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ANTHER CULTURE RESPONSE OF DIFFERENT SALT TOLERANCE GENOTYPES IN BREAD WHEAT

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ABSTRACT: The present study aimed to investigate the anther culture response of different salt tolerance genotypes in bread wheat as well as to determine the optimum hormone balance for anther culture. Four Egyptian bread wheat cultivars, i.e., Gemeiza 7, Gemeiza 9, Sakha 8 and Sakha 93 were selected on the basis of salt tolerance differences in addition to four hybrids between them were used. Salt tolerant variety Sakha 8 Possessed higher response than susceptible variety Gemeiza7, meanwhile varieties possessed intermediate response to anther culture. The hybrid Gem. 7X Sak. 8 showed higher frequency of anther culture response than the other hybrids.

Medium I (P4+1.5mg/L 2.4.D +0.5mg/L BAP) considered as a better hormone balance than the other media in the varieties, in contrast medium III (P4+1.5/L NAA+ 0.5mg/L Kin) gave higher frequency of anther culture response than other media for hybrids.

Highly difference of interaction between genotypes X media was found. Highly significant differences were reported for anther culture response between Sakha 8 X Medium I, as well as hybrid (Gem. 7X Sak. 8) X medium III.

In general, the mean values for varieties and hybrids of seed germination percentage and Plant height under salinity applications were lower than under normal conditions.

Number of tillers consider as a good indicator for screening of salt tolerant genotypes, as well as Sakha 8 followed Sakha 93 consider as a good donor for salt tolerance genes to other genotypes.

The regression coeffient between anther culture response and number of tillers were 7.958 and 41.269, Correlation coeffient between anther culture response and percentage of seed germination were 0.038 and 0.53 under normal and salinity condition, respectively. These findings may be suggest that salt stress condition increase of anther culture response as well as, salt tolerant genotypes (Sakha 8) possessed higher response of anther culture, callus induction and plant regeneration. In conclusion, the above relationships require further intensive researches to insure that idea.

Key words: Anther culture, wheat, *Triticum aestivum*, salt tolerance.

INTRODUCTION

Wheat is a major staple food for more than one third of the world population, in Egypt, which cover more than 1 million hectare with an annual Production of 6.6 million tons (Ministry of Agric., Egypt, 2003) that supply only 50% of the fast growing Population demand. Thus, wheat occupies a unique position in the Egyptian agricultural economy.

Wheat production is confronted by many problems. Diseases such as rust, smuts and root rots as well as a biotic stress as drought, heat, and salinity stresses etc., are serious problems in Egypt. Breeding of new cultivars is the lonely safety and most effective way to raise and sustain the wheat productivity. Salinity negative effect could arise in salinity affected soils and or in area depending on saline infected drainage water or mixed water so breeding for salinity tolerance remains an important objective for wheat production. Several earlier activities were carried out and succeeded to release salt tolerant cultivars, e.g., Sakha 8 and Sakha 93, that enabled the introduction of wheat production up to northern coast of Nile- Delta and raised the wheat production in several area in the country. These two salt tolerant varieties, e.g., Sakha 8 and Sakha 93 suffer to many problems as infection with rust and smut deseases and susceptible to aphid insects and subsequently, the research of new sources of salinity tolerance are needed for wheat growing at salt- affected land.

The development of *in vitro* technologies have complemented the conventional methods of wheat breeding in generating genetic variability necessary for creating novel cultivars with desirable

characters. Anther culture is one of the methods commonly used for induction of haploid plants. It's application in breeding programs of crops is great agronomic importance sense it allows fast achievement of genetic homozygosity which is primary importance in production of new breeding lines and varieties. The use of anther culture to facilitate the selection of salt tolerant cereal crops was succeeded in barley (Ye, *et al.*, 1987). Also, similar efforts were carried out in rice (Zapata *et al.*, 1991; Binh, et *al.*, 1992 and Draz, *et al.;* 1994). In wheat, several studies were carried out to use anther culture technique in screening for salt tolerance (Kardimo Va 1990; Barakat and Abdel-Latif 1995, and El-Hennawy 1996). However, such kind of work needs more efforts, (Abdel Hafez *et al.*; 1999 a, b) studied the ability of genotypes and media for anther culture in Egyptian wheat as a pre-stage of work for screening of salt tolerance.

The Induction of androgenesis from wheat anther is strongly controlled by genetical and environmental factors with significant interaction between them (lazer *et al.*, 1990; Moieni *et al.*, 1997 and Stober and Hess, 1997). The present study was carried out to investigate the androgenetic response of different salt tolerance genotypes under different concentrations of growth regulators as well as relationships between salt tolerance and anther culture response of different wheat genotypes.

MATERIALS AND METHODS

The present investigation was carried out at the biotechnology, Res. Lab. and Greenhouse of Genetics Department, Faculty of Agriculture, Zagazig University.

Plant Materials

Four Egyptian spring bread wheat cultivars *(Triticum aestivum* L.) namely Gemeiza 7 (Gem7), Gemeiza 9 (Gem9), Sakha 8 (Sk8) and Sakha 93 (Sk93) were selected on the basis of salt tolerance differences between them. The following four hybrids were obtained, i.e., Gem9 X Sk93, Gem7 x Gem9, Gem7 X Sk8 and Gem7 X Sk93 at spring season 2006. The four varieties and four hybrids were grown at green house at spring season 2007. The pedigree and characterization of salt tolerance Egyptian bread wheat cultivars under study showed in Table 1.

Methods

Anther Culture Procedure

Spikes collection

Plants (varieties and hybrids) were grown in the experimental field at 2007 and spikes were collected when the microspore were in the mid- to late uninucleate stage of developed, and subjected to a cold pre treatment for 5 days at 4°C.

Media preparation

Callus induction media

Anthers were cultured on Potato 4 media (Ouyang *et al.*, 1983) with three different concentrations of growth regulators as follows:

a- P4 basic medium + 1.5 mg/l 2,4.D + 0.5 mg/l BAP

b- P4 basic medium + 1.5 mg/l NAA + 0.5 mg/l BAP

c- P4 basic medium + 1.5 mg/l NAA + 0.5 mg/l Kinetin

Plant regeneration medium

Anther callus were transferred to 190-2 regeneration medium (Zhuang and Jia 1983), at 4-6 weeks age with 30 g sucrose, 0.5 mg/l each of Kinetin and NAA, 0.7% agar. The culture was maintained at 26°C with 16 hours photoperiod for 4-5 weeks. Green plants were transplanted into pots and placed in growth chamber at 20°C with 16 hours photoperiod.

Table 1. Breeding history and pedigree of Egyptian wheat varieties

Entry	Name	Pedigree	Salt tolerance
1	Sakha 8	Indus66/.norteno"s"PK3418-6s-1sw-0s	Tolerant
2	Sakha 93	Sakha92/TR810328-s8871-/s-2s-/s-0s	Mod. Tolerant
3	Gemeiza 7	CMH74A.630/sx//seri82/3/Agent-CGM4611-2GM-1GM-0GM	Susceptible
4	Gemeiza 9	Ald"s"/Huac"s"//CMH74A.630/sx-CGM4583-5GM-1GM-0GM	Mod. susceptible

Salinity Studies

Field lysimeter experiments

Grains for cultivars and their hybrids were sown in tank at greenhouse (2007). Salinity treatment was imposed by adding NaCl to soil of tanks. The concentration of NaCl was 5000ppm in the soil. One row of each cultivar and hybrid (under normal and saline soil conditions) was planted in lysimeter. Rows were spaced 20 cm apart, with the grainss placed 10 cm apart within the rows.

Data Collection

Anther culture

The response frequencies were calculated as using the following equation:

1. Anther culture response frequency was calculated as follows:

$$\frac{\text{No. of anther responded}}{\text{Total no. of anthers cultured}} \times 100$$

2. Callus induction frequency

$$\frac{\text{No. of calli formation}}{\text{Total no. of anthers cultured}} \times 100$$

3. Plant regeneration frequency was calculated as.

$$\frac{\text{No. of plantlet regenerated}}{\text{Total no. of calli transfered}} \times 100$$

Salinity studies

Seed germination, plant height, and number of tillers were measured after 2 months from sowing.

Statistical Analysis

Data on anther response frequencies was analyzed using factorial experiment with two factors i.e., genotypes and growth regulators, mean sum of square were estimated for genotypes, growth regulators and their interaction, as well as average mean and LSD for them were calculated also Waller and Duncan (1969). Heritability in broad sense was estimated for anther culture response according to Singh and Chaudhary (1977). Simple regression and correlation were calculated also.

RESULTS AND DISCUSSION

Anther Culture Response

Effect of genotypes

Highly significant differences for anther culture response were found among varieties and significant among hybrid Table 2 subsequently large difference affects in response to anther culture among all varieties and hybrids were recorded Table 3. Salt tolerant variety (Sakha 8) possessed

Table 2. Analysis of variance of anther culture response for varieties and hybrids under different media in bread wheat

SOV	df	MS (Mean Squere)		Fc		F_t	
		varieties	hybrids	varieties	hybrids	0.05	0.01
Replicate	2	0.014	0.016	1.136	1.269	3.456	5.758
Genotypes (A)	3	0.129	0.094	9.92 **	3.357 *	3.064	4.854
Media (B)	2	0.052	0.274	4.00 *	9.785 **	3.456	5.758
A X B	6	0.036	0.023	2.769 *	8.821 **	2.564	3.79
Error	22	0.013	0.028	-	-	-	-
h^2		0.580	0.711				

* Significant at 0.05

** Significant at 0.01

h^2 in broad sense

Table 3: Average mean and least significant difference (LSD) of anther culture response for varieties, media and their interaction in bread wheat

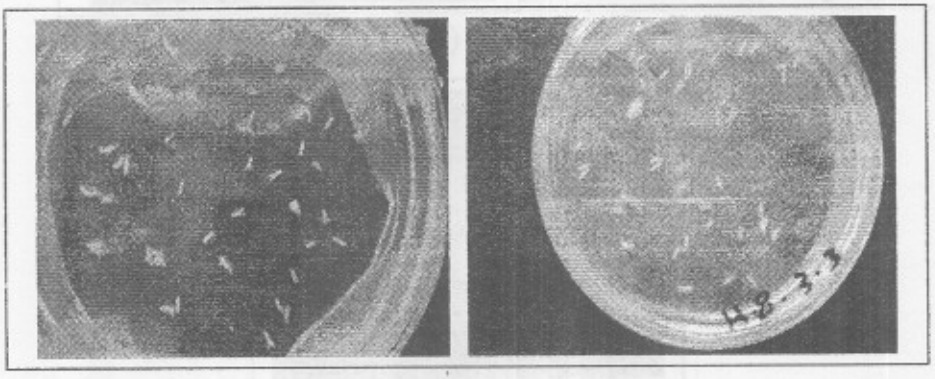
Media	Genotypes				
	Sak 93	Gem 9	Sak 8	Gem 7	Mean
Medium I	3.6	20.33	23.00	3.8	12.683
Medium II	11.33	4.33	16.33	2.33	8.58
Medium III	5.33	4.33	12.00	3.00	6.165
Mean	6.753	9.663	17.11	3.043	

L.S.D 0.05= 0.193

L.S.D 0.01 = 0.262

higher value than susceptible variety (Gemiza7), other varieties possessed intermediate response of anther culture. Their hybrid (Gem7 x Sak 8) showed that higher value of anther culture response than rest hybrids. These results indicated that salt tolerance character may be possessing relationship with anther culture response in bread wheat genotypes. These result confirmed that sakha8 (salt tolerant) consider as a very good donor for anther culture response criterion of other different genotypes, which conforming the above way, that unique sakha8 gave callus and green plant regenerate as well as Sakha 93 (moderate tolerant) possessed callus which lack green plant regenerate (Fig. 1,2).

Many outhers (Han-min *et al.;* 1990, Hassawi. *et al.*, 1990; Bruins and Snijders 1995, Ahmed *et al.,* 1996; Machii *et al.* 1998, Kang *et al.*, 2003 and Tersi Maria *et al.*, 2005) have reported marked effects of genotypes on anther culture responses. Some investigators used, a large number of wheat genotypes were investigated, for example, (Anderson *et al.* 1987) obtained a good response form 93 out 215 winter wheat cultivars (*T. aestivum* L.). However Foroughi- Wehr and Zeller, 1990, investigated 75 commercial German spring and winter wheat cultivars, they found that response of winter wheat to anther culture was higher than spring wheat.



Sakha 93 Sakha 8

Fig. 1. Response of callus formation for sakha8 and Sakha 93

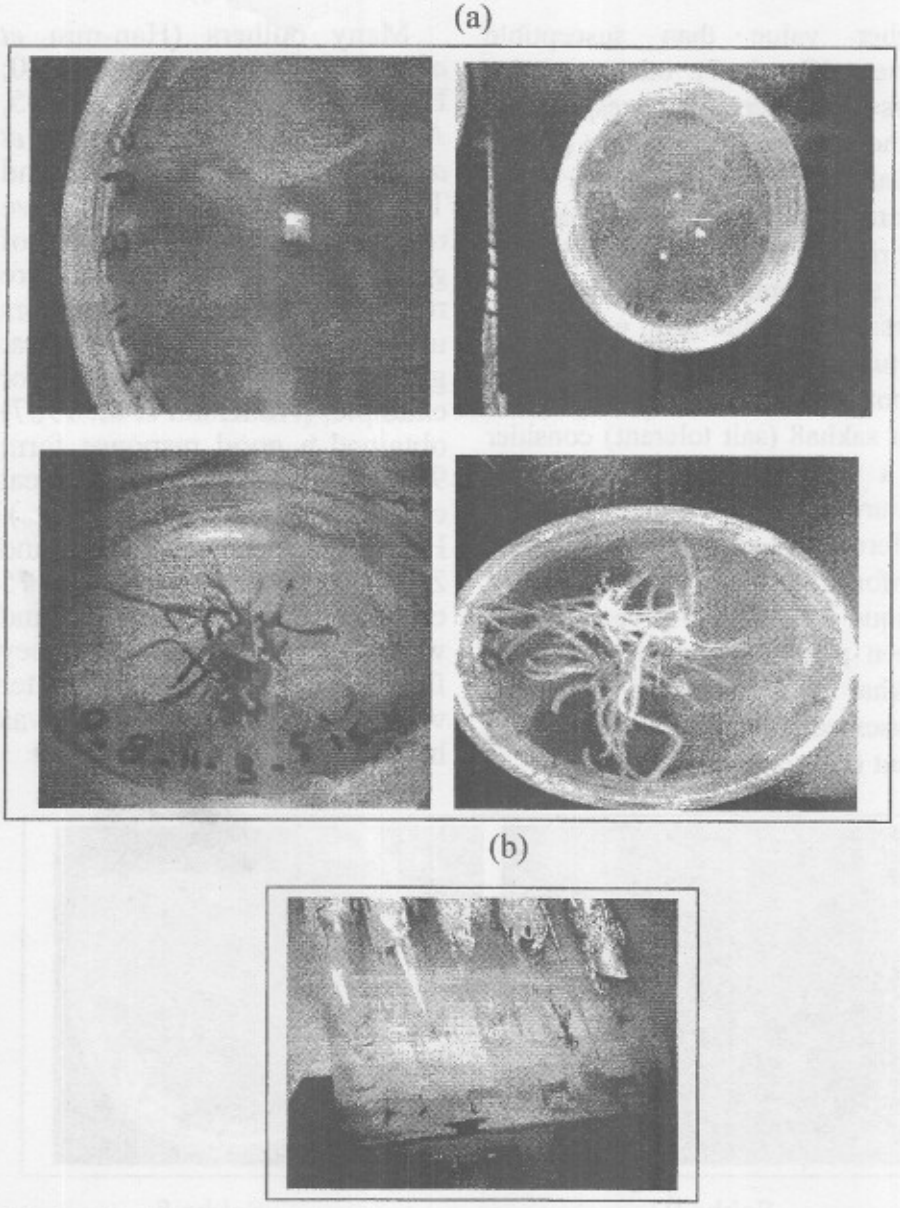


Fig. 2. (a) Response of plant regeneration for Sakha 8 after four weeks from transferring of callus to plant regeneration medium and (b) plantlet at test tube

Similar results were obtained by Abdel-Hafez, *et al.,* 1999 (b), in Egypt, they studied 14 genotypes (8 varieties and 6 hybrids) and reported that Sakha 8 (salt tolerant) possessed higher frequencies (2.51) than other genotypes, (1.52 mean).

The behavior of four-F_1 hybrid confirmed that salt tolerance character may be playing an important role for the response of genotypes for anther culture response Fig 3. Over dominance were recorded of Gem7 (salt susceptible) X Sak8 (salt tolerant) hybrid. This results confirmed that Sakha 8 consider as a very good donor for anther culture response to other genotypes. In contrast, other hybrids showed negative heterosis (Gem 9 X Sak 93) (Gem7 X Gem 9) and partial dominance (Gem7 x Sak93).

These results appeared in agreement with the findings of (Nazan Dagiistii, 2008), he studied diallel analysis of anther culture response in wheat (*Tritcium aestivam* L.). The results indicated that parents, which give rise to highly improvement of hexaploid wheat is possible through selection. High X low responding crosses generated F1's that were intermediate in response. Response of wheat crosses involving salt tolerance genotypes to anther culture were studies by Sehrawat, Anita *et al.,* 2002 they reported that crosses differed significantly, and the best cross with respect to callus formation and green plantlet regeneration was Kharchia 375 (Salt tolerant) X HD2009.

Effect of media composition

Highly significant differences were registered between three media, as well as significant difference for interaction between varieties and media were reported also Table 2. Medium I (P4+1.5 mg/L 2.4. D + 0.5 mg/L BAP) consider as a better hormone balance than the other media with varieties, in contrast Medium III (p4+1.5mg/L NAA + 0.5mg/L Kin) gave higher anther culture response than the other media with hybrids. Large difference of interaction between genotype X media was found. Better anther culture response between Sakha 8 x Medium I, as well as (Sak 8 x Gem7) x medium III were observed. These results confirmed the Importance of Sakha 8 as a donor to anther culture response and salt tolerance criteria in the same time into other genotypes.

Many Investigations recorded a major effect of media composition, i.e., basic medium, hormone balance and other components

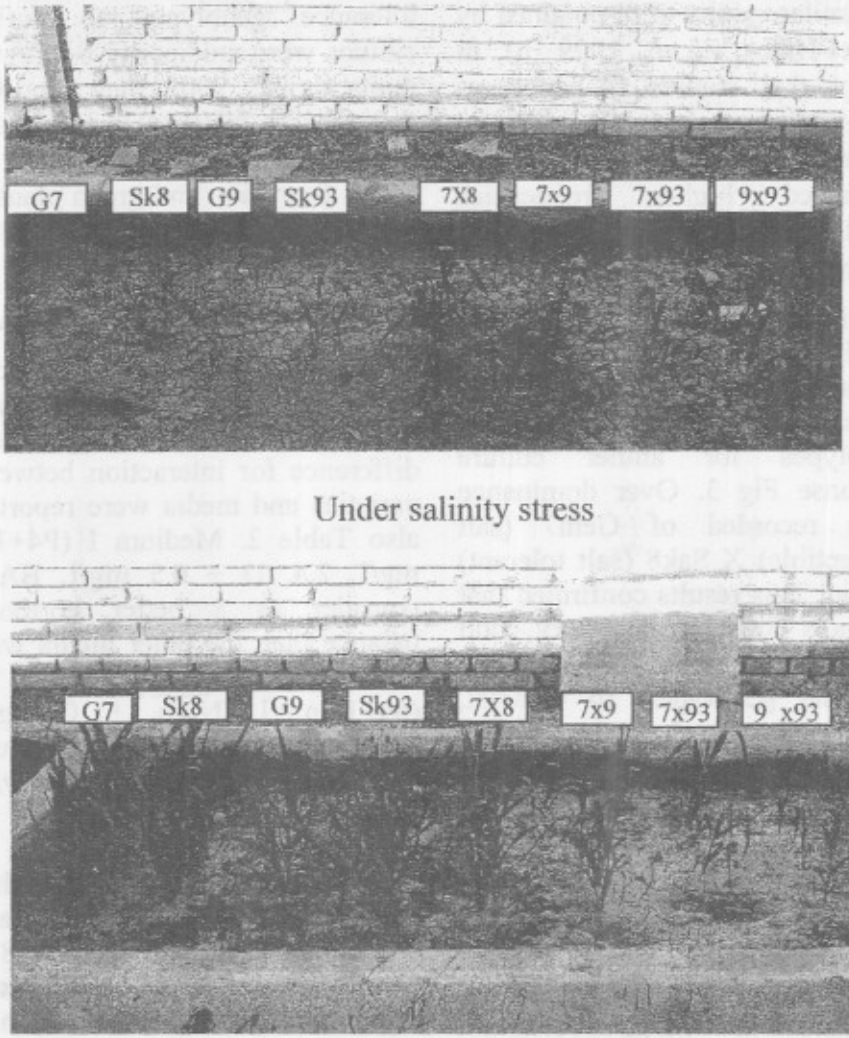


Fig. 3. Salinity experiment at lysimeter in green house for varieties and hybrids

(Chaghamirza and Arzani, 1999; Puolimatka and Pauk, 2000; Kang *et al.*, 2003; Tersi *et al.*, 2006 and Amina Pedha and Talaat 2008) on anther culture response frequencies. Similar results were recorded by several Investigators such as, Zhou and Konzak, 1997, studied the influence of genetic and environmental factors on anther culture response of wheat and they found that there is a significant variation within genotypes for anther culture response were observed in wheat varieties, chis, yecwa Rogo, WA7176 and Edwail, indicating the presence of genetic heterozygosity in commercial cultivars and the potential for improving anther culture responses by *in vitro*, screening. Liquid potato 4 medium gave the best results compared to agar. Solidified medium. Massiah *et al*, 2001 also studied hormone balance and other factors was made by They found that cold pretreatment of spikes excised from donors plants and addition of 2.4-D together with either Kinetin or BA in the callus induction medium improves the anther culture response. Sehrawat Anita *et al.*, 2002 studied the response of wheat crosses involving salt tolerant genotypes to anther culture and they stated that different crosses were significantly better in liquid media and the most effective medium overall was P_2 followed by p_4, Bac1 and Heh. Other way for improving the anther culture response was recorded by Kang *et al.*, 2003. They studied the effect of asmotic potential on anther culture in spring wheat (*T. aestivum*).

The Behavior of Hybrids and their Parents under Normal and Salinity Stress Conditions

The present study depended on the estimation of salt tolerance for different varieties and their hybrids on salt tolerance related criteria (seed germination%, Plant height and number of tillers at 2 months from sowing) under lysimeter at green house Fig 3. Generally, the mean values for varieties and their hybrids of seed germination% and plant height expect number of tiller under Salinity were lower than the mean values under normal conditions Table 3 and 4. Flacution behavior of some hybrids under normal and salinity conditions were reported Fig 4, b,c and d, for example Gem7 X Sak 8 possessed over dominance under normal and partial dominance under salinity

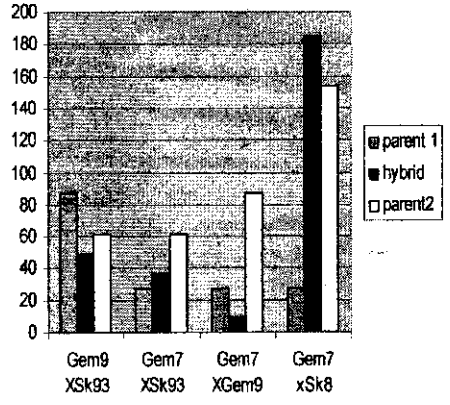
regarding seed germination % as well as partial dominance under normal and over dominance under salinity were showed regarding plant height. These change of dominance degrees with change of the condition from normal to salinity stress condition, may be due to change of gene expression or induction of new genes for controlling of these characters under salinity stress. In contrast no change or little change of the F_1 behaviors under normal than salinity stress conditions, were reported for number of tillers per plant, especially Gem 7 X Sak 8 possessed complete dominance under both conditions, as well as Gem 9 X Sak 93 also possessed little change under both condition also. These results confirmed the importance of number of tillers as a good indicator of salt tolerance, for example Sak 8 (salt tolerant) and Sak 93 (moderate tolerant) possessed the same mean value under both conditions, in the same way Gem 7 as susceptible for salinity gave lower number of tillers under salinity than under normal conditions. Therefore, number of tillers consider as a good indicator for screening of salt tolerance between genotypes under segregation generation as well as Sak 8 followed Sak 93 consider as a good donor for salt tolerance in breeding program for development of new salt tolerance lines.

Table 4. Average mean and least significant difference (LSD) for anther culture response of hybrids, media and their interaction in bread wheat

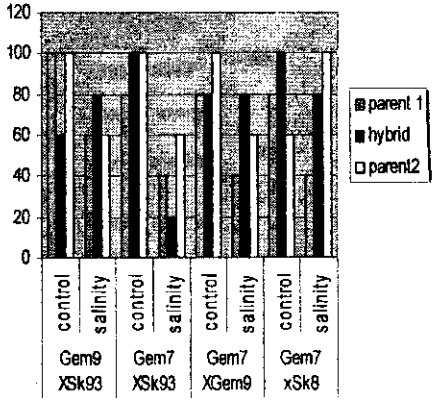
Media	Genotypes				Mean
	Gem 9 x Sak 93	Gem 7 x Sak 93	Gem 7 x Gem 9	Gem 7 x Sak 8	
Medium I	13.33	1.33	1.0	19.66	8.83
Medium II	2.0	1.0	1.0	17.0	5.25
Medium III	1.0	10.0	1.0	25.0	9.25
Mean	5.443	4.11	1.0	20.553	

L.S.D 0.05= 0.193

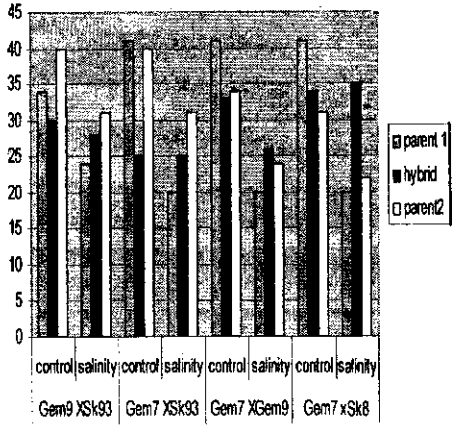
L.S.D 0.01 = 0.262



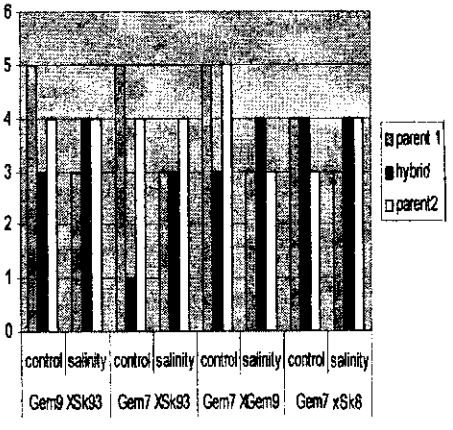
(a) Anther culture response



(b) Seed germination %



(c) Plant height after 2 months



(d) Number of tiller after 2 months

Fig. 4. Average mean of F1hybrids and the behavior for F1 hybrids and their parent (a) anther culture response (b) seed germination % (c) plant height (d) number of tiller at tow months under normal and salinity stress condition in bread wheat

These results confirmed with several Investigations (El-Hendawy *et al.*, 2005) evaluated thirteen wheat genotypes from Egypt, Germany, Australia and India for salt tolerance and their results showed that tiller number was more affected by salinity than leaf number and leaf area at the vegetative stages. Salinity decreased dry weight per plant significantly at all growth stage. According to cluster analysis with multiple agronomic parameters at all growth stages, the Egyptian genotypes sakha8 and sakha93 and Indian genotypes Kharehia were ranked as the most tolerant to salinity.

A change in salt tolerance with growth stages was observed for Sids1, Gemeiza 7 and Westonia. Drysdale and Sakha 69 were ranked as moderate tolerant, the remaining genotypes showed the lowest tolerance to salinity at all growth stages. We conclude that an increase in tiller number per plant and spikelet per spike will improve the salt tolerance of wheat genotypes in breeding programs. In the same trend, (Eugene *et al.*, 1994) showed that gain yield are highly depended upon the number of spike- bearing tillers produced per plant. Salinity decreased the number of primary and secondary tillers in both cultivars, i.e., Anza and Yecora Rojo. Higher salinity reduced the percentage of tillers with spikes, but not as much as the reduction in tillers. Adjusting planting densities to increase the number of anticipated spike-bearing culms per unit area could help to maintain yields as salt affected soils.

Other studies on the effect of NaCl salinity on wheat (*T. aestivum* L.) cultivars were carried out by (Rahman *et al.*, 2008) and they founed that a decrease in water uptake and germination of all cultivars. Increase salt concentration also affected the early seedling growth. Among the cultivars under investigation Zarlasht cultivar appeared to be more sensitive at germination stage, however, it performed quite satisfactor at seedling stage.

Relations among Anther Culture Response and Some Vegetative Criteria

Relationships between anther culture response and three criteria, i.e. seed germination%, Plant height and number of tillers under normal and salinity stress conditions were showed in Table 5, Change relationships between anther

Table 5. The relationships between anther culture response and vegetative characters i.e., seed germination, plant height and number of tillers at two months estimated as regression (b) correlation coeffient (r) under normal and salinity stress condition in bread wheat

Anther culture response		Regression (b)	Correlation coeffient (r)					
		Anther culture response	Seed germination		Plant height		No. of tillers	
			Control	salinity	Control	salinity	Control	salinity
			0.0380	0.53	-0.062	0.399	0.166	0.341
Seed Germination	Control	2.682		-0.566	0.155	0.406	0.123	-0.389
	Salinity	25.891			0.021	0.235	0.170	0.808*
Plant height	Control	-0.739				0.014	0.838**	0.0265
	Salinity	5.128					-0.034	0.574
No. of tillers	Control	7.958						-0.105
	Salinity	41.267						

culture response and three criteria were recorded under normal and salinity stress conditions. Regression coefficients (b) were higher under salinity stress than under normal condition. For example, the regression coefficient (b) between anther culture response and number of tillers were 7.958 and 41.269 under normal and salinity stress conditions, respectively as well as of seed germination% and plant height.

Generally insignificant correlation coefficient (r) between anther culture response and three vegetative criteria under study was recorded Table 5. The change in the value of (r) under normal than under salinity stress conditions were cleared, with increasing value of (r) toward salinity stress, for example the correlation coefficient (r) between anther culture response and seed germination percentage were 0.038 and 0.53 under normal and salinity stress conditions respectively. As well as plant height and number of tillers showed the same relationships. These results may be probable the genes controlling salt tolerance affect on anther culture response in the same time (pleoitropic effects) or induction of new gene expressions under salinity stress of salt tolerant genotypes for salinity stress. Correlation coefficient (r) between all studied characters change under salinity stress than under normal conditions were observed also. These relationships between anther culture response and salt tolerance of genotypes or salt stress conditions require more recent investigation, for confirming of these important remarkable, especially Sakha 8 (salt tolerant variety) possessed higher anther culture response than other genotypes. These findings confirmed with (Mehmet *et al.*, 2008). They studied the relationship between anther culture and morphological traits. Correlation coefficient analysis showed significantly positive correlation among anther culture traits. Tillering capacity and morphological characters of tillers had generally negative effects on anther culture ability of wheat. Higher spikelet number and spike length were significantly correlated with callus number and plant regeneration. By morphological markers, it seems to be possible to select highly producing wheat lines in anther culture.

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استجابة تراكيب وراثية مختلفة التحمل للملوحة لزراعة المتوك فى قمح الخبز

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تهدف هذه الدراسة الى دراسة مدى استجابة تراكيب وراثية مختلفة التحمل للملوحة من قمح الخبز لزراعة المتوك وكذلك تحديد انسب إتزان هرمونى لزراعة المتوك.

أشتملت هذه الدراسة على أصناف مصرية من قمح الخبز جميزة 7، جميزة ٩ وسخا٨ وسخا ٩٣ تم أختيارهم على أساس اختلافهم لتحمل الملوحة وكذلك ٤ هجن ناتجة من التهيجات بينهم.

الصنف سخا ٨ (المقاوم للملوحة) امتلك اعلى استجابة لزراعة المتوك بالمقارنة بالصنف جميزة ٧ (الحساس للملوحة)، باقى الاصناف اعطت استجابة متوسطة، الهجين (جميزة ٧ x سخا ٨) اظهر اعلى تكرار لاستجابة المتوك عن باقى الهجن.

وجد أنه البيئة I (p4 + 1.5mg/L 2.4.D + 0.5mg BAP) تعتبر أفضل اتزان هرمونى لاستجابة الاصناف لزراعة المتوك عن بقية البيئات، بينما كانت البيئة III (P4 +1.5mg/L NAA + 0.5 Kin) أعطت أعلى تكرار بالنسبة لاستجابة الهجن لزراعة المتوك عن باقى البيئات.

وقد وجد أختلافات كبيرة للتفاعل بين التراكيب الوراثية × البيئة حيث كانت هناك اختلافات عالية المعنوية بين سخا 8 × البيئة 1 وكذلك الهجين (جميزة 7 × سخا 8) × البيئة III

بشكل عام قيمة المتوسطات للاصناف والهجن بالنسبة لنسبة الانبات وطول النبات تحت الظروف الملوحه أقل من مثيلتها تحت الظروف الطبيعية، عدد الخلفات يعتبر دليل جيد بالنسبة للانتخاب لتحمل الملوحة بين التراكيب الوراثية تحت ظروف الملوحة وكذلك يعتبر الصنف سخا 8 وبلية سخا ٩٣ اباء جيدة لنقل صفة مقاومة الملوحة للتراكيب الوراثية الاخرى. معامل الانحدار بين الاستجابة لزراعة المتوك وعدد الخلفات ٧,٩٥٨، ٤١,٢٦٩ وكذلك معامل الارتباط بين الاستجابة لزراعة المتوك ونسبة الإنبات ٠,٠٣٨ ، ٠,٥٣ تحت الظروف الطبيعية والملوحه على التوالى. من هذه النتائج يتضح أن ظروف الملوحة تزيد من الاستجابة لزراعة المتوك وكذلك التراكيب الوراثية متحملة الملوحة (سخا 8) أمتلكت استجابة عالية لزراعة المتوك وأستحداث الكالس وإنتاج نباتات جديدة. الاستنتاج ان هذه العلاقة السابقة تحتاج لدراسات مكثفة فى السنوات القادمة.