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GENETICAL ANALYSIS OF ANTHER CULTURE RESPONSE IN RICE UNDER DROUGHT STRESS

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ABSTRACT

Breeding for drought tolerance is one of the main concern of rice breeding. This investigation aimed to evaluated some rice (*Oryza sativa* L.) genotypes under *in vitro* conditions and to study their genetic behavior under three different concentrations of PEG as a tool to asses their drought tolerance. The genetic materials used in this investigation were four parental varieties named and their six F_1 hybrids, which were produced from half diallel crosses mating design. The parental varieties were Giza 177, Sakha 101, Sakha 104 and IRAT 112. Results revealed that genotypic mean squares were highly significant for all studied *in vitro* traits, indicating the presence of real differences among genotypes. Furthermore, the variation due to PEG levels and genotypes x levels interactions were also highly significant for all *in vitro* traits except for green point percentage. This indicates that these genotypes gave different responses at different PEG levels. The magnitude of general combining ability (GCA) effects for each variety indicated that the varieties Sakha 104 and Sakha 101 were the best, while Giza 177 and IRAT 112 were inferior for studied traits. The crosses Sakha 104 x IRAT 112 and Sakha 101 x IRAT 112 were the best combinations for all *in vitro* studied traits except for green point percentage. The magnitudes of dominance genetic variance were larger than their corresponding variances of additive for all *in vitro* traits, indicating that the major role of dominance genetic variance in the inheritance of these traits. The results were emphasized by dominance degree ratio, which were more than unity for all studied traits, revealing the importance of over dominance in the genetic expression of these traits.

It could be concluded that most of studied *in vitro* traits are mainly controlled by non-additive genes in addition to the minor role of additive gene action under drought stress levels. Thus, it could be suggested that one of the best methods of breeding to improve these genotypes for tolerance to drought is the recurrent selection program.

INTRODUCTION

Rice, *Oryza sativa L.*, is the major food crop of about one half of the world's population. In Egypt, rice is considered as one of the most important field crops, since it contributes about 20% of the total cereal consumption and also as a cash export crop. Annually, more than 1.5 million feddans are cultivated with rice, producing 6.5 million tons of rice, with an average of 4.2

ton/ fed (10 ton/ha) (Proceeding of 2005, Researchers at RRTC) this average ranked the first among the rice producing countries in the world. This production satisfies the needs of local consumption, and the increase is exported abroad. But, with the expected increase of population, the production should be increased.

Drought is the major factor determining production of rice in the world, which about 90% of the world's rice producing areas suffer from moisture stress and water deficit. For this reason, breeding for drought tolerance is becoming of high priority in rice breeding program, especially in Egyptian conditions. Drought tolerance are a complex traits, which controled by several genes. Therefore, these traits are greatly effected by environmental factors, and take along time to be transferred. Thus, to over come this problem, the traditional methods, up to date breeding methodology such as tissue culture and genetic engineering are recommended.

Haploid plants were first described and obtained by Blakslee *et al.,* (1922) through parthenogenesis in datura. A doubling of chromosome number leads to the creation of pure lines in one step. So, the haploid approach eliminates many generation required for achieving homozygosity. In addition, recessive genes are more readily expressed at the plant level. However, research into haploid and their utilization in breeding were limited by their low frequency. In the recent years, many haploid techniques especially *in vitro* anther culture have been developed to induce large number of haploid plants which are subsequently reduplicated to obtain the doubled haploid (DH) pure lines. Since the first successful production of haploid plants from anther culture in rice was achieved by Niizeki and Oono (1968). This method has been used as anew technique for rice breeding. This technique made the *in vitro* selection for biotic and a biotic stress are available. Thus, the present investigation was planned to determine the genetic parameters of some *in vitro* traits of rice under drought stress

MATERIALS AND METHODS

In this investigation four rice varieties belong to the species (*Oryza sativa,* L.) were used. These varieties were Giza 177, Sakha 101, Sakha 104 and IRAT 112. Three of these varieties are Egyptian including one sensitive (Giza 177) and two moderate (Sakha 101 and Sakha 104) for drought tolerance. The fourth variety was introduced by INGER, which recognized as highly tolerated for drought. All possible combinations excluding reciprocals among these parental varieties were made according to a half diallel crosses. At maturity, the hybrid seeds were obtained. Therefore the genetic materials included four parental varieties and their six F_1 hybrids. The four parents and their six F_1 hybrid seeds were sown in May 15th, during 2005. Panicles of the reproductive stage were collected from the primary tillers when the distance of the flag leaf auricle to that of the next leaf is between five and nine centimeters. This distance would approximately at the mid- uninucleate to early binucleate stage of pollen development. The boots were washed roughly with tap water, then wrapped in towel paper which moistened with

distilled water. The boots were incubated in the dark at 8C° for eight days. Panicles were sterilized by 70% alcohol (ethanol) for one minuet and followed by 20 % chlorox (sodium hypochlorite 5.25 %) for 20 minutes. Then, it was rinsed three times with sterilized distilled water The based part of the spikelets were cut out using sterilized scissors with ethanol and flame. Then, anthers were excised with sterilized forceps from the spikelets in the middle of the panicles. About 100 anthers were inoculated in Petri dish (60 X 15 mm) containing induction medium.

The induction medium used in this study was recommended modified Fj medium for rice anther culture, according to Gamborg 1970. This medium was supplemented with three different concentration of polyethylene glycol 6000 (PEG), which were 0% as control, 5% and 10%(w/v). The pH was adjusted to 5.8 before outoclaving at 121C° for 20 minuets. The dishes were sealed with parafilm and incubated in darkness at 25+ 1 C° for 30 days. Then, the calli and/ or embryoid were measured and transferred to regeneration medium. For plant regeneration, the calli formed about 2- 3 mm in diameters were transferred to Petri dishes containing plant regeneration medium. The regeneration medium was MS medium as described by Murashiage and Skoog (1962). The same concentration of PEG in induction medium were used in the regeneration medium. These dishes were kept in the light (16 hours light and eight hours dark) at 25- 27C° for (13- 27) days. Then, green point were counted and transferred to big test tubes with the same regeneration medium. When the regenerated plants become have normal roots and shoots were carefully transferred to the culture solution for two weeks under normal day length for their adaptation to the field condition. The experimental design was completely randomized with three replicates. In each replicate the anthers of three spikes were distributed over three Petri dishes 10 cm in diameter. Each Petri dish with induction medium for each level of PEG 6000 contain about 100 anthers was considered an experimental unit. Subsequently, the produced calli were transferred to regeneration medium. The data were recorded on each replicate for the following traits: Responding anthers: this trait was determined as the ratio of the number of responded anthers (producing at least one embryoid or callus) to total number of anthers plated. Callus fresh weight (mg): it was recorded as the weight of fresh callus derived from one anther after 60 days from plating. Callus size (mL): it was measured by using the sterile distilled water in a 10 cm^3 cylinder and it was calculated by the following formula, *Callus size* = $v_2 - v_1$

Where: v_1 = water volume without callus

V_2 = water volume with callus

Green point percentage: which is the ratio of the number of green point to the number of calli, were transferred to regeneration medium. In order to normalize the distribution of the percentage data which fall between 0.00 to 1.00, these data were transformed by using arcsine $X^{1/2}$ function prior to statistical analysis for the *in vitro* studied traits which were responding anthers, callus size and green point percentage. A combined analysis of variance for genotypes over the PEG levels was made for the studied *in vitro*

traits according to Steel and Torrie (1980). The amounts of heterosis were determined as the percentage increase of F_1 hybrid means over their mid-parents (M.P) and/or their better parent (B.P).

The combining ability analysis of variance for the combined data over the three PEG levels was carried out to determine the GCA, SCA and their interactions with PEG levels. The statistical analysis were performed according to Griffing's method II (1956) as described by Singh and Chaudhary (1985). On the basis of the expected mean squares, estimates of GCA, SCA and their interactions with PEG levels variances. These estimates could be expressed in terms of the covariance among the two types of relatives in a diallel cross. However, general combining ability variance (σ^2g) is equivalent to the covariance among half-sibs and the specific combining ability variance (σ^2s) is equivalent to the covariance among full-sibs minus twice of covariance among half-sibs (Hallauer and Miranda, 1988). The covariance of relatives were translated into appropriate genetic components of variance as outlined by Matzinger and Kempthorne, (1956) and Cockerham (1963). Degree of dominance, heritability in broad and narrow senses were calculated for all studied traits.

RESULTES AND DISCUSSION

The data obtained from the three PEG levels for parental lines and their F_1 hybrids were setup in a combined analysis of variances and the obtained results are presented in Table 1. Significance testes on the mean squares of genotypes were highly significant for all studied traits. These findings indicated the presence of real differences among the genotypes. Therefore, the planned comparisons between them as well as the partition of these genotypic variance to its components in order to understanding the nature of variation through diallel crosses analysis are valied. Furthermore, levels and genotype by levels interaction mean squares were highly significant with respect to all studied *in vitro* traits except for green point percentage. This indicates that these genotypes gave different response at different PEG levels. Thus, these genotypes may differed in their tolerant to drought stress at *In vitro* levels. These results are in agreement with the results obtained by Abd El-Khalek (2001).

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

S. O. V	d. f	R. A.	C. W.	C. S.	G. P. %
Levels (L)	2	2218.64**	837.06**	404.48**	456.69**
Reps / L	6	3.180	0.065	0.613	3.847
Genotypes (G)	9	393.87**	310.22**	163.12**	64.55**
G x L	18	39.93**	21.23**	6.785**	18.44
Error	54	2.179	0.434	0.508	10.71

Note: , ** significant at 0.01 levels of probability.
The percentage data were transformed using arcsin x1/2 perior to statistical analysis.

Since, these genotypes which included parents and their F_1 hybrids gave different performances with different PEG levels for the studied *in vitro* traits. So, the combined data over the three PEG levels could be more precise to present information concerning the performance of these genotypes. Therefore, the means of five parents and their F_1 hybrids were combined from the data over the three levels of PEG and the obtained results are shown in Table 2. In spite of, significant differences were observed among most of parental varieties for studied *in vitro* traits, greatest mean frequencies of responding anthers, callus weight and callus size were observed in Sakha 104 (P_3) with means of 28.07, 24.53 and 29.99, respectively. Furthermore, this variety recorded the second one for green point percentage after Giza 177 (P_1) which not differed significantly than it. Therefore, it could be concluded that from the previous results, Sakha 104 is the best variety among this group for drought tolerance at *in vitro* levels. In general the greatest overall values responding anthers was observed in the cross Sakha 101 x IRAT 112 ($P_2 \times P_4$) with means of 37.76. Whereas, the greatest overall values of callus weight and green point percentage were recorded in cross Sakha 101 x Sakha 104 ($P_2 \times P_3$) with means of 25.70 and 17.36, respectively. The cross Giza 177 x Sakha 101 ($P_1 \times P_2$) was the highest value of callus size with means of 30.80. The lowest hybrids for responding anthers and green point percentage were Giza 177 x Sakha 104 ($P_1 \times P_3$) with means of 27.10 and 9.44, respectively. While the crosses Giza 177 x IRAT 112 ($P_1 \times P_4$) gave the lowest values of means for callus weight and callus size with means of 14.50 and 21.75, respectively. These results are in agreement with the results obtained by Joyeeta *et al.* (2002) and Vaithiyalingan and Nadarajan (2005).

Table 2: Mean performance of parental varieties and their F_1 hybrids for all *in vitro* traits form the combined data over the three levels of PEG

Genotypes	R. A.	C. W.	C. S.	G. P. %
Giza 177 (P_1)	23.21	15.13	24.60	15.44
Sakha 101 (P_2)	26.91	20.20	26.77	14.49
Sakha 104 (P_3)	28.07	24.53	29.99	14.82
IRAT 112 (P_4)	15.58	7.86	17.16	9.50
$P_1 \times P_2$	27.67	24.93	30.80	10.33
$P_1 \times P_3$	27.10	16.10	23.41	9.44
$P_1 \times P_4$	28.62	14.50	21.75	11.93
$P_2 \times P_3$	33.63	25.70	28.76	17.36
$P_2 \times P_4$	37.76	22.10	27.99	13.21
$P_3 \times P_4$	37.40	23.70	28.64	11.97
LSD 5%	1.430	0.714	0.754	3.300
1%	1.961	0.979	1.034	4.521

The percentage data were transformed using arcsin x1/2 perior to statistical analysis.

Heterosis relative to mid-parents (M.P) and better parents (B.P) for all *in vitro* traits were estimated from the combined data over the three levels of PEG and the obtained results are presented in Table 3. The results revealed that most of studied crosses exhibited different heterotic values at the different levels of PEG and their combined, which could be due to the different behavior of the genotypes on the different levels of PEG. This finding indicates to the different tolerant levels with respect studied genotypes. However, high heterotic values for responding anthers were observed in (P_1 x P_4), (P_2 x P_4) and (P_3 x P_4) crosses at the three levels and their combined. These values from the combined data were 47.60%, 77.77% and 71.40% for these combinations, respectively. While, in the case of callus weight, five crosses exhibited positive and high significant at the three levels and their combined data with values ranged from 14.93% (P_2 x P_3) to 57.51% (P_2 x P_4) for the combined data. In addition, for combinations exhibited high significant positive heterosis for callus size at the three levels and their combined data with values ranged between 4.17% (P_1 x P_4) and 27.45% (P_2 x P_4) from the combined data. On the other hand, there were no significant positive heterosis at all levels and the combined data in the case of green point percentage. Furthermore, the amounts of heterosis relative to better parent (B.P) for all *in vitro* traits at the three levels of PEG and their combined data were determined and the obtained results are presented in Table 4. Due to the different values and signs of heterotic effect with respect to the three levels of PEG, it could be more precise the exhibition of the results obtained from the combined data. Thus, four, three and two out of the studied six crosses showed positive and significant heterotic values for anther responding, callus weight and callus size, respectively. These values ranged from 19.8% (P_2 x P_3) to 40.31% (P_2 x P_4), 4.77% (P_2 x P_3) to 23.41% (P_1 x P_2) and 4.55% (P_2 x P_4) to 15.05% (P_1 x P_2) for the previous traits, respectively. On the other hand, no crosses exhibited positive significant heterosis for green point percentage. These results are in agreement with the results obtained by Draz *et al.* (1992), Abd El Khalek (2001) and Gomez and Ragnsamy (2003).

Table 3: Estimates of heterosis relative to mid-parents (M.P) and better parent (B. P) for *in vitro* traits from combined data over the three PEG levels.

Crosses	R. A.		C. W.		C. S.		G. P. %	
	M.P	B.P	M.P	B.P	M.P	B.P	M.P	B.P
P_1 X P_2	10.41	2.82	41.17	23.41	19.93	15.05	-30.94	-33.09
P_1 X P_3	5.69	-3.45	-18.80	-34.36	-14.21	-21.94	-37.60	-38.86
P_1 X P_4	47.60	23.30	26.08	-4.16	4.17	-11.58	-4.33	-22.73
P_2 X P_3	22.33	19.80	14.93	4.77	1.34	-4.10	18.50	17.14
P_2 X P_4	77.77	40.31	57.51	9.40	27.45	4.55	10.10	-8.83
P_3 X P_4	71.40	33.16	46.29	-3.38	21.51	-4.50	-1.56	-19.23
LSD 5 %	1.238	1.430	0.618	0.714	0.653	0.754	2.855	3.300
1 %	1.698	1.961	0.848	0.979	0.895	1.034	3.915	4.521

P_1, P_2, P_3 and P_4 are: Giza 177, Sakha 101, Sakha 104 and IRAT 112 varieties, respectively.
***, ** Significant at 0.05 and 0.01 levels of probability, respectively.**

Mean squares for combining ability variances from the combined analysis over the three levels of PEG for all *in vitro* traits are shown in Table 4. The results indicated that both GCA and SCA mean squares were highly significant in all *in vitro* traits. These findings suggest that both additive and non-additive genetic action contributed in the genetic expression of these traits. Furthermore, the interactions between the GCA by levels of PEG (GCA x L) and SCA by levels of PEG (SCA x L) were highly significant for all *in vitro* traits. This results explain the different performance of these genotypes on different levels of PEG. These results agreed with the results obtained by Chen *et al.* (1999) and Gomez and Rangsamy (2003).

Table 4:Analysis of combining ability variance and mean squares for all *in vitro* traits from the combined data over three PEG levels

S. O. V	d. f	R. A.	C. W.	C. S.	G. P. %
GCA	3	78.905**	188.890**	93.777**	19.663**
SCA	6	157.503**	60.127**	34.672**	22.440**
GCA x L	6	95.971**	208.597**	98.648**	48.678**
SCA x L	12	208.841**	82.932**	42.010**	35.597**
Error	54	0.696	0.174	0.193	3.697

** Significant at 0.01 level of probability.
The percentage data were transformed using arcsin x1/2 perior to statistical analysis

The estimates of GCA effects for each parental variety, which obtained from the combined data over the three levels are shown in Table 5. Positive values indicate a contribution towards response, while negative values represent the opposite. The results showed that Sakha 104 and Sakha 101 were the best general combiners which exhibited positive and highly significant values in cases of responding anthers, callus weight and callus size, while positive but non significant values for green point percentage. On the other hand, Giza 177 and IRAT 112 were the inferior combiners, which had negative values for all studied traits.

Table 5: General combining ability (g_i) effects of parental varieties from combined data over the three PEG levels for all in *vitro traits*.

Parents	R. A.	C. W.	C. S.	G. P. %
Giza 177 (p_1)	-2.214**	-1.931**	-0.797**	-0.275
Sakha 101 (p_2)	1.670**	2.625**	1.861**	0.939
Sakha 104 (p_3)	1.902**	2.864**	1.809**	0.692
IRAT 112 (p_4)	-1.357**	-3.558**	-2.873**	-1.356
S. E (g_i)	0.295	0.147	0.155	0.680

** significant at 0.05 and 0.01 levels of probability, respectively.
The percentage data were transformed using arcsin x1/2 perior to statistical analysis.

The estimates of SCA effects from the combined data over the three levels of PEG were determined and the obtained results are presented in Table 6. The results revealed that, the best combinations for responding anthers, callus weight and callus size were Sakha 101 x IRAT 112 and Sakha

104 x IRAT 112, which had positive and highly S_{ij} values. However, Giza 177 x Sakha 101 was one of the best combiners for callus weight and callus size traits. Therefore, from these results, it could be suggested that the good combinations resulted from the hybridization between good x bad general combiners. These results are similar to the results obtained in rice by Chen *et al.* (1999) and Sanjay *et al.* (2005).

Table 6: Specific combining ability effects (S_{ij}) for each cross from the combined data over the three PEG levels for *in vitro* traits.

Crosses	R. A.	C. W.	C. S.	G. P. %
$P_1 X P_2$	-0.405	4.762**	3.751**	-3.182
$P_1 X P_3$	-1.210	-4.310**	-3.590**	-3.825
$P_1 X P_4$	3.576**	0.512	-0.567	0.714
$P_2 X P_3$	1.683*	0.734	-0.899	2.874
$P_2 X P_4$	8.825**	3.557**	3.014**	0.780
$P_3 X P_4$	8.240**	4.918**	3.723**	-0.217
S. E (s_{ij})	0.714	0.356	0.376	1.646

* , ** significant at 0.05 and 0.01 levels of probability, respectively.
The percentage data were transformed using arcsin x1/2 perior to statistical analysis.

The additive (σ^2A) and non-additive including dominance (σ^2D) genetic variances in addition to heritability in broad (H_b) and narrow (H_n) sense as well as, dominance degree ratio (D.d) were estimated for all studied *in vitro* traits from the combined data over the three PEG levels and the obtained results are shown in Table 7. The results revealed that the magnitude values of non-additive genetic variance were larger than the corresponding values of additive genetic variance for all *in vitro* traits. This indicated the predominance of non-additive gene action in the genetic control for all traits. This result could be emphasized by dominance degree ratio, which were more than unity for all studied traits, revealing the importance of over dominance in the genetic expression of these traits. Furthermore, the results also showed that both variances due to additive (σ^2A x L) and non-additive (σ^2D x L) by levels interactions were positive in all studied *in vitro* traits except for (σ^2A x L) in the case of responding anthers. This finding explain the non-stability of genotypes on different levels of PEG for the studied traits. Also, the results showed that heritability in broad sense was higher than the corresponding estimates in narrow sense with respect to all studied *in vitro* traits. These values ranged from 31.93 to 47.10 for green point percentage and callus size, respectively in broad sense, while it ranged from zero to 18.85 for responding anthers and callus weight, respectively in narrow sense. This finding insures again the role of dominance gene effects expression of the *in vitro* traits. These results are similar to the results obtained in rice by Ammar (1997), Abd El-Khalek (2001) Sanjay *et al.* (2005).

In conclusion, the previous results indicated that, most of studied *in vitro* traits are mainly controlled by non-additive genes in addition to the minor role of additive gene action under drought stress levels. Thus, it could be suggested that one of the best way for improvement the genotypes for their

tolerance to drought stress is recurrent selection program at *in vitro* level as a tool to select the promise genotypes for drought tolerance and subsequently it will be valid to corporate in applied field program.

Table 7: Estimates of relative magnitudes of different genetic parameters for *in vitro* traits obtained from the combined data over the three levels

Genetic parameters	R. A.	C. W.	C. S.	G. P. %
σ^2 A	-26.20	42.92	19.70	-0.926
σ^2 D	156.80	59.95	34.47	18.74
σ^2 A x L	-37.62	41.89	18.88	4.36
σ^2 D x L	208.14	82.76	41.82	31.90
σ^2 e	0.696	0.174	0.193	3.697
Hb %	42.88	45.18	47.10	31.93
Hn %	00.00	18.85	17.12	00.00
D. d	> 1.00	1.182	1.323	> 1.00

Note: negative values were considered equal to zero during the calculation of heritability. The percentage data were transformed using arcsin x1/2 perior to statistical analysis.

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التحليل الوراثى للإستجابة لزراعة المتوك فى الأرز تحت ظروف الجفاف

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هذه الدراسة تهدف الى تقييم بعض التراكيب الوراثية ودراسة سلوكها الوراثى للإستجابة لزراعة المتوك فى الأرز تحت ظروف الجفاف بإستخدام مستويات مختلفة من البولى إثيلين جليكول . ومن أجل هذا الغرض أستخدمت أربعة أصناف من الأرز بالإضافة الى ستة هجن ناتجة بنظام التزاوج النصف دائرى بينهم. وكانت النتائج المتحصل عليها تتلخص فى التالى:

متوسط المربعات للتراكيب الوراثية كانت عالية المعنوية لكل الصفات المدروسة وتشمل معدل الإستجابة، نسبة الكالوس، وزن الكالوس، حجم الكالوس و معدل البقع الخضراء مما يشير الى أن هذه التراكيب الوراثية بينها إختلاف حقيقى فى هذه الصفات. علاوة على ذلك تبين أن الإختلافات الراجعة لمستويات البولى إثيلين جليكول والتداخل بينه وبين التراكيب الوراثية عالى المعنوية لكل الصفات فيما عدا معدل البقع الخضراء مما يشير الى أن هذه التراكيب تستجيب بدرجات مختلفة لزراعة المتوك بإختلاف مستويات الإجهاد المائى.

وبدراسة تأثير القدرة العامة على التآلف لكل صنف من الأصناف المستخدمة فى هذه الدراسة تبين أن أفضل الأصناف قدرة على التآلف هى سخا ١٠٤ و سخا ١٠١ لكل الصفات المدروسة.

وعلى الجانب الآخر تبين ان الهجن سخا ١٠٤ X IRAT112 و سخا ١٠١ X IRAT112 كانت أفضل الهجن لقدرتها الخاصة على التآلف بكل الصفات المدروسة فيما عدا معدل البقع الخضراء. وبدراسة الفعل الجينى لهذه الصفات أظهرت النتائج أن التباين السيادى له الدور الرئيسى والأكثر أهمية مقارنة بالتباين الأضافى فى توريث هذه الصفات. وبتقدير قيم درجة السيادة تبين أنها تزيد عن الواحد الصحيح لكل الصفات مما يؤكد دور السيادة الفائقة فى التعبير الوراثى لهذه الصفات.

ويمكن أن نستخلص من هذه النتائج أن الطريقة المناسبة لتحسين مثل هذه التراكيب الوراثية لتحملها الجفاف يكون عن طريق الإنتخاب المتكرر للأجيال المتقدمة للهجن الناتجة من التهجين بين الأصناف عالية التحمل للجفاف مع الهجن الحساسة على المستوى المعملى وبالتالى يمكن التوصل الى الطرز الواعدة بتحملها الجفاف لإستخدامها للتطبيق الحقلى عن طريق طرق التربية التقليدية.