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Combining ability of anther culture response in some rice crosses under drought stress conditions

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Abstract

Anther culture response of ten rice parental genotypes and their 24 F_1 crosses was studied to estimate combining ability effects for callus induction and plant regeneration under drought stress conditions. The analysis of variance showed that drought stress levels mean squares were found to be highly significant for all *in vitro* studied traits. The percentage of anthers that developed calli ranged from 12.92% for the IRRI 148 to 45.00% for the cross $L_1 \times T_3$ among the genotypes across the four different stress levels of PEG. The control (PEG-free medium) gave the highest mean value of callus induction (41.33%). On the other hand, the medium containing 30 g/l of PEG gave the lowest one (14.28%) across all genotypes. Results showed that the three crosses ($L_1 \times T_2$, $L_1 \times T_3$ and $L_3 \times T_1$) gave the highest mean values of plant regeneration (9.50%, 8.00 and 7.92% respectively). The percentage of plant regeneration from the control medium was 7.12%, while the medium containing 30 g/l of PEG gave the lowest one across all genotypes (3.76%). The ratio of GCA/SCA was found less than unity for all *in vitro* studied traits under the four stress levels, revealing the predominant role of dominance gene action in the inheritance of these traits. Results showed that the IRGC (T_3) followed by Giza 177 (L_1) were the best combiners for callus induction under varying concentrations of PEG due to their highly significant positive GCA values. For plant regeneration, one parental genotype Giza 177 (L_1) was the best combiner for plant regeneration under varying concentrations of PEG due to their highly significant positive GCA values. The two crosses; $L_3 \times T_1$ and $L_1 \times T_2$ recorded the highest SCA effects for plant regeneration and green plant regeneration under the four stress levels.

Keywords: rice, anther culture, combining ability, drought stress.

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1. Introduction

Rice (*Oryza sativa* L.) is a widely grown global grain that feeds most of the world's population and will continue to play an important role in global food and livelihood security. The requirement for sustainable strategy to improve rice production remains an immense problem due to limited water resources and diverse biotic and abiotic stresses. The total production in Egypt reached 4.44 million tons produced from an area of about 1.18 million feddan (CAPMAS, 2020). Rice consumes around 20% of Egypt's total water resources during the summer season and occupies about 22% of the entire growing area. Because Egypt's water supplies are restricted, the total water requirements for rice crops are a severe challenge due to the limited irrigation water accessible from the Nile. Some rice-growing areas, particularly those near the termini of terminal irrigation canals in the northern section of the Nile Delta, have irrigation water shortages at various phases of growth, which is regarded one of Egypt's most important restrictions to rice production (Abd Allah *et al*., 2009). Therefore, one of the major aims of rice breeding for increasing rice production in Egypt is the development of water stress-tolerant genotypes with high yield potential. Traditional methods of breeding for water stress tolerance are time-consuming and inefficient. Tissue culture as a promising technology for obtaining the desired characteristics of variants can lead to the expected outcomes. In contrast, the likelihood of achieving *in vitro* selection is dependent on the availability of an effective regeneration system in combination with an effective selective agent (Dita *et al.*, 2006; Jain, 2001, Predieri, 2001; Predieri and Virgilio, 2007; Rai *et al.*, 2011). One of the most essential techniques in plant breeding and genetic analysis is haploid production. This is important, especially when it is used with common plant breeding approaches to produce desired features. Anther cultures, one of the haploid plant generation methods, are now widely used in rice breeding. Rice pure lines are produced utilizing the anther culture method in a very short time and with assurance in the genetic purity of the derived lines. Many factors influence the effectiveness of the anther culture reaction for androgenic calli and plant regeneration, including genotype, physiological age of the donor plant, and culture media (Mercy, 1990). Callus induction from anther culture is influenced by the culture medium and genotype. The genotypic number of chromosomes in haploid plants is the same as the number of chromosomes in a sporophyte. Different ways can be used to produce haploidy, with microspore androgenesis being the most promising and successful. Using biotechnological techniques like anther culture, many novel rice cultivars have been produced (Brown and Thorpe, 1995; Zapata *et al*., 2004). In rice and other cereal crops, however, poor regeneration and low

