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Invitro Heterosis for Osmotic Relative Tolerance in Wheat

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ABSTRACT
To predict early heterosis and inbreeding depression for some callus traits in 4 bread wheat hybrids produced from 7 parents, 2 different experiments were achieved by using tissue culture technique in order to study the response of the exposed populations to water stress due to adding mannitol to MS media. Thus, the 1st experiment was a preface to decide which concentration of 2 or 2.5 mg/l of 2,4 Dichloro phenoxy acetic acid hormone (2,4-D) beside 0.5 mg/l of naphthalene acetic acid (NAA) for each concentration, will be more suitable undertaken in this study. Consequently, regarding the 2nd experiment, the selected concentration was reliable to use in the main experiment, discriminations were callus induction % and callus fresh weight (mg) for the 1st one. While, for the 2nd which exhibited the effect of ascorbic acid (AsA) in the tolerance improvement to stress of the tested populations, callus growth index (CGI), *Invitro* tolerance (INTOL), relative tolerance percentage (Rt %) and reduction percentage (R%) were used as osmotic stress tolerance indices.
Key words: bread wheat, drought stress, mature embryo, osmotic relative tolerance, callus characters, heterosis, inbreeding depression and potance ratio.

INTRODUCTION

Within the 20th century, world`s population has increased fourfold rapidly to be about 6 billion people, it may become more than 8 billion by 2030 (United nations, 2006). Conversely, earth`s resources are now under severe strain, while, the environmental and climatic changes are attacking the agronomic development. So, challenge is to confront the incessant increasing by adequate nourishment using the same amount of the available water and land.

Human civilization knows cereals as a satisfied source for about 50% of calories and amino acids required. Wheat is one of the three major cereal crops around the world and plays a key role in the global trading and economics, moreover, it is the dominant crop in temperate countries being used for human food and livestock feed, its success depends partly on its adaptability and high yield potential but also on the gluten protein fraction which confers the viscoelastic properties that allow dough to be processed into several products, it also contributes some minerals, vitamins, beneficial phytochemicals and dietary fibre components (Shewry, 2009). Wheat is an attractive option as a 'first generation biofuel' as the high content of starch is readily converted into sugars (saccharification) which can then be fermented into ethanol. (Murphy and Power, 2008). High adaptation of bread wheat (*Triticum aestivum* L.) and its diverse consumption in the human nutrition lead to be presented as the most important cereal in the world, especially in the developing countries (Farzi and Bigloo, 2010).

In fact, abiotic stresses such as salinity, water and heat stress can cause a dramatic loss in the international income. Global warming and concomitant increase in drought affected areas limit plant production which restricted by drought exposed areas, this loss lead to considerable economic and social problems (Ilker *et al*., 2011). Drought is the most significant environmental stress in wheat production worldwide, water stress can inhibit most bio-chemical and morpho-physiological processes, yet, improving yield under drought conditions is a major goal of plant breeding in the arid and semi-arid regions (Battah, 2007 and Belal *et al*., 2009).

Traditional breeding techniques are often losing a lot of time, effort and cost with uncertain results. In addition to the classical method of breeding, modern technologies of biotechnology have been developed in support of the classical breeding method in research on plant tolerance to drought (Galovic *et al*., 2010). *Invitro* traits can be used in combination with other agronomic important traits for crop improvement programs. Furthermore, *Invitro* selection is mainly associated with shorter time and lower cost as compared to conventional breeding methods (Abd El-Hady, 2006). One of such biotechnological techniques is tissue culture, which becoming increasingly popular as an alternative means of plant propagation and mass production (Shah *et al*., 2009). Tissue and callus culture creates a wide range of genetic variation in plant species which can be combined in breeding programs. (Mercado *et al*., 2000; Jain, 2001; El-Aref, 2002). Wheat mature embryos have a high frequency of callus induction (Ozgen *et al*., 1996).

The most important aspect of embryo culture is determining the culture medium that will provide the regular growth of embryos (Hu and Zanettini, 1995). Medium components and osmotic agents have been undertaken by many workers such as Zhang *et al*. (2000), Wang *et al*. (2008) and Rashid *et al*. (2009). Various osmotic agents have been employed in appropriate nutrient media to screen germplasm for drought tolerance; researchers have been able to control

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the drought environment more precisely using *Invitro* selection techniques (Maruyama *et al*., 2008; He *et al*., 2009; Srinivasan *et al*., 2010). Osmotic solutions of NaCl, mannitol, sorbitol or Polyethylene Glycol (PEG) have been used as *Invitro* stress factors for selecting salt and drought tolerant genotypes in screening procedures for seed germination of wheat (Almansouri *et al*., 2001). Ascorbic acid (AsA) can alleviate the harmful effects of osmotic stress on wheat calli and improve their tolerance (Ibrahim, 2009).

There is increasing evidence that genetic factors are the main contributors to the *Invitro* response of cereal tissues in culture (Hartmann *et al*., 1989). Genotypic difference in callus induction and callus fresh weight has been demonstrated in wheat (Ahloowalia, 1982 and Battah, 2014). Tissue culture responses using callus induction of wheat are influenced by some factors and their interaction such as genotypes and media (Zale *et al.,* 2004). Genotype is one of the main significant factors for successful induction percentage from wheat embryos under drought stress, hence, callus induction is genotype dependent and can be considered as an index for *Invitro* screening drought tolerant plants (Farshadfar *et al*., 2014).

Tolerance indices such as callus growth index (CGI), *Invitro* tolerance (INTOL) relative tolerance percentage (Rt%) and reduction percentage (R%) have been used by some investigators such as Barakat and Abd El-Latif, (1996); El-Hennawy (1996); Abd El-Samad *et al*. (2007); Elyasi *et al*. (2012); Farshardfar *et al*. (2012); Soliman and Hendawy (2013) and Farshardfar *et al*. (2014).

Complementary to the phenomenon of inbreeding depression is its opposite, "hybrid vigor" or heterosis. When inbred lines are crossed, their progeny shows an increase of those traits that previously suffered a reduction from inbreeding. In general terms, the fitness which was lost by inbreeding depression can be restored by crossing. The heterosis amount is the difference between the crossbred and inbred means (Falconer, 1989). Wheat shows hybrid vigor when hybridization occurs between varieties. Heterosis in tissue cultures of wheat was reported by Haggag and El-Hennawy (1991); Dornelles *et al*. (1997); Abd El-Hafez and Hamad, (2000); Abdel-Hady (2006) and Dağüstü (2008). Degree of dominance can also be calculated Invitro for callus characteristics, dominant effects were reported for the callus formation in wheat (Dağüstü, 2008).

The main objective of this present investigation was to predict early heterosis, inbreeding depression and dominance degree of some osmotic tolerance indices for the tested bread wheat populations.

MATERIALS AND METHODS

This study was achieved at the tissue culture laboratory, Faculty of Environmental Agricultural Science (FEAS), Al-Arish, Suez Canal University (SCU), Egypt in 2014 in order to predict early heterosis, inbreeding depression and dominance for some mature embryo callus characters of some bread wheat (*Triticum aestivum* L.) populations.

Genetic materials

The genetic materials used in this investigation included seven bread wheat genotypes. These genotypes were chosen on the basis of their genetic diversity for several agronomic traits. The seven genotypes were used as crossed parents in a six population model. The name, pedigree and origin of these parental genotypes are presented in Table (1). Hence, four crosses have been derived from the mentioned before parents, whereas, mature embryos of only the two parents and their F_{1s} as well as their F_{2s} of each cross were included without the two backcrosses (Bc_{1s} & Bc_{2s}) of the six populations model. While, the 4 crosses have been designed as follows:

Cross 1 = Amna-2 X Damara-6
Cross 2 = Rama-2 X Sakha 95
Cross 3 = Alshoroq-3 X Sakha 95
Cross 4 = Salah-1 X Babaga-3

Table (1); Name, pedigree and origin of the parental genotypes.

