results also showed that heritability in broad sense (H_b%) was low (less than 75%) for all traits in the high concentration salinity (6000 ppm).

Table 6. Mean performance of all genotypes for all studied traits at each level of salinity and the combined data over all the four levels (S_0, S_1, S_2 and S_3).

Genotype		Na^+%	K^+%	Na^+/K^+	Cl^-%	Sugars	Proline	EL	RGR	W.C
122	S_0	0.15^{C}	0.97^{C}	0.17^{B}	0.21^{C}	6.52^{A}	21.53^{A}	44.96^{A}	7.30^{B}	95.37^{A}
	S_1	0.63^{B}	0.94^{B}	0.71^{A}	0.37^{B}	10.76^{A}	24.94^{B}	53.43^{A}	6.57^{B}	94.90^{A}
	S_2	1.11^{A}	0.83^{B}	1.34^{A}	0.44^{A}	10.26^{A}	28.00^{C}	61.09^{A}	4.60^{B}	94.17^{A}
	S_3	1.79^{AB}	0.96^{A}	1.90^{AB}	0.60^{AB}	11.22^{AB}	36.87^{B}	63.86^{B}	2.10^{B}	93.03^{A}
	Com	0.92^{B}	0.92^{C}	1.03^{A}	0.41^{B}	9.69^{A}	27.84^{B}	55.83^{B}	5.14^{B}	94.37^{A}
168	S_0	0.23^{B}	1.20^{B}	0.19^{B}	0.24^{B}	2.39^{B}	21.99^{A}	31.26^{B}	29.53^{A}	94.00^{A}
	S_1	0.75^{AB}	1.19^{B}	0.63^{A}	0.35^{B}	4.41^{B}	35.78^{A}	34.38^{B}	26.15^{A}	93.63^{B}
	S_2	1.20^{A}	1.24^{A}	0.97^{B}	0.46^{A}	3.24^{B}	40.40^{B}	40.28^{B}	18.10^{A}	92.03^{B}
	S_3	2.02^{A}	0.98^{A}	2.11^{A}	0.65^{A}	4.34^{C}	45.71^{A}	56.55^{C}	7.73^{A}	90.70^{B}
	Com	1.05^{A}	1.15^{B}	0.98^{A}	0.42^{B}	3.59^{C}	35.97^{A}	40.61^{C}	20.38^{A}	92.60^{B}
173	S_0	0.51^{A}	1.76^{A}	0.29^{A}	0.26^{B}	6.41^{A}	13.03^{B}	30.43^{B}	3.33^{C}	93.93^{A}
	S_1	0.97^{A}	1.22^{AB}	0.82^{A}	0.33^{B}	10.51^{A}	35.88^{A}	37.04^{B}	3.03^{BC}	92.73^{C}
	S_2	1.27^{A}	1.27^{A}	1.01^{B}	0.46^{A}	10.63^{A}	47.50^{A}	40.82^{B}	1.35^{BC}	91.90^{B}
	S_3	1.83^{AB}	1.21^{A}	1.50^{AB}	0.61^{AB}	13.29^{A}	49.42^{A}	61.03^{BC}	0.59^{C}	91.27^{B}
	Com	1.15^{A}	1.37^{A}	0.90^{A}	0.42^{B}	10.21^{A}	36.46^{A}	42.33^{C}	2.07^{C}	92.46^{B}
Pioneer	S_0	0.24^{B}	1.34^{B}	0.18^{B}	0.31^{A}	7.29^{A}	11.47^{B}	45.32^{A}	1.27^{C}	94.83^{A}
	S_1	0.72^{AB}	1.51^{A}	0.48^{A}	0.45^{A}	10.07^{A}	11.71^{C}	55.20^{A}	0.47^{C}	92.63^{C}
	S_2	1.19^{A}	1.46^{A}	0.83^{B}	0.49^{A}	6.00^{B}	33.17^{C}	65.06^{A}	0.23^{C}	91.30^{C}
	S_3	1.52^{B}	1.11^{A}	1.43^{B}	0.58^{B}	10.08^{B}	36.73^{B}	73.17^{A}	0.12^{C}	91.03^{B}
	Com	0.92^{B}	1.36^{A}	0.73^{B}	0.46^{A}	8.36^{B}	23.27^{C}	59.69^{A}	0.52^{D}	92.45^{B}

Note: Means followed by the same letter in the same column are not significantly different at the 0.05 level of probability.
S_0: M.S medium with 0.000 ppm NaCl S_1: M.S medium with 1500 ppm NaCl
S_2: M.S medium with 3000 ppm NaCl S_3: M.S medium with 6000 ppm NaCl

T7

The results showed that increasing concentrations of salinity led to decrease in heritability for most traits. Thus, it could be more precise to estimate this parameter from the data combined over four levels of salinity. Therefore, the relative magnitudes of these parameters were estimated for the same studied traits from the combined data over the four levels and the obtained results are shown in Table 8. The results revealed that the genetic variations were low and positive for all cases except for K^+ ratio. This finding was emphasized by the heritability values, which were less than 55% for all cases except for K^+ ratio. In addition, the values of genetic by levels interaction variations were high and positive in all traits except for K^+ ratio. This finding explains the low values of heritability in these traits as well as the genes control these traits are highly affected by salinity levels.

Table 8: Estimates of relative magnitudes of different genetic parameters for Na^+%, K^+ %, Na^+/K^+ ratio and Cl^-% over all the four levels of salinity from the combined data.

Genetic parameters	K^+%	Na^+%	Na^+/k^+	Cl^- %
σ^2g	0.0370	0.0084	0.0083	0.0002
σ^2gL	0.0191	0.0093	0.0237	0.001
σ^2e	0.0191	0.0155	0.0359	0.0005
σ^2ph	0.0481	0.0159	0.0262	0.0006
$H_{b\%}$	76.92	52.83	31.68	33.33

The genetic variation and heritability in broad (Hb%) sense were estimated within each salinity level and over all levels from the combined data for organic solutes accumulation (sugars, proline mg/g f.wt) and the results are presented in Table 9.The results revealed that the magnitude of genetic variation was positive for organic solutes accumulation at the four levels of salinity. The results showed that genetic variation was high with respect to the four levels of salinity (0.0ppm, 1500 ppm, 3000 ppm and 6000 ppm). These results were confirmed by the values of heritability in broad sense(H_b%), which was high (more than 85%) for two traits. Thus, it could be more precise to estimate these parameters from the data combined over four levels of salinity. The results revealed that the genetic variation were high and positive for studied traits. This finding was emphasized by the heritability values, which were more than 70% for studied. In addition the values of genetic by levels interaction variations were high and positive in two studied traits, especially in the cases of proline (mg/g f.wt). This finding explained the low values of heritability in these traits as well as the genes control these traits are highly affected by media composition. The results are in agreement with Talei *et al.,*(2013)who found high broad-sense heritability of proline which mean that trait is under the control of genes with additive and non-additive effects.

T9-10

The genetic variation and heritability in broad (H_b%) sense were estimated within each salinity levels for electrolyte leakage and relative growth rate as well as water content and the obtained results are presented in Table 10. The results revealed that the magnitude of genetic variation was positive for all traits at the four levels of salinity. The results showed that genetic variation was high for all traits with respect to the four levels of salinity (0.0 ppm, 1500 ppm, 3000 ppm and 6000 ppm) except for water content at level S_0 (0.0 ppm). These results were confirmed by the values of heritability, which ranged from 49.63 to 99.42 for water content at level S_0 and relative growth rate at level S_0. Thus, it could be more precise to estimate this parameter from the data combined over four levels of salinity.

Therefore, the relative magnitudes of these parameters were estimated for all traits from the combined data over the four levels and the obtained results are shown in Table 11. The results revealed that the genetic variation were high and positive for studied traits. This finding was emphasized by the heritability values, which were more than 80% for studied traits. In addition the values of genetic by levels interaction variations were positive in studied traits.

Genotypic (r_g) and phenotypic (r_{ph}) correlation among different pairs of the studied traits were studied from the data combined over the four salinity levels and the obtained results are shown in Table 12. The results revealed that both phenotypic (above) and genotypic (below) correlation coefficient values were close with respect to most of studied traits. Although, most of pairs of studied traits exhibited positive or negative but non-significant correlation coefficient values. A negative and significant correlation was found between water content and K^+ content. This indicates that K^+ content increase with decreasing water content. A negative and significant correlation was found between Na^+/k^+ and Cl^- content. This indicates that increasing that Na^+/k^+ ratio will decrease Cl^- content. Proline showed a high significant and negative correlation with electrolyte leakage. This indicates that with increasing proline membrane damage will decrease. Also, Na^+ showed a high significant and negative correlation with electrolyte leakage. Suggesting that comparementation of Na^+ in vacuoles is an essential mechanism to cope with salinity.

Table 11: Estimates of relative magnitudes of different genetic parameters for electrolyte leakage, relative growth rate and water content ratio over all the four levels of salinity from the combined data

Genetic parameters	Electrolyte leakage	Relative growth rate	Water content
$\sigma^2 g$	87.9913	78.3592	0.8140
$\sigma^2 gL$	10.9420	17.0957	0.1173
$\sigma^2 e$	8.1039	3.0099	0.3839
$\sigma^2 ph$	93.4281	83.6364	0.9713
$H_{b\%}$	94.18	93.69	83.81

Table 12: Phenotypic (above) and genotypic (below) correlations among pairs of traits over the four levels of salinity.

	K^+	Na^+	Na^+/k^+	Cl^-	Sugar	proline	EL	RGR
K^+	---							
Na^+	0.45 0.48	---						
Na^+/k^+	-0.80 -0.80	0.17 0.11	---					
Cl^-	0.65 0.73	-0.29 -0.37	-0.92 -0.99**	---				
Sugars	0.10 0.12	-0.05 -0.07	-0.14 -0.17	-0.19 -0.23	---			
proline	0.08 0.08	0.90 0.94	0.52 0.56	-0.52 -0.58	-0.31 -0.32	---		
EL	-0.15 -0.15	-0.89 -0.95*	-0.43 -0.47	0.39 0.42	0.44 0.44	-0.98* -0.99**	---	
RGR	-0.35 -0.36	0.20 0.21	0.52 0.57	-0.21 -0.23	-0.91 -0.92	0.54 0.55	-0.62 -0.62	---
W.C	-0.91 -0.96*	-0.52 -0.57	0.65 0.75	-0.64 -0.71	0.32 0.33	-0.28 -0.29	0.39 0.41	-0.07 -0.07

***,** Significant at 0.05 and 0.01 of probability, respectively.**

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التباين الوراثى فى سلوك كالس أصناف الذرة الناتج من مزارع الأنسجة تحت مستويات مختلفة من الملوحة

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تم اختبار أربعة هجن من الذرة هى:122 و 168 و173 و بايونير لاظهار دور التركيب الوراثى فى الاستجابة لتكوين الكالوس باستخدام 2,4-D و مقاومة الملوحة المعملية باستخدام ملح كلوريد الصوديوم. تم تكوين الكالوس من الأجنة الناضجةبزراعتها على بيئة موراشيج و سكوج مضافا اليها صفر،2 ،4 ملغم/لتر من 2,4-D. ثم تعريض الكالس الناتج من البيئة التى تحتوى على 2 ملغم/لتر 2,4-D لكل تركيب وراثى لمستويات مختلفة من الملوحة هى صفر،6000،3000،1500 جزء فى المليون من ملح كلوريد الصوديوم لتقييم الاستجابة الفسيولوجية للكالس الناتج من الهجن المدروسة. أظهرت النتائج أن متوسط مربعات التراكيب الوراثيةفى الثلاث تركيزات من 2,4-D كان عالى المعنوية, مما يدل على وجود اختلافات معنوية بين التراكيب الوراثية بالنسبة الصفات المتعلقة بالثلاث مستويات من الملوحة. علاوة على ذلك فان متوسط مربعات مستويات 2,4-D و التداخل بينهما و بين التراكيب الوراثية كان عالي المعنوية فيما يتعلق بالصفات السابقة. هذا يدل على ان هذة التراكيب الوراثية أعطت استجابات مختلفة على المستويات المختلفة من -2,4. Dوكان التباين الوراثى عاليا و موجبا فيما يتعلق بالصفات المدروسة لكل مستوى من 2,4-D. هذا يدل على أن هذة الصفات تتحكم بها الجينات بصفة اساسية.هذة النتائج مؤكدة من خلال قيم معامل التوريث والتى كانت أكبر من 80% لكل الصفات المدروسة فى كل مستوى من 2,4-D. وفيما يتعلق باستجابة الكالس للاجهاد الملحى, أكدت النتائج وجود اختلافات معنوية بين التراكيب الوراثية بالنسبة للمحتوى الأيونى فى خلايا الكالس من البوتاسيوم و الصوديوم و نسبة الصوديوم الى البوتاسيوم وكذلك الكلوريد فيما يتعلق بالمستويات الأربعة من الملوحة ماعدا النسبة المئوية للبوتاسيوم للمستويين S_1,S_3 والنسبة المئوية للصوديوم للمستويات S_1, S_2, S_3 والنسبة بين عنصرى الصوديوم والبوتاسيوم فى المستويات S_0, S_1, S_3 و النسبة المئوية لعنصر الكلوريد فى المستويات S_2, S_3.بالنسبة لتراكم المواد العضوية المذابة (السكريات و البرولين ملغم/غرام للوزن طازج)فان متوسط مربعات التراكيب الوراثية كان معنويا فى الأربعة مستويات من الملوحة ماعدا البرولين فى المستوى S_3.بالنسبة لنفاذية الغشاء الخلوى،معدل النمو النسبى و المحتوى المائى متوسط مربعات التراكيب الوراثية كان معنويا فى الأربعة مستويات من الملوحة ماعدا S_0, S_3 للمحتوى المائى. وقد أظهرت النتائج أن التباين الوراثى كان عاليا و ايجابيا لكل من النسبة المئوية لعنصر الصوديوم و البوتاسيوم و نسبة الصوديوم الى البوتاسيوم و الكلوريد, تراكم المواد العضوية (السكريات و البرولين) ، نفاذية الغشاء, معدل النمو النسبى و كذلك المحتوى المائى فيما يتعلق بالأربعة مستويات من الملوحة. هذة النتائج مؤكدة من خلال قيم معامل التوريث والتى كانت أكبر من 80% لأغلب الصفات المدروسة.

Table 5: Combined analysis of variance and the mean squares of genotypes, levels and their interactions for all studied traits.

S.O.V	d.f	K^+%	Na^+%	Na^+/k^+	Cl^-%	Sugars	Proline	Electrolyte leakage	Relative growth rate	Water content
Levels (L)	3	0.128	4.901**	5.026**	0.27**	38.42*	1501.97**	1432.77**	141.59**	20.879**
Genotypes (G)	3	0.519*	0.145*	0.206	0.006*	109.12**	496.93**	1096.83**	994.60**	10.504**
G X L	9	0.076*	0.044	0.107*	0.004**	5.01	109.64**	40.93**	54.29**	0.736
Erorr	32	0.022	0.016	0.043	0.001	1.74	10.36	8.53	3.01	0.355

Note: *, Significant at 0.05 and 0.01 levels of probability, respectively.**
S_0: M.S medium with 0.000 ppm NaCl
S_1: M.S medium with 1500 ppm NaCl
S_2: M.S medium with 3000 ppm NaCl
S_3: M.S medium with 6000 ppm NaCl

Table 7: Estimates of relative magnitudes of different genetic parameters for Na^+%, K^+ %, Na^+/K^+ ratio and Cl^-% at the four levels of salinity (S_0, S_1, S_2 and S_3)

Genetic parameters	K^+%				Na^+%				Na^+/k^+				Cl^-%			
	S_0	S_1	S_2	S_3	S_0	S_1	S_2	S_3	S_0	S_1	S_2	S_3	S_0	S_1	S_2	S_3
$\sigma^2 g$	0.1067	0.0485	0.0640	0.0049	0.0242	0.0158	0.0014	0.0297	0.0023	0.0092	0.0433	0.0732	0.0017	0.0023	0.0002	0.0005
$\sigma^2 e$	0.0118	0.0219	0.0145	0.0285	0.0012	0.0152	0.0092	0.0366	0.0020	0.0343	0.0099	0.0973	0.0002	0.0004	0.0005	0.0008
$\sigma^2 ph$	0.1106	0.0558	0.0688	0.0144	0.0246	0.0209	0.0045	0.0419	0.0030	0.0206	0.0466	0.1056	0.0018	0.0024	0.0004	0.0008
$H_{b\ \%}$	96.47	86.92	93.02	34.03	98.37	75.60	31.11	70.88	76.67	44.66	92.92	69.32	94.44	95.83	50.00	62.50

S_0: M.S medium with 0.000 ppm NaClS_1: M.S medium with 1500 ppm NaCl
S_2:M.S medium with 3000 ppm NaClS_3: M.S medium with 6000 ppm NaCl

Table 9: Estimates of relative magnitudes of different genetic parameters for sugars and proline (mg/g f.wt) ratio at the four levels of salinity (S_0, S_1, S_2 and S_3) and over all the four levels from the combined data

Genetic parameters	Sugars (mg/g f.wt)					Proline(mg/g f.wt)				
	S_0	S_1	S_2	S_3	Com.	S_0	S_1	S_2	S_3	Com.
$\sigma^2 g$	4.4301	8.2774	11.8850	14.1039	8.6762	30.1204	129.0344	68.4307	36.2046	32.2746
$\sigma^2 gL$	-	-	-	-	0.9979	-	-	-	-	33.6730
$\sigma^2 e$	1.3932	2.7006	2.1325	1.8164	2.0107	1.4714	6.9893	11.8325	14.1759	8.6173
$\sigma^2 ph$	4.8945	9.1776	12.5958	14.7094	9.5959	30.6117	131.3642	72.3749	40.9299	43.5653
$H_{b\%}$	90.51	90.19	94.36	95.88	90.42	98.40	98.23	94.55	88.46	74.08

S0: M.S medium with 0.000 ppm NaCl, S1: M.S medium with 1500 ppm NaCl, S2 :M.S medium with 3000 ppm NaCl, S3: M.S medium with 6000 ppm NaCl

Table 10: Estimates of relative magnitudes of different genetic parameters for electrolyte leakage, relative growth rate and water content ratio at the four levels of salinity (S_0, S_1, S_2 and S_3)

Genetic parameters	Electrolyte leakage				Relative growth rate				Water content			
	S_0	S_1	S_2	S_3	S_0	S_1	S_2	S_3	S_0	S_1	S_2	S_3
$\sigma^2 g$	67.3097	113.87	169.27	45.29	168.700	134.450	66.576	12.0930	0.2364	1.0500	1.5550	0.8855
$\sigma^2 e$	2.7751	9.69	7.817	12.129	2.942	5.099	3.507	0.4906	0.7197	0.1642	0.0414	0.6064
$\sigma^2 ph$	68.2347	117.10	171.87	49.331	169.681	136.150	67.745	12.2565	0.4763	1.1047	1.5688	1.0876
$H_{b\%}$	98.64	97.24	98.48	91.80	99.42	98.75	98.27	98.67	49.63	95.05	99.12	81.42

S_0: M.S medium with 0.000 ppm NaCl,S_1: M.S medium with 1500 ppm NaCl,S_2: M.S medium with 3000 ppm NaCl,S_3: M.S medium with 6000 ppm NaCl