haploid efficiency have been the most important limiting factors. Although plants naturally produce haploids, *in vitro* anther culture or androgenesis is the most efficient and straightforward method for producing haploids or doubling haploids in a lot of species (Forster *et al.*, 2007). Doubled haploid breeding by anther culture has emerged as an intriguing and potent technology for crop development, as well as a practical alternative to traditional methods (Purwoko *et al.*, 2010). Doubled haploids provide a number of advantages, including a shorter breeding cycle due to fast homozygosity fixation, high selection efficiency, increased genetic variety via gametoclonal variations, and early expression of recessive genes appropriate for breeding (Devaux and Pickering 2005). There are numerous reviews that explain how androgenesis produces double haploids and how they are used (Germana, 2011; Touraev *et al.*, 2009; Seguí-Simarro 2010). According to Kim *et al.* (1991) the best response to anther culture came from a hybrid of Japonica × Japonica, Indica × Japonica, and finally Indica × Indica rice cultivars. Callus induction and green plant regeneration are strongly affected by genotype and medium× genotype interaction, according to a study conducted to determine the callus induction and green regeneration heredity of genotypes derived via diallel analysis of four rice cultivars (Quimio and Zapata, 1990). One of the most critical variables in callus formation from cultured anthers is the culture media. Androgenesis and the growth of created embryos have separate nutritional requirements (Zapata *et al*., 1990). One of the most common methods is to use high molecular weight osmotic chemicals such as polyethylene glycol (PEG). PEG was used to select callus and plantlets for *in vitro* drought stress induction. PEG 6000 is a non-permeable, non-toxic osmotic material that has been used to imitate drought stress in cultured plant tissues by lowering the water potential of the culture medium (Hamayun *et al.*, 2010). The development of efficient and reliable callus induction and plant regeneration technologies is required for *in vitro* selection for abiotic stress resistance (Wani *et al.*, 2010). Several crops, including rice, have been successfully *in vitro* selected for drought tolerance utilizing polyethylene glycol (PEG), sorbitol, mannitol, and agar as selection agents (Biswas *et al.*, 2002). Water is withdrawn not only by the cell but also by the cell wall since PEG does not enter the apoplast. In comparison to other low molecular weight osmotica, PEG simulates soil drying in a comparable way and has been used to simulate drought stress in plants as well as the selection of tolerant cell lines (Nepomuceno *et al*., 1998). Drought tolerance breeding necessitates a thorough understanding of gene function as well as the ability of drought characteristics and yield components to combine underwater stress and non-stress situations. Given that the success of any

plant breeding program is determined not only by the parents chosen but also by the breeding procedures used (Can *et al.*, 1997; Torres and Geraldi, 2007). Therefore, appropriate breeding methodology should be devised. The knowledge of combining ability is useful to assess nicking ability among genotypes and, at the same time, explicate the nature and magnitude of gene actions involved (Dar *et al*., 2014). Line × tester (Kempthorne, 1957) mating designs provide dependable information about the general and specific combining ability (GCA and SCA) of parents and their cross combinations and are helpful in estimating various types of gene actions (Verma, 2003). The objective of the present investigation was to study the anther culture response of some rice parental genotypes and their F_1 crosses under drought stress treatments as well as to gather information on the genetic behavior of anther culture response under PEG stress treatments in a total of ten parents and their crosses of rice.

2. Materials and methods

The present investigation was carried out at the Cell and Tissue Culture Laboratory, Agronomy Department, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt and the Experimental Farm and the laboratories of the Rice Research and Training Center, Agricultural Research Center, Sakha, Kafr El-Sheikh, Egypt during the period from 2018 to 2020. Ten rice genotypes were selected in this study, namely; Giza 177, Giza 178, Sakha104, Sakha107, IRRI 148, IRAT 112, IRGC 1165, N22, IRRI 163 and Azucena, which represented a wide range of diversity for several traits. In season 2018, the ten parental genotypes were crossed according to line × tester mating design (Kempthorne, 1957). The first four genotypes were used as female parents (Lines) and the last six genotypes were utilized as male parents (Testers), respectively. In the 2019 season, the ten parental genotypes and their crosses (24 F_1 crosses) were planted at the Experimental Farm to obtain the needed anthers. The ten genotypes and their 24 F_1 crosses were evaluated in anther culture under the four stress levels from polyethylene glycol 6000 (PEG) for their ability to initiate callus and plant regeneration. For evaluation of the anther culture responses of ten parental genotypes and 24 F_1 crosses under drought stress conditions, panicles were gathered, and microspores were microscopically examined. panicles carrying anthers with microspores at the mid to late uninucleate stage were surface sterilized with 20% chlorax solution commercial bleach (1.05% NaOCl) for 20 minutes and washed three times in sterile distilled water. Excised anthers were then placed on the N6 induction medium supplemented with various levels of polyethylene glycol 6000 (0, 10, 20, and 30 g/l PEG). The experimental design was a completely

randomized design including 34 genotypes and 10 replicates for each genotype. After 5-6 weeks of culture, the number of responsive anthers was recorded. When the calli induced from the anthers reached the cotyledonary stage, they were transferred to jars containing MS regeneration medium (Murashige and Skoog, 1962) supplemented with 2.5 mg/l Kin, 0.5 NAA mg/l and 30 g/l sucrose, and 8 g/l agar with various levels of polyethylene glycol 6000 (0, 10, 20, and 30 g/l PEG). These jars were incubated for 5–6 weeks at 25–27 °C with 16 h of light. Well-developed shoots were transferred to 1/2 MS basal medium supplemented with 0.2 NAA mg/l for root initiation, elongation and their development. The number of regenerated shoots and plantlets was counted. Plantlets with adequate root formation were transplanted to small pots with a mixture of soil, sand, and compost under plastic cover for three weeks in a growth chamber maintained at 18 ºC and 16 h light per day. The recorded data were subjected to the ordinary analysis of variance (Steel and Torrie, 1980) to determine the significant differences among crosses and parents' response under drought stress condition. Combining ability analysis was done using line x tester method (Kempthorne, 1957).

3. Results and Discussion

3.1 Effect of different drought stress treatments on the anther culture response of rice genotypes

The analysis of variance for the studied traits under the four drought stress treatments (0, 10, 20 and 30 g/l PEG) and their combined data is presented in Table (1). Results showed that drought stress levels mean squares were found to be highly significant for all the studied traits, revealing that performance of the tested genotypes differed under the four stress levels. Similar results were also found by Wani *et al.* (2010). Mean squares due to genotypes, parents, crosses, line, tester and line × tester were highly significant for all the studied traits under the four stress levels and their combined data. Also, highly significant mean squares due to the interaction of genotypes, parents, crosses, line, tester and line × tester with PEG treatments were detected for all the studied traits except albino plant regeneration, indicating that these genotypes varied in their response to PEG stress levels for most of the studied traits. These results are in agreement with those obtained by Akte *et al*. (2016), and Sabesan and Saravanan (2016). Parent *vs*. crosses mean squares (Table 1) as an indication to average heterosis overall crosses were found to be highly significant for all the studied traits under the four stress levels and the combined data. Moreover, the interaction of parents *vs*. crosses with drought stress levels was found to be highly significant for all *in vitro* studied traits except albino plant regeneration.

Table (1): Mean squares of single (S) and combined (Comb.) analysis of variance for the studied traits of rice genotypes under PEG - stress levels (L).

Source of variation	df.		PEG - stress levels g/l									
			Callus induction (%)					Plant regeneration (%)				
	S	Comb.	Control	10	20	30	Comb.	Control	10	20	30	Comb.
PEG-stress levels (L)	-	3	-	-	-	-	4252.70**	-	-	-	-	65.12**
Genotypes(G)	33	33	141.65**	56.47**	40.80**	16.53**	143.81**	4.81**	2.19**	1.20**	1.06**	7.58**
Parents (P)	9	9	57.48**	52.45**	26.45**	8.11**	113.65**	2.31**	1.83**	0.56**	0.40**	3.52**
Crosses (C)	23	23	56.58**	43.21**	48.15**	19.82**	108.13**	4.93**	2.20**	1.36**	1.26**	8.28**
P vs. C	1	1	2856.01**	397.60**	0.99**	16.83**	1235.81**	24.43**	5.24**	3.19**	2.37**	27.87**
Lines	3	3	39.15**	64.40**	105.25**	38.34**	205.79**	11.61**	5.42**	4.14**	4.10**	23.37**
Testers	5	5	85.59**	92.38**	64.33**	17.49**	183.78**	2.99**	1.62**	0.52*	0.68**	4.36**
Lines × Testers	15	15	50.39**	22.58**	31.33**	16.89**	63.37**	4.24**	1.75**	1.08**	0.88**	6.57**
G × L	-	99	-	-	-	-	37.23**	-	-	-	-	0.56**
P × L	-	27	-	-	-	-	10.28**	-	-	-	-	0.53**
C × L	-	69	-	-	-	-	19.90**	-	-	-	-	0.49**
Lines × L	-	9	-	-	-	-	13.77**	-	-	-	-	0.63**
Testers × L	-	15	-	-	-	-	25.31**	-	-	-	-	0.48**
Lines × Testers × L	-	45	-	-	-	-	19.32**	-	-	-	-	0.46**
P vs. C × L	-	1	-	-	-	-	2035.35**	-	-	-	-	7.37**
Error	297	1188	0.14	0.17	0.17	0.16	0.16	0.24	0.22	0.18	0.13	0.20
Source of variation	d.f.		PEG - stress levels g/l									
	S	Comb.	Green plant regeneration (%)					Albino plant regeneration (%)				
			Control	10	20	30	Comb.	Control	10	20	30	Comb.
PEG -stress levels (L)	-	3	-	-	-	-	40.02**	-	-	-	-	12.77**
Genotypes (G)	33	33	4.46**	1.93**	1.22**	0.85**	6.73**	0.95**	0.56**	0.47**	0.42**	1.88**
Parents (P)	9	9	2.85**	2.09**	0.62**	0.47**	4.59**	0.53**	0.40*	0.40*	0.50**	1.62**
Crosses (C)	23	23	4.39**	1.52**	1.01**	0.83**	5.94**	1.05**	0.46**	0.31	0.29*	1.47**
P vs. C	1	1	20.39**	9.93**	11.48**	4.56**	44.19**	2.50**	4.29**	4.65**	2.66**	13.72**
Lines	3	3	9.06**	4.82**	2.54**	2.36**	16.60**	4.28**	2.19**	1.20**	0.68**	7.26**
Testers	5	5	2.64**	0.21**	0.69**	0.55**	2.05**	0.65**	0.19	0.04	0.30	0.56*
Lines × Testers	15	15	4.05**	1.30**	0.80**	0.62**	5.10**	0.54**	0.20	0.22	0.21	0.61**
G × L	-	99	-	-	-	-	0.59**	-	-	-	-	0.18
P × L	-	27	-	-	-	-	0.48**	-	-	-	-	0.07
C × L	-	69	-	-	-	-	0.62**	-	-	-	-	0.23
Lines × L	-	9	-	-	-	-	0.62*	-	-	-	-	0.45*
Testers × L	-	15	-	-	-	-	0.70**	-	-	-	-	0.19
Lines × Testers × L	-	45	-	-	-	-	0.59**	-	-	-	-	0.19
P vs. C × L	-	1	-	-	-	-	2.63**	-	-	-	-	0.28
Error	297	1188	0.19	0.25	0.21	0.18	0.21	0.19	0.18	0.20	0.17	0.19

* and ** denote significant at 0.05 and 0.01 levels of probability, respectively.

The responses of the anther culture of ten rice parents and their F_1 crosses studied under different drought stress (PEG) treatments are presented in Table (2). Results showed that callus induction varied among the rice genotypes studied. The percentage of anthers that developed calli ranged from 12.92% for the IRRI 148 (T_1) to 45.00% for the cross $L_1 \times T_3$ among the genotypes across the four different stress levels of PEG (Table 2). The response of callus induction varied according to different concentrations used, indicating that the control (PEG-free medium) gave the highest mean value of callus induction (41.33%). On the other hand, the medium containing 30 g/l of PEG gave the lowest one (14.28%) across all genotypes (Table 2, and Figures 1 and 2). Results presented in Table (2) showed that the cross ($L_2 \times T_1$) gave the highest percentage of callus induction (63.33%) in control, while the cross ($L_3 \times T_1$) gave the lowest one (6.67%) with medium containing 30 g/l of PEG. Our results showed that callus

induction frequency response under PEG treatments was genotype dependent. The genetic constitution appeared to play a major role in callus induction under water stress. A decrease in callus induction is a typical response of the explants of species, including rice when subjected to PEG simulated drought stress (Wani *et al.*, 2010). Anther derived calli upon subjection to different concentrations of PEG exhibited necrosis and reduction in survival percentage compared to control, suggesting the response to water stress is due to water shortage in the cells, which leads to a decrease in cell turgor and eventually cell growth effect of PEG (Sakthivelu *et al.*, 2008). In the present investigation, callus induction percentage decreased with increasing concentrations of PEG in all genotypes (Figures 1 and 2). The decrease in callus induction, however, varied from one genotype to another and also between cultures of the same genotype. Biswas *et al.* (2002), Wani *et al*. (2010) and Tripathy (2015) reported similar results in rice. Mon *et al.* (2020) reported that the anther culture ability (callus induction and green plant regeneration) was dependent on genotypes. Therefore, the anther culture ability could be improved by crossing with high responsive genotypes.