No.	Name	Pedigree	Origin
1	Amna-2	CHIL-1//VEE'S'/SAKER'S' ICW99-0026-2AP-0AP-0AP-3AP-0AP	Syria
2	Damara-6	VEE/PJN//2*KAUZ/3/PLK70/LIRA'S'//CNO79*2/PRL ICW99-0427-8AP-0AP-0AP-3AP-0AP	Australia
3	Rama-2	BOOMA-2/BOCRO-4 ICW99-0351-1AP-0AP-0AP-5AP-0AP	South Africa
4	Sakha 95	Under registration	Egypt
5	Alshoroq-3	BOCRO-4/3/MAYON'S'//CROW'S'/VEE'S' ICW99-0368-18AP-0AP-0AP-22AP-0AP	Syria
6	Salah-1	LFN/II58.57//PRL/3/HAHN/4/KAUZ/5/KAUZ/6/TOWPE ICW99-0425-8AP-0AP-0AP-22AP-0AP	Syria
7	Babaga-3	CHEN/AE.SQ//2*OPATA/3/BABAX CMSS98Y00585S-040Y-0B-0MXI-0AP-0AP-8AP-0AP	Syria

Methods

Herein, two successive experiments in ten replications were carried out on the mature embryos of the tested bread wheat genotype seeds: one for hormone concentrations and the other for osmotic stress.

Hormone concentrations experiment

Two concentrations of 2,4 Dichloro phenoxy acetic acid hormone (2,4-D) e.g.: 2 and 2.5 mgl^{-1} supplied with 0.5 mgl^{-1} of Naphthalene acetic acid (NAA) for each concentration were tested to select the best and suitable one to use in callus induction medium.

Callus induction medium

Murashige and Skoog (1962) media (MS) with vitamins was used as reported by Mohamed (2003) and supplemented with 30 g/l sucrose, 0.5 mg/l NAA and 0.1 g/l of myo-inositol. The 2,4-D hormone was added once by a concentration of 2 mg/l for a half number of the used mature embryos for each genotype, and once else by a concentration of 2.5 mg/l for the other half number of the used mature embryos for each genotype. In another words, the same media amount have been divided into the two above mentioned concentrations of 2,4-D, while, pH was adjusted to 5.7 and solidified with 3.5 g/l phytagel. Then, the two types of MS media were distributed in jars (10 cm. in diameter) which were autoclaved at 121˚C for 20 minutes.

Sterilization, isolation and culture of the wheat mature embryos

Wheat grains were surface sterilized for 3 min in 70% ethanol, followed by 30 min. in sodium hypochlorite 5.25% (commercial bleach: Clorox) containing one drop of tween twenty, then rinsed five times with sterilized distilled water, and soaked in sterile distilled water for 20 h. Under the hood, mature embryos were dissected from the endosperm and placed embryos axis-side down in full contact with the medium which was autoclaved in the glass jars. Embryos were incubated in an incubation chamber under fluorescent light (40 µE m^{-2} S^{-1}) at 24˚C and a 16/8 h. (light/dark). After seven days of culture, the embryos started germination and callus formation. By the second week, embryos were division rapidly. The comparison between the two concentrations of 2,4-D hormone was carried out by the determination of:

1- Callus induction percentage.
2- Callus fresh weight (mg).

Accordingly, MS medium contained 2,4-D by a concentration of 2.5 mg/l was considered better than medium contained 2 mg/l only of 2,4-D. Therefore, embryos which grown on the best media was subcultured and divided into two parts; the first part was the control and the second part treated with mannitol as a drought stress agent, whereas, osmotic potential was 0.4 M. After another two weeks, subculture have been achieved.

Osmotic stress experiment

Subculture was achieved by using ascorbic acid - (AsA) to alleviate the harmful effects due to mannitol stress and to improve the tolerance for osmotic stress. At the second subculture, half of the treated medium by mannitol was supplemented with ascorbic acid (1000 ppm) according to Ibrahim, 2009 to compare with the another half which still stressed and non-treated with ascorbic acid by the next fourteen days, the following traits were determined:

Callus fresh weight (mg)

For the three treatments; control, mannitol and mannitol + AsA.

Callus growth index (CGI)

Calculated twice, once for (C/M) concerning the effect of osmotic stress due to mannitol (M) contrary to the control (C), and the second for (M/M+AsA) concerning the alleviation treatment using M+AsA contrary to the stress treatment due to mannitol (M) as the following:

CGI= $(w1 - w0)/w0$**)**, (Abd El-Samad *et al.*, 2007).

Where: W0 and W_1 are fresh weight before treatment and final fresh weight after 2 weeks of treatment for each of the two treatments of stress and alleviation.

Invitro tolerance

(INTOL)= $\mathbf{RGR_{treatment} / RGR_{control}}$**,** (Al-Khayri and Al-Bahrany, 2000).

Where: RGR= relative growth rate = $[L_nW_2 - L_nW_1]/GP$ (**Birsin and Ozgen, 2004**), W_1 and W_2 are the initial and final weight of callus (only between control and stress treatments) and GP is the growth period = 14 days.

Relative tolerance percentage

$$(Rt\%)=\frac{Value\ under\ stress}{Value\ under\ non\ stress}x100$$

according to (Abd El-Samad *et al.*, 2007) betweencontrol and stress treatments only.

Reduction percentage (R%)

only between control and stress treatments according to Abd El-Samad *et al.*, 2007.

Statistical and genetical analysis

Records were subjected to analysis of variance via randomized complete design according to the method outlined by Steel and Torrie, (1980). Least significant difference (LSD) method at confidence intervals of 0.95 have been used to compare means of each of treatment, genotype and their interaction. Computations were fulfilled conformably with MSTAT computer program package. In each cross, mean and variance were calculated for each of P_1, P_2, F_1 and F_2 populations. Means and variances were used to estimate genetical parameters and significance.

Heterosis and heterobeltiosis

Heterosis was expressed as deviation of F_1 generation mean from mid-parent (MP) or better parent (BP= heterobeltiosis) values, as follows:

Heterosis over mid-parent (MP) %

$$= \frac{(\overline{F1} - \overline{MP})}{\overline{MP}} X\ 100$$

Heterosis over the better-parent (BP) %

$= ((\overline{F_1} - \overline{BP}) / \overline{BP}) \times 100$

To test the significance of the above estimate of heterosis, the variance of heterosis was calculated as a linear function of three variances according to Wynne *et al.* (1970).

Variances of heterosis over mid-parent deviation = $\overline{VF_1}$ + ¼ $\overline{VP}_1$ + ¼ $\overline{VP}_2$

Variances of heterosis over better-parent deviation = $\overline{VF}_1 + \overline{V}BP$

Heterosis significance determined by least significance difference value (L.S.D) at 0.05 level of probability according to Steel and Torrie (1980) by the following formula:

L.S.D. 0.05 = t 0.05 (edf) x Sd

Where: $t_{0.05}$ is the tabulated value of "t" for the error degrees of freedom (edf) at 0.05 level. Sd = standard deviation.

Sd for mid– parents heterosis = $\sqrt{3MSe / 2r}$

Sd for better – parents heterosis = $\sqrt{2MSe / r}$

Where: MSe = the mean squares of experimental error from the analysis of variance table, and r = number of replications.

Inbreeding depression (I.D %)

I.D % = $(\overline{F_1}-\overline{F_2}/ \overline{F_1}) \times 100$

To test the significance of inbreeding depression the variance of (I.D.) deviation was calculated as following formula: Variances of I.D deviation = $VF_1 + VF_2$

Potence ratio

The nature and degree of dominance were measured by calculating the potence ratio according to the formula given by Smith (1952) as follows:

$$P = \frac{\overline{F_1} - \overline{MP}}{½ (\overline{P_2} - \overline{P_1})}$$

Where; F_1= First generation mean, P_1= The mean of the lower parent, P_2= The mean of the higher parent and MP = The mean of the two parent = $P_1 + P_2 / 2$.

Complete dominance is indicated when potence ratio is equal ± 1.0. Partial dominance is indicated when the ratio falls between + 1.0 and -1.0 except the zero value which indicates absence of dominance. Over dominance is indicated if potence ratio exceeds + 1.0 or less than -1.0. It should be pointed out here that potence values indicate the average dominance of the whole gene set of one parent on the other parent and did not indicate actual dominance of individual genes.

RESULTS AND DISCUSSION

Hormone concentrations experiment

Hormonal effect and initiation

Callus induction percentage

Analysis of variance in Table (2) for callus induction percentage clearly indicated that there were significant differences between the two tested concentrations of the used phyto-hormone, also among the evaluated genotypes of the seven parents and their F_1 hybrids of bread wheat plant. However, the interaction between the concentrations and the genotypes was insignificant. Data presented in Table 3 pointed out that the callus induction percentage have been increased as a result to the increasing of the concentration of the 2,4-D hormone from 2.00 to 2.50 mg/l in the culture medium of MS.

Table (2): Analysis of variance for callus induction percentage (CI%) and callus fresh weight (CFW) (mg) in mature embryo callus of the tested bread wheat (*Triticum aestivum* L.) parents and their F_1 hybrids.

SOV	Df	Mean square	
		CI%	CFW
Concentrations (C.)	1	628.48**	150690.00**
Genotypes (G.)	10	1029.01**	43182.75**
C. X G.	10	13.12^{ns}	5970.04**
Error	198	15.90	742.10
Total	219		
C.V%		4.41	12.93

Thus, significant differences among genotypes are indicating the presence of genetic variability, different responses of genotypes to callus induction and possible selection of callus induction in bread wheat genotypes at *Invitro* level using mature embryos of wheat (Table 3). Racz *et al*. (1993) studied the effect of sucrose, 2,4-D and different vitamins on callus induction in winter wheat mature embryos. Solid MS medium was optimum

for mature embryo culture of wheat supplement with different combinations of plant growth regulators (Mendoza and Kaeppler, 2002). Mature embryos of all seven parents and their four F_1 hybrids were inoculated in two different concentrations of 2,4-D in the MS callus induction medium, concentrations were 2 and 2.5 mg/l beside 0.5 mg/l of Naphthalene acetic acid (NAA) for each concentration.

Embryos of all genotypes swelled up after about 3 - 4 days of inoculation and calli emerged afterwards. Embryogenic calli produced were nodular and compact, which is in agreement with the finding of Ozias-Akins and Vasil, (1982) who identified these nodular structures as embryoids. Remarkable effect on callus induction was recorded due to the concentration 2.5 mg/l as the 2,4-D was increased from 2 mg/l to 2.5 mg/l of 2,4-D production of embryogenic calli increased from 94.20 % to 98.30 % regarding to the highest value which obtained by Babaga-3, followed by Salah1, by a percentage from 91.10 to 96.70 concerning parents, therefore, concerning the F_1 hybrids, the fourth hybrid Salah-1 X Babaga-3 recorded the highest percentage which increased from 98.69 to 99.87 due to this increasing in the hormone concentration. It could be observed that 2.5 mg/l may be the best concentration of 2,4-D with 0.5 mg/l of naphthalene acetic acid (NAA) in the MS induction media, so that this study suggests that genotypic factor seems to be operating in response of particular concentrations of growth hormones. Our results revealed the difference in the ability to callus induction among wheat genotypes and confirmed that callus induction is genotype dependant. Similar results obtained by Ozgen *et al.* (1996) and Farshadfar *et al.* (2012).

Table (3): Concentration effect of 2,4-D hormone in the MS media on callus induction percentage and callus fresh weight (mg) in mature embryo callus of the tested bread wheat (*Triticum aestivum* L.) parents and their F_1 hybrids as well as their combined (Com.).

Genotypes	Callus induction %			Callus fresh weight (mg)		
	2.5 g 2,4-D	2.0 g 2,4-D	Com.	2.5 g 2,4-D	2.0 g 2,4-D	Com.
Amna-2	96.10	90.60	93.35	214.94	149.50	182.22
Damara-6	95.40	92.20	93.80	222.53	154.80	188.67
Rama-2	88.90	87.10	88.00	201.85	182.80	192.33
Alshoroq-3	82.20	77.30	79.75	183.48	87.90	135.69
Sakha 95	92.00	89.40	90.70	225.39	128.40	176.90
Salah-1	96.70	91.10	93.90	265.21	216.40	240.81
Babaga-3	98.30	94.20	96.25	289.74	234.00	261.87
Amna-2 X Damara-6	99.37	98.02	98.69	257.82	259.21	258.52
Rama-2 X Sakha 95	81.48	78.96	80.22	218.73	188.90	203.81
Alshoroq-3 X Sakha 95	83.82	79.38	81.60	228.41	139.92	184.16
Salah-1 X Babaga-3	99.87	98.69	99.28	296.84	287.34	292.09
General mean	92.19	88.81	90.50	236.81	184.47	210.64
LSD (0.05)						
Concentrations (C)			6.83^{*}			46.67^{*}
Genotypes (G)	4.81^{*}	4.22^{*}	2.81^{*}	11.78^{*}	24.59^{*}	19.19^{*}
Interaction (CX G)			3.97^{ns}			27.14^{*}

Com; Combined data.

It is worthy to note that, in common, F_1 hybrids have a better mean performance than that of the parents. Current results are also agree more or less with the findings of some investigators such as Shah *et al*., (2003) who found excellent callus induction in wheat at 3.5 mg/l and good callus induction at 3 mg/l of 2,4-D. While, Turhan and Baser, (2004) who obtained best results by using 4 mg/l of 2,4-D. In addition, results obviously revealed that culture response was influenced by the wheat genotypes and also emphasized a profound effect of genotype on callus induction capacity which is in agreement with the reports of callus induction in bread wheat (Hess and Carman, 1998). Results are consistent with other studies of several workers with a respect of osmotic stress, such as Galovic *et al.* (2010) who found that cultivars differed significantly from 24% to 100% in percentage of induced calli. In general, callus induction used as an efficient character for assessment of culture responses from mature embryo in wheat genotypes, the callus fresh weight is provided a more concise quantitative character for the development rate of callus, in plant culture, a desirable genotype is expected to possess high callus induction (Abdelsamad *et al.*, 2007).

Callus fresh weight (mg)

Analysis of variance in Table 2 for callus fresh weight (mg) clearly indicated that there were significant differences between the two tested concentrations of the used phyto-hormone, and among the evaluated genotypes of the seven parents and their F_1 hybrids of bread wheat plant, as well as, the interaction between the concentrations and the genotypes. Data presented in Table 3 pointed out that the callus fresh weight (mg) increased as a result to the concentration increasing of the 2,4-D hormone from 2.00 to 2.50 mg/l in the culture medium of MS. Thus, significant differences among

genotypes are indicating the presence of genetic variability, different responses of genotypes to callus fresh weight (mg) and possible selection of callus fresh weight (mg) in bread wheat genotypes at *Invitro* level using mature embryos of wheat (Table 3). Similarly, remarkable effect on callus fresh weight (mg) was recorded due to the concentration 2.5 mg/l as the 2,4-D was increased from 2 mg/l to 2.5 mg/l of 2,4-D production of embryogenic calli increased from 234.00 mg to 289.74 mg regarding to the highest value which obtained by Babaga-3, followed by Salah-1, by a value from 216.40 mg to 265.21 mg concerning parents, therefore, concerning the F_1 hybrids, the fourth hybrid Salah-1 X Babaga-3 recorded the highest weight (mg) which increased from 287.34 mg to 296.84 mg due to this increasing in the hormone concentration.

Consequently, It could be observed that 2.5 mg/l may be the best concentration of 2,4-D with 0.5 mg/l of naphthalene acetic acid (NAA) in the MS induction media, so that this study suggests that genotypic factor seems to be operating in response of particular concentrations of growth hormones.

Generally, It is of interest to declare that F_1 hybrids have a better mean performance than the parents mean performance. These results are agree more or less with some previous investigators such as Shah *et al*. (2003) who found excellent callus induction and weight in wheat at 3.5 mg/l and good callus induction at 3 mg/l of 2,4-D, and Turhan and Baser, (2004) who obtained best results by using 4 mg/l of 2,4-D. These results are also go on line completely with the findings of Abdelsamad *et al.* (2007), Shah *et al. (*2009) and Farshadfar *et al. (*2012).

Genetical analysis of callus traits

Inheritance behaviour of plant studies can be detected through a good observation about some biometrical parameters such as means and variances in addition to some other indicators of gene action and hybrid vigor such as percentages of heterosis, heterobeltiosis, and inbreeding depression as well as through the identification of both the nature and the degree of the dominance by different manners such as the potence ratio. Hereinafter, callus induction percentage and callus fresh weight (mg) in view of the mentioned before parameters. Data illustrated in Table (4) referred to means, mid-parents and variances for 4 types of the six populations model, *i.e.* P_1, P_2, F_1 and F_2 of the four crosses in a comparison between the two concentration of 2,4-D phyto-hormone for callus induction percentage. Results clearly showed that the F_1 mean values for the first and the fourth crosses; (Amna-2 X Damara-6) and (Salah1 X Babaga-3) into the two hormone concentrations were higher, together, than the mid-parent values. This indicated the presence of heterotic effects towards the higher parent for callus induction percentage. Otherwise, the F_1 mean values for the second and the third crosses; (Rama-2 X Sakha 95) and (Alshoroq-3 X Sakha 95) into the two hormonal levels of concentration were lower, together, than the mid-parent values. This indicated the presence of heterotic effects towards the lower parent for this character. The highest F_1 mean value obtained by cross 4 (Salah-1 X Babaga-3) under the increased hormone concentration conditions, meanwhile, the lowest F_1 mean value obtained by cross 2 (Rama-2 X Sakha 95) under the decreased concentration of the 2,4-D hormone conditions. In this context, it is obvious that the F_2 mean values were more than the F_1 means in the four crosses under the two hormonal levels, except cross 2 (Rama-2 X Sakha 95) under the decreased concentration conditions. These results indicated that the divers of genetic background of the parents involved.

Table (4); Mean ($\bar{X}$) and variance (S^2) for P_1, P_2, F_1 and F_2 as well as mid-parent (MP) value of callus induction percentage in mature embryo callus for the four crosses of bread wheat (*Triticum aestivum* L.*)* under the two concentrations of 2,4-D hormone in the MS media.

Cr	Cn	I	P_1	P_2	MP	F_1	F_2
Cross 1	2.5 g	$\bar{X}$	96.10	95.40	95.75	99.37	99.65
		S^2	4.10	2.49		7.51	11.25
	2.0 g	$\bar{X}$	90.60	92.20	91.40	98.02	97.48
		S^2	15.16	22.18		20.49	32.49
Cross 2	2.5 g	$\bar{X}$	88.90	82.20	85.55	81.48	83.71
		S^2	22.54	13.73		27.13	49.28
	2.0 g	$\bar{X}$	87.10	77.30	82.20	78.96	81.50
		S^2	4.10	2.46		6.81	16.02
Cross 3	2.5 g	$\bar{X}$	92.00	82.20	87.10	83.82	84.63
		S^2	0.89	13.73		8.57	15.96
	2.0 g	$\bar{X}$	89.40	77.30	83.35	79.38	84.22
		S^2	19.82	2.46		13.28	26.74
Cross 4	2.5 g	$\bar{X}$	96.70	98.30	97.50	99.87	99.89
		S^2	3.79	1.12		4.01	9.12
	2.0 g	$\bar{X}$	91.10	94.20	92.65	98.69	99.31
		S^2	4.32	5.29		7.91	12.17

Cr.; Cross, Cn; Concentration, I; Index, Cross 1; Amna-2 X Damara-6, Cross 2; Rama-2 X Sakha 95, Cross 3; Alshoroq-3 X Sakha 95 and Cross 4; Salah-1 X Babaga-3.

Likewise, Data illustrated in Table (5) referred to means, mid-parents and variances for 4 types of the six populations model, *i.e.* P_1, P_2, F_1 and F_2 of the four crosses in a comparison between the two concentration of 2,4-D phyto-hormone for callus fresh weight (mg). Results clearly showed that the F_1 mean values for all crosses into the two hormone concentrations were heavier than the mid-parent values. This indicated the presence of heterotic effects towards the heavier parent for callus fresh weight (mg). The heaviest F_1 mean value obtained by cross 4 (Salah-1 X Babaga-3) under the increased hormone concentration conditions, meanwhile, The lowest F_1 mean value obtained by cross 3 (Alshoroq-3 X Sakha 95) under the decreased concentration of the 2,4-D hormone conditions. In this context, it is obvious that the F_2 mean values were more

than the F_1 means in the four crosses under the two hormonal levels, except cross 1 (Amna-2 X Damara-6) at the decreased concentration conditions. These results indicated that the divers of genetic background of the parents involved.

Table (5): Mean ($\bar{X}$) and variance (S^2) for P_1, P_2, F_1 and F_2 as well as mid-parent (MP) value of callus fresh weight (mg) in mature embryo callus for the four crosses of bread wheat (*Triticum aestivum* L.*)* under the two concentrations of 2,4-D hormone in the MS media.

Cr	Cn	I	P_1	P_2	MP	F_1	F_2
Cross 1	2.5 mg	$\bar{X}$	214.94	222.53	218.74	257.82	263.38
		S^2	84.48	166.72		253.87	579.42
	2.0 mg	$\bar{X}$	149.50	154.80	152.15	259.21	233.22
		S^2	472.72	274.62		540.21	946.16
Cross 2	2.5 mg	$\bar{X}$	201.85	183.48	192.67	218.73	221.02
		S^2	85.30	127.40		304.64	633.80
	2.0 mg	$\bar{X}$	182.80	87.90	135.35	188.90	190.61
		S^2	25.73	147.43		257.32	533.80
Cross 3	2.5 mg	$\bar{X}$	225.39	183.48	204.44	228.41	230.66
		S^2	67.07	127.40		192.21	229.03
	2.0 mg	$\bar{X}$	128.40	87.90	108.15	139.92	211.49
		S^2	165.16	147.43		301.93	629.03
Cross 4	2.5 mg	$\bar{X}$	265.21	289.74	277.48	296.84	313.64
		S^2	113.05	235.33		276.45	588.91
	2.0 mg	$\bar{X}$	216.40	234.00	225.20	287.34	292.21
		S^2	174.04	215.56		265.34	388.91

Cr.; Cross, Cn; Concentration, I; Index, Cross 1; Amna-2 X Damara-6, Cross 2; Rama-2 X Sakha 95, Cross 3; Alshoroq-3 X Sakha 95 and Cross 4; Salah-1 X Babaga-3.

In this regard, variances have been calculated separately for each population in each cross are given in Tables (4-5). The highest magnitude of variances were registered by the F_2 generations in the four studied crosses for callus induction percentage and callus fresh weight (mg) at the two treatments of the 2,4-D, which is logic due to the fact that the maximum heterogeneity exists in the F_2 generation.

On the other hand, the lowest variance magnitude was manifested by parents (P_1 and P_2) and F_1 populations, which also is expected from the breeding point of view due to the homogeneity of such populations. Thus, variances observed in the populations of (P_1, P_2 and F_1) of the investigated bread wheat (*Triticum aestivum* L.) mature embryo callus are may be due to the environmental factors. Concerning the hybrid vigor and gene action, Table (6) previously showed each of the heterosis percentage over both mid and better parent, inbreeding depression percentage and potence ratio for callus induction and weight.

Whereas, for heterosis, the desirable heterosis for the two characters in all cases at the two levels of the hormone concentration was the positive values. Results demonstrated that heterosis relative to the mid-parents was significant for 9 cases out of 16 for both callus induction percentage and callus fresh weight in the four crosses under the two treatment conditions.

In details, 8 cases of the positive and significant heterosis values over the mid-parents were concerning the all cases of the callus fresh weight (mg) character addition to the case of the first cross at the 2.5 g/l level of the 2,4-D hormone concentration. While, four significant negative values of mid-parent heterosis were found for callus induction percentage in both crosses 2 and 3 at both concentrations. Other 3 cases were positive and insignificant.

Meanwhile, 5 significant positive estimates of heterosis over the better parent were found only in callus fresh weight (mg) in cross 1 at the two levels of hormone concentrations, cross 2 at the 2.5 g/l level, cross 3 and cross 4 at the 2.0 g/l level. Meantime, 4 significant negative values of better parent heterosis were found for callus induction percentage also in both crosses 2 and 3 at both concentrations. The other remaining 7 cases of the heterosis over better parent estimates were positive and insignificant. The most useful heterosis estimate were recorded for callus fresh weight at the level of 2.0 g/l of 2,4-D hormone concentration in cross 2 over the mid-parent and cross 1 over better parent.

Regarding to inbreeding depression, percentages are measured as the percentage deviation of F_2 from F_1 mean performance. The desirable inbreeding depression for all cases of both callus induction and weight at the two hormonal treatments is the negative value. Significance was found in 7 cases; 4 of them were in callus induction percentage for the four crosses at the 2.0 g/l concentration of 2,4-D, another 2 cases were detected at the same level for callus fresh weight (mg) in both crosses 1 and 3, while, the other 1 case was estimated for the last trait at the level of 2.5 g/l in cross 4. The remaining cases are insignificant.

Nature and degree of dominance have been demonstrated in this study using the potence ratio. The results indicated that the degree of dominance was less than unit for callus induction percentage in cross 2 at the 2.0 g/l level of hormone concentration and in cross 3 at both concentration levels. These results indicated the important role of the partial dominance gene effects in controlling these cases, indicating that selection might be more effective for such these cases. Opposite trend, for all other cases for callus induction percentage and callus fresh weight (mg) at the two hormonal concentration treatments, results indicated that the degree of dominance was ranged between less than -1 and more than +1, except the values of 0 and 1. These results indicated the important role of the over dominance gene effects in controlling callus initiation and formation via both induction percentage and fresh weight.

These results are go on line with those findings of several workers such as Haggag and El-Hennawy

(1991); Abd El-Hady (2006) and Farshadfar *et al*. (2012) who reported that significant differences among genotypes and media are may indicating the presence of the genetic variability. Furthermore, Bahman *et al*. (2012) pointed out to the relatively high correlation between callus induction and final volume, therefore, genotypes have high callus induction % also represent bigger callus and favourable for tissue culture approaches. While, the existence of heterobeltosis in most of the hybrid combinations and dominance by large degrees, suggest the exploitation of dominance gene action for the improvement of callus traits (Etedali *et al*., 2011).

Significant and positive heterosis and over-dominance have been also indicated by Lange *et al*. (1995) and Dornelles *et al*. (1997).

Table (6): *Invitro* heterosis, inbreeding depression and potence ratio for callus induction percentage and callus fresh weight (mg) in mature embryo callus for the four crosses of bread wheat (*Triticum aestivum* L.) under the two concentrations of 2,4-D hormone in the MS media.

Trait	Cross	Conc.	Heterosis %		Inbreeding depression %	Potence ratio
			M.P	B.P		
Callus induction %	1	2.5 g/l	3.78*	3.40	-0.28	10.34
		2 g/l	2.56	1.67	-3.99*	2.92
	2	2.5 g/l	-4.76*	-8.35*	-2.73	-1.21
		2 g/l	-3.94*	-9.35*	-3.22*	-0.66
	3	2.5 g/l	-3.77*	-8.89*	-0.97	-0.67
		2 g/l	-4.76*	-11.21**	-6.10*	-0.66
	4	2.5 g/l	2.43	1.60	-0.02	2.96
		2 g/l	2.97	1.27	-4.10*	1.77
Callus fresh weight	1	2.5 g/l	17.87**	19.95**	-2.16	-10.30
		2 g/l	27.14**	24.96**	-20.56**	15.58
	2	2.5 g/l	13.53**	8.36*	-1.05	2.84
		2 g/l	39.56**	3.34	-0.91	1.13
	3	2.5 g/l	11.73**	1.34	-0.99	1.14
		2 g/l	29.38**	8.97*	-51.15**	1.57
	4	2.5 g/l	6.98*	2.45	-5.66*	1.58
		2 g/l	27.60**	22.80**	-1.69	7.06

Osmotic stress experiment

Effect of mannitol on callus fresh weight (mg) and use of ascorbic acid (AsA) in order to alleviation

Mannitol as a drought stress agent has been added to MS medium in order to create an osmotic stress during the callus growth and formation. Callus fresh weight (mg) after subcultures have been undertaken to assess such stress.

Ascorbic acid (AsA) have been used in a try of alleviation for a harmful or oxidative effect of stress caused by mannitol. Analysis of variance in Table (7) for callus fresh weight (mg) for the 7 parents and their 4 F_{1s} at the control condition and under both treatments of osmotic stress and its alleviation, exhibited that there were significant differences among treatments and among genotypes as well as the interaction between treatments and genotypes.

Table (7): Analysis of variance of callus fresh weight (CFW) (mg) in mature embryo callus of the tested bread wheat (*Triticum aestivum* L.) parents and their F_1 hybrids under control and both of osmotic stress and alleviation conditions.

SOV	df	Mean square
Treatments (T.)	2	833585.43**
Genotypes (G.)	10	70318.11**
T. X G.	20	9683.13**
Error	297	257.75
Total	329	
C.V%		7.83

It is understandable fact that osmotic stress has an oxidative and damaged effects on the structure of plant cells and all bio-process due to the accumulation of the free radicals in tissues. In view of the results in Table 8, no doubt that osmotic stress due to mannitol affected callus fresh weight (mg) negatively and significantly.

Since, general mean under the control conditions have been decreased from 267.42 mg to 106.09 mg by a percentage of 58.36%. These results are go in line with those of Zhang *et al*., (2000), Almansouri *et al*., (2001), Wang *et al*., (2008) and Rashid *et al*., (2009) and agree completely with Galovic *et al.* (2010) when applied

highly osmotic nutritive medium that contained induction medium reagents enriched with 0.4 M sorbitol as an inter mediate step in the preparation of callus tissue for transformation of mature embryo-derived wheat with major stress-modulated antioxidant target gene.

To alleviate osmotic stress effect and improve the osmotic stress tolerance, ascorbic acid (AsA) as a non enzymatic antioxidant which do as scavengers of the free radicals to counteract these harmful effects. Therefore, use of MS medium supplemented with AsA over duplicated the general mean of callus fresh weight (mg) under osmotic stress from 106.09 mg t0 223.28 mg to become about 83.49% of a callus fresh weight (mg) under the control conditions. Several studies are confirm these results. Ushimaru *et al.,* 2005 stated that L-ascorbic (vitamin C) is important for antioxidative and metabolic functions in both plants and humans, ascorbate itself is oxidized to dehydroascorbate during the process of antioxdation and dehydroascorbate reductase (DHAR) re-reduces the oxidized ascorbate, therefore this enzyme is assumed to be critical for ascorbate recycling.

Thus, stress tolerant genotypes have different mechanisms of tolerance and can preserve their growth rate better than sensitive genotypes (Zhu, 2000 and Ghanadha *et al*., 2005). Furthermore, the significant differences among genotypes and media are may indicate the presence of genetic variability, different responses for stress and alleviation treatment. In this context, Ibrahim, 2009 formulated different media for mature embryos from wheat to determine the effective concentration of ascorbic acid (AsA) which might alleviate the harmfull effects of salinity on wheat calli. She found that the effective concentration of AsA was at 1000 ppm which increased salt stress condition tolerance of wheat plants. In addition, the medium of MS which contained Naphthalene acetic acid (NAA) beside the 2,4-Dichlorophenoxy acetic acid hormone was better than MS media which contained 2,4-D only. She also explained the effectiveness of the ascorbic acid (AsA) which alleviated the harmful effects of salinity stress on wheat plants, due to its protected role on the treated wheat cells concerning the oxidation effects.

Table (8): Callus fresh weight (mg) in mature embryo callus of the tested bread wheat (*Triticum aestivum* L.) parents and their F_1 hybrids as well as their combined (Com.) under control and both of osmotic stress and alleviation conditions.

Genotypes	Callus fresh weight (mg)			
	Control	**Mannitol**	**Mannitol+AsA**	**Com.**
Amna-2	229.99	104.65	188.59	174.41
Damara-6	238.11	108.36	190.49	178.98
Rama-2	215.98	127.96	181.42	175.12
Alshoroq-3	196.32	61.53	139.39	132.41
Sakha 95	241.17	89.88	192.93	174.66
Salah-1	283.77	151.48	246.88	227.38
Babaga-3	310.02	163.80	263.52	245.78
Amna-2 X Damara-6	307.36	93.45	273.17	224.66
Rama-2 X Sakha 95	265.03	60.83	205.73	177.20
Alshoroq-3 X Sakha 95	276.76	60.48	230.40	189.21
Salah-1 X Babaga-3	377.06	144.55	343.60	288.40
General mean	267.42	106.09	223.28	198.93
LSD (0.05)				
Treatments (T)				9.31*
Genotypes (G)	9.92*	11.63*	7.68*	9.24*
Interaction (T X G)				14.98*

Com; Combined data, AaA; Ascorbic acid.

Indices of osmotic stress tolerance

Invitro osmotic stress tolerance can be evidenced by using some calculated formulas, which related to drought physiology during the growth inspections proceeding depending on callus fresh weight such as callus growth index (CGI), *Invitro* tolerance (INTOL), relative tolerance percentage (Rt%) and reduction percentage (R%). Notification, In this study, in order to study the direction of callus growth index under the stress conditions which caused by mannitol treatment contrary to the treatment of alleviation using AsA, determination of the callus growth index (CGI) have been done towards two opposite trends; once concerning control (C) and mannitol (M) treatments in a relationship of CGI (C/M) and the other one was concerning mannitol (M) and mannitol with ascorbic acid (M+AsA) treatments in a relationship of CGI (M/M+AsA). Analysis of variance in Table (9) indicated that genotypes varied significantly concerning each evidence of osmotic stress. Thus, significant differences have been observed among genotypes in Table (10) which showed that values of CGI (C/M) with negative trend due to mannitol stress ranged from -0.78, recorded by the third F_1 hybrid (Alshoroq-3 X Sakha 95) to – 0.41, recorded by the third parent (Rama-2). On the contrast, values with positive trend have been determined regarding to CGI (M/M+AsA) as an improving response to the role of ascorbic acid in such osmotic tolerance which exhibited in callus fresh weight (mg), range recorded 0.42 – 2.90 by (Rama-2) and

(Alshoroq-3 X Sakha 95), respectively. It is worthy to notice that these two genotypes are limited the range, whatever their ranks due to the arithmetic trend (-/+), this detection is true for all of the studied indices, except INTOL.

Whereas, the fourth parent (Alshoroq-3) recorded the lowest value of INTOL (-17.07), meanwhile, the highest value (-1.77) have been recorded by the fourth F_1 hybrid (Salah-1 X Babaga-3). In this regard, range of 22.17 – 59.32 have been estimated for Rt% by each of (Alshoroq-3 X Sakha 95) and (Rama-2), respectively. Dissimilarity, (Rama-2) and (Alshoroq-3 X Sakha 95) recorded the range of 40.68 – 77.83 for R%, respectively.

These results are take the same trend of those results which obtained by Lutts *et al*. (2004); Turhan and Baser (2004); Abdelsamad *et al*. (2007); Ibrahim (2009); Shah *et al.* (2009) and Farshadfar *et al.* (2012). Whereas, osmotic stress apparently decreased indices of osmotic stress tolerance. While, hybridization breeding procedure using some superior plant materials supplemented with *Invitro* selection of osmotic tolerance might be beneficial for improving bread wheat characters. Consequently, it is obvious that *Invitro* selection can be used as an effective tool to screen a large number of bread wheat genotypes to osmotic stress. Actually, more studies are needed to corroborate this thought (Farshadfar *et al*., 2014).

Table (9): Analysis of variance of callus growth index (CGI) for both stress (C/M) and alleviation (M/M+AsA) treatments, *Invitro* tolerance (INTOL), relative tolerance percentage (Rt%) and reduction percentage (R%) in mature embryo callus of the tested bread wheat (*Triticum aestivum* L.) parents and their F_1 hybrids

SOV	df	Mean square				
		CGI C/M	CGI M/M+AsA	INTOL	Rt%	R%
Replications	9	0.05**	1.79**	28.99**	500.79**	500.79**
Genotypes	10	0.16**	6.06**	230.21**	1546.66**	1546.66**
Error	90	0.002	0.19	3.19	24.07	24.07
Total	109					
C.V%		8.28	13.81	22.1	12.25	8.18

C/M; Value concerning control/mannitol treatment, M/M+AaA; value concerning mannitol/ mannitol+AsA treatment.

Table (10): Callus growth index (CGI) for both stress (C/M) and alleviation (M/M+AsA) treatments, *Invitro* tolerance (INTOL), relative tolerance percentage (Rt%) and reduction percentage (R%) for callus fresh weight in mature embryo callus of the tested bread wheat (*Triticum aestivum* L.) parents and their F_1 hybrids.

Genotypes	Callus growth index		INTOL	Rt%	R%
	C/M	M/M+AsA			
Amna-2	-0.54	0.83	-10.75	45.63	54.37
Damara-6	-0.54	0.77	-10.69	45.57	54.43
Rama-2	-0.41	0.42	-6.73	59.32	40.68
Alshoroq-3	-0.68	1.55	-17.07	31.79	68.21
Sakha 95	-0.63	1.28	-14.07	37.39	62.61
Salah-1	-0.47	0.64	-8.29	53.46	46.54
Babaga-3	-0.47	0.61	-8.44	52.95	47.05
Amna-2 X Damara-6	-0.70	1.62	-3.07	30.43	69.57
Rama-2 X Sakha 95	-0.77	2.35	-3.92	23.40	76.60
Alshoroq-3 X Sakha 95	-0.78	2.90	-4.16	22.17	77.83
Salah-1 X Babaga-3	-0.62	1.12	-1.77	38.47	61.53
General mean	-0.60	1.28	-8.09	40.05	59.95
LSD (0.05)	0.05*	0.43*	1.78*	4.89*	4.89*

C/M; Value concerning control/mannitol treatment and M/M+AaA; value concerning mannitol/ mannitol+AsA treatment.

Genetical analysis of tolerance indices

Inheritance behavior of such these measurements could be detected through good observation about some biometrical parameters such as means and variances in addition to some other indicators of gene action and hybrid vigor such as percentages of heterosis, heterobeltiosis, and inbreeding depression as well as through the identification of both the nature and the degree of the dominance by different manners such as the potence ratio. Hereinafter, osmotic stress tolerance indices in view of the mentioned before parameters.

Data illustrated in Tables 11 and 12 are referred to each of means, mid-parents and variances for 4 types of the six populations model, *i.e.* P_1, P_2, F_1 and F_2 of the

four crosses. For callus growth index (CGI) in both trends; (C/M) and (M/M+AsA), results in Table 11 clearly showed that with a respect of negative values of CGI (C/M) duo to mannitol stress vs control, the F_1 mean values for the four crosses were less than the mid-parent values. This indicated the presence of heterotic effects towards the lower parent for CGI (C/M).

The highest F_1 mean value obtained by cross 4 (Salah-1 X Babaga-3), meanwhile, the lowest F_1 mean value obtained by cross 3 (Alshoroq-3 X Sakha 95). In this context, it is obvious that the F_2 mean values were more than the F_1 means in the four crosses, except cross 4 (Salah-1 X Babaga-3) which equaled F_1value. These results indicated the divers of genetic background of the parents involved.

In this respect, regarding the other side of CGI in positive trend duo to alleviated role of AsA against the osmotic stress, results revealed that the F_1 mean values for the four crosses were more than the mid-parent values. This indicated the presence of heterotic effects towards a higher parent for CGI (M/M+AsA). The highest F_1 mean value obtained by cross 3 (Alshoroq-3 X Sakha 95), meanwhile, the lowest F_1 mean value obtained by cross 4 (Salah-1 X Babaga-3). In this context, it is obvious that the F_2 mean values were less than the F_1 means in the four crosses, except cross 4 (Salah-1 X Babaga-3). These results indicated the diverse of genetic background of the parents involved.

Table (11): Mean ($\bar{X}$) and variance (S^2) for P_1, P_2, F_1 and F_2 as well as mid-parent (MP) value of callus growth index (CGI) for both stress (C/M) and alleviation (M/M+AsA) treatments for the four crosses of bread wheat (*Triticum aestivum* L.*)*.

Index	Cross	Statis.	P_1	P_2	MP	F_1	F_2
CGI C/M	1	$\bar{X}$	-0.54	-0.54	-0.54	-0.70	-0.62
		S^2	0.005	0.002		0.006	0.009
	2	$\bar{X}$	-0.41	-0.68	-0.54	-0.77	-0.65
		S^2	0.005	0.002		0.004	0.009
	3	$\bar{X}$	-0.63	-0.68	-0.65	-0.78	-0.62
		S^2	0.005	0.002		0.004	0.009
	4	$\bar{X}$	-0.47	-0.47	-0.47	-0.62	-0.62
		S^2	0.005	0.008		0.009	0.010
CGI M/M+AsA	1	$\bar{X}$	0.83	0.77	0.80	1.62	1.13
		S^2	0.060	0.034		0.079	0.111
	2	$\bar{X}$	0.42	1.55	0.98	2.35	2.14
		S^2	0.261	0.552		1.523	3.010
	3	$\bar{X}$	1.28	1.55	1.41	2.90	0.96
		S^2	0.261	0.552		1.523	3.010
	4	$\bar{X}$	0.64	0.61	0.62	1.12	1.24
		S^2	0.491	0.201		0.513	0.908

Cross 1; Amna-2 X Damara-6, Cross 2; Rama-2 X Sakha 95, Cross 3; Alshoroq-3 X Sakha 95 and Cross 4; Salah-1 X Babaga-3

For INTOL, the F_1 mean values for the four crosses were higher than the mid-parent values. This indicated the presence of heterotic effects towards the higher parent for INTOL. The highest F_1 mean value obtained by cross 4 (Salah-1 X Babaga-3), meanwhile, the lowest F_1 mean value obtained by cross 3 (Alshoroq-3 X Sakha 95). In this context, it is obvious that the F_2 mean values were more than the F_1 means in the four crosses. These results were indicated the divers of the genetic background of the parents involved. The F_1 mean values of Rt% for the four crosses were lower than the mid-parent values. This indicated the presence of heterotic effects towards the lower parent for Rt%. The highest F_1 mean value obtained by cross 4 (Salah-1 X Babaga-3), meanwhile, the lowest F_1 mean value obtained by cross 3 (Alshoroq-3 X Sakha 95). In this context, it is obvious that the F_2 mean values were more than the F_1 means in the four crosses, except cross 4 (Salah-1 X Babaga-3). These results indicated the divers of the genetic background of the parents involved.

In this regard, variances have been calculated separately for each population in each cross are given in

Tables (11-12). The highest magnitude of variances were registered by the F_2 generations in the four studied crosses for osmotic stress tolerance indices, which is logic due to the fact that the maximum heterogeneity exists in the F_2 generation. On the other hand, the lowest variance magnitude was manifested by parents (P_1 and P_2) and F_1 populations, which also is expected from the breeding point of view due to the homogeneity of such populations. Thus, variances observed in the populations of (P_1, P_2 and F_1) of the investigated bread wheat (*Triticum aestivum* L.) mature embryo callus are may be due to the environmental factors.

Table (11): Mean ($\bar{X}$) and variance (S^2) for P_1, P_2, F_1 and F_2 as well as mid-parent (MP) value of callus growth index (CGI) for both stress (C/M) and alleviation (M/M+AsA) treatments for the four crosses of bread wheat (*Triticum aestivum* L.*)*.

Index	Cross	Statis.	P_1	P_2	MP	F_1	F_2
CGI C/M	1	$\bar{X}$	-0.54	-0.54	-0.54	-0.70	-0.62
		S^2	0.005	0.002		0.006	0.009
	2	$\bar{X}$	-0.41	-0.68	-0.54	-0.77	-0.65
		S^2	0.005	0.002		0.004	0.009
	3	$\bar{X}$	-0.63	-0.68	-0.65	-0.78	-0.62
		S^2	0.005	0.002		0.004	0.009
	4	$\bar{X}$	-0.47	-0.47	-0.47	-0.62	-0.62
		S^2	0.005	0.008		0.009	0.010
CGI M/M+AsA	1	$\bar{X}$	0.83	0.77	0.80	1.62	1.13
		S^2	0.060	0.034		0.079	0.111
	2	$\bar{X}$	0.42	1.55	0.98	2.35	2.14
		S^2	0.261	0.552		1.523	3.010
	3	$\bar{X}$	1.28	1.55	1.41	2.90	0.96
		S^2	0.261	0.552		1.523	3.010
	4	$\bar{X}$	0.64	0.61	0.62	1.12	1.24
		S^2	0.491	0.201		0.513	0.908

Cross 1; Amna-2 X Damara-6, Cross 2; Rama-2 X Sakha 95, Cross 3; Alshoroq-3 X Sakha 95 and Cross 4; Salah-1 X Babaga-3

Concerning the hybrid vigor and gene action, Table 13 previously showed each of the heterosis percentage over both mid and better parent, inbreeding depression percentage and potence ratio for osmotic stress tolerance indices; CGI (C/M), CGI (M/M+AsA), INTOL, Rt% and R%. Whereas, for heterosis, the desirable heterosis for the tolerance indices was the positive values.

Results demonstrated that heterosis relative to the mid-parents was significant for 12 cases out of 20 for all indices in the four crosses. The twelve cases of the positive and significant heterosis values over the mid-parents were concerning the all crosses of CGI (C/M and M/M+AsA) and R%. While, other 8 significant negative values of mid-parent heterosis were found for INTOL and Rt% in all crosses. Likewise, the same trend and the same results were true concerning the estimation of heterosis over the better parent for all osmotic stress tolerance in all crosses.

The most useful heterosis estimate were recorded in cross 2 (Rama-2 X Sakha 95) for CGI (M/M+AsA) concentration over the mid-parent and cross 1 (Amna-2 Damara-6) over the better parent for the same indice. Regarding to inbreeding depression, percentages are measured as the percentage deviation of F_2 from F_1 meanperformance. It is of interest to mention that the superior cross 4 (Salah-1 X Babaga-3) had no ID% for CGI (C/M)

because F_1 value equaled F_2 value, the same cross also recorded negative ID% for CGI (M/M+AsA) and R% as well as positive ID% for INTOL and Rt%.

The desirable inbreeding depression for all cases of all osmotic stress tolerance indices is the positive value, except the three crosses 1, 2 and 3 for Rt% with negative significance out of 13 cases. Other 10 significant cases were positive and found in crosses 2 and 3 for CGI (C/M), crosses 1 and 3 for CGI (M/M+AsA) and R% as well as crosses 1, 2 and 3 for INTOL. The remaining cases are insignificant.

Nature and degree of dominance have been demonstrated in this study using the potence ratio. The results indicated that absence of dominance was found in crosses 1 (Amna-2 X Damara-6) and 4 (Salah-1 X Babaga-3) for CGI (C/M). Meantime, over dominance have been recorded concerning other cases. These results indicated the important role of the over dominance gene effects in controlling osmotic stress tolerance indices. Negative degree of dominance have been recorded in crosses 2 and 3 for CGI (C/M) and in all crosses for Rt%

Table (12): Mean ($\bar{X}$) and variance (S^2) for P_1, P_2, F_1 and F_2 as well as mid-parent (MP) value of *Invitro* tolerance (INTOL), relative tolerance percentage (Rt%) and reduction percentage (R%) for callus fresh weight in mature embryo callus for the four crosses of bread wheat (*Triticum aestivum* L.*)*.

Index	Cross	Statis.	P_1	P_2	MP	F_1	F_2
INTOL	1	$\bar{X}$	-10.75	-10.69	-10.72	-3.07	-1.89
		S^2	4.76	2.34		4.13	8.00
	2	$\bar{X}$	-6.73	-17.07	-11.90	-3.92	-1.81
		S^2	1.30	1.86		1.98	3.88
	3	$\bar{X}$	-14.07	-17.07	-15.57	-4.16	-1.64
		S^2	1.30	1.86		1.98	3.88
	4	$\bar{X}$	-8.29	-8.44	-8.36	-1.77	-1.60
		S^2	1.17	1.30		1.72	2.58
Rt %	1	$\bar{X}$	45.63	45.57	45.60	30.43	37.53
		S^2	5.05	3.97		4.96	7.09
	2	$\bar{X}$	59.32	31.79	45.56	23.40	35.44
		S^2	4.95	9.00		5.22	10.44
	3	$\bar{X}$	37.39	31.79	34.59	22.17	37.73
		S^2	4.95	9.00		5.22	10.44
	4	$\bar{X}$	53.46	52.95	53.21	38.47	38.34
		S^2	5.21	8.25		8.95	12.08
R %	1	$\bar{X}$	54.37	54.43	54.40	69.57	62.47
		S^2	25.05	23.97		21.96	50.09
	2	$\bar{X}$	40.68	68.21	54.44	76.60	64.56
		S^2	40.95	29.00		55.22	74.44
	3	$\bar{X}$	62.61	68.21	65.41	77.83	62.27
		S^2	40.95	29.00		55.22	74.44
	4	$\bar{X}$	46.54	47.05	46.79	61.53	61.66
		S^2	15.21	13.25		18.95	28.08

Cross 1; Amna-2 X Damara-6, Cross 2; Rama-2 X Sakha 95, Cross 3; Alshoroq-3 X Sakha 95 and Cross 4; Salah-1 X Babaga-3**.**

Table (13): *Invitro* heterosis, inbreeding depression and potence ratio for callus growth index (CGI (C/M) and(M/M+AsA)), *Invitro* tolerance (INTOL), relative tolerance percentage (Rt%) and reduction percentage (R%) for callus fresh weight in mature embryo callus for the four crosses of bread wheat (*Triticum aestivum* L.*)*.

Index	Cross	Heterosis %		Inbreeding depression %	Potence ratio
		M.P	B.P		
CGI C/M	1	29.63**	29.63*	11.43	0.00
	2	42.59**	87.81**	15.48*	-1.70
	3	20.00*	23.81*	20.51*	-5.20
	4	31.92*	31.92*	0.00	0.00
CGI M/M+ AsA	1	99.50**	95.18**	30.25*	27.33
	2	99.80**	51.61**	8.94	2.43
	3	99.67**	87.10**	66.90**	11.04
	4	80.65**	75.00**	-10.71	33.33
INTOL	1	-71.35**	-71.27**	38.44*	55.00
	2	-67.08**	-41.77**	53.74**	1.54
	3	-73.28**	-70.45**	60.49**	7.60
	4	-78.79**	-78.60**	9.89	90.76
Rt %	1	-33.26*	-33.30*	-23.32*	-55.53
	2	-48.64**	-60.56**	-51.49**	-1.61
	3	-35.92*	-40.72**	-70.21**	-4.44
	4	-27.70*	-28.04*	0.34	-58.47
R %	1	27.88*	27.94*	10.20*	55.67
	2	40.70**	88.29**	15.73*	1.61
	3	19.00*	24.32*	19.99*	4.44
	4	31.08*	32.20*	-0.21	57.80

*; significant at 0.05 level of probability, **; significant at 0.01 level of probability and ns; non-significant.
Cross 1; Amna-2 X Damara-6, Cross 2; Rama-2 X Sakha 95, Cross 3; Alshoroq-3 X Sakha 95 and Cross 4; Salah-1 X Babaga-3 M.P; Mid-parent, B.P; Better parent, C/M; Value concerning control/mannitol treatment and M/M+AaA; value concerning mannitol/ mannitol+AsA treatment.

These results are supported by many studies and investigators who reported also such desirable of heterosis and dominance for callus growth rate and other tolerance indices. Haggag and El-Hennawy, 1991 reported positive heterosis for callus growth rate and suggested that breeding and selection could result in the use of callus growth rate as genetic marker. While, Abd El-Hady, 2006 with a regard of positive heterosis for callus growth rate, stated that heterosis at cellular level may be relative to heterotic effects for grain yield. Meanwhile, the existence of heterobeltosis in most of the hybrid combinations and dominance by large degrees, suggest the exploitation of dominance gene action for the improvement of callus traits (Etedali *et al*., 2011).

Significant and positive heterosis and over-dominance have been also indicated by Lange *et al*. (1995) and Dornelles *et al*. (1997). However, high over-dominance value for callus characters may be the result of pseudo-over-dominance caused by correlated gene distributions among the parents, so that partial and/or complete dominance become pseudo-over-dominant (Hayman, 1954 and Dehghanpour *et al.*, 1996). Measurements of such tolerance indices which involve time as a variable are considered efficient factors for the evaluation of stress tolerance in callus level (Yue *et al.*, 2001).

In addition, hybridization breeding procedure using some superior plant materials supplemented with *Invitro* selection of osmotic tolerance indices might be beneficial for improving bread wheat traits. Consequently, *Invitro* selection can be used as an effective tool to screen wheat genotypes to osmotic tolerance indices. In fact, more studies and researcher effort are needed to corroborate this thought (Farshadfar *et al*., 2014).

CONCLUSION

It could be concluded that 2.50 g/l of the 2,4-D hormone was suitable concentration in this study to callus initiation. Both of callus induction percentage and callus fresh weight (mg) were genotype dependents. Results also indicated that osmotic stress due to mannitol decreased callus fresh weight significantly and ascorbic acid (AsA) alleviated the harmful effects of stress. Osmotic stress also affected tolerance indices negatively. While, positive desirable of heterosis and over dominance have been detected for most cases.

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قوة الهجين للتحمل الأسموزى النسبى في قمح الخبز معملياً

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الملخص العربي

إنما يُعد تكثيف الجهود البحثية تلبيةً لإحدى توصيات العديد من أبحاث علوم البيئة التطبيقية و تنميتها المستدامة استناداً لأسس و تقنيات الوراثة و التكنولوجيا الحيوية فى ظل التغيرات المناخية العالمية المصاحبة لمحدودية الموارد الأرضية و تراجع الموارد المائية مقابل الزيادة المطردة فى التعداد السكانى عاماً بعد عامٍ تعظيماً لمشكلةٍ اقتصاديةٍ بتزايد الطلب فى مجتمع الندرة مما يضاعف الفجوة الغذائية و لاسيما القمحية عمقاً و اتساعاً حيث "الإنتاج كسر الاستهلاك" إحدى سمات العالم النامى. قد يلتمس هذا البحث بعضَ تحقيقٍ لتلك التوصية عند مجابهة الإجهاد البيئى غير الحيوى بسبب الجفاف أو الملوحة أو كلاهما. لذلك فإنه استهدف التنبؤ المبكر باستخدام تكنيك زراعة الأنسجة و ذلك لقوة الهجين و التدهور الناشئ نتيجة التربية الداخلية و كذلك طبيعة و درجة السيادة لعدد من دلائل تحمل الإجهاد الأسموزى بفعل المانيتول المضاف إلى بيئة النمو MS ، فضلاً عن محاولة تلافى الآثار الضارة المترتبة على إحداث الإجهاد الأسموزى باستخدام حمض الأسكوربيك على الكالس المتكشف عن الأجنة الناضجة لأربع هجن مبشرة من قمح الخبز نتجت من تهجين سبعة آباء تميز أداؤها تحت ظروف الإجهاد و ذلك باستخدام "موديل العشائر الست" بتحليل متوسط الأجيال لصفتى النسبة المئوية لتكون الكالس و الوزن الطازج (ملليجرام) للكالس و ذلك من خلال تجربتين معمليتين: إحداهما تمهيدية فى مرحلة التأسيس لاختيار التركيز الأفضل من هرمون 2,4-D المضاف لبيئة النمو بالإضافة إلى تركيز ثابت من إندول حامض النفثاليك (٠.٥ جم لتر$^{-١}$) ، و الأخرى كانت للإجهاد الأسموزى و تلافى آثاره. أظهرت النتائج فروقاً معنويةً بين كلٍ من التراكيب الوراثية الإحدى عشر و بين معاملتى الهرمون ، كما أسفرت النتائج عن تفوق الهجن المبشرة غن آبائها فى صفتى الكالس بينما أدت زيادة تركيز 2,4-D من ٢ حتى ٢.٥ جم لتر$^{-١}$ إلى تحسن صفتى الكالس المدروستين. كذلك فقد بيَّن التحليل الوراثى أن توريث هاتين الصفتين يعتمد على التركيب الوراثى ، فى حين أشار تحليل التباين لمعاملات الإجهاد الأسموزى إلى تدهور صفة الوزن الطازج للكاس معنوياً نتيجة الإجهاد. كما أوضحت النتائج المتحصل عليها دور حمض الأسكوربيك فى محاولة التغلب على آثار الإجهاد الذى أثَّر كذلك سلبياً و معنوياً غلى دلائل تحمل الإجهاد الأسموزى ، كما أظهرت التحليلات الوراثية الاتجاه الموجب لقوة الهجين و السيادة الفائقة تحت ظروف الإجهاد و الكنترول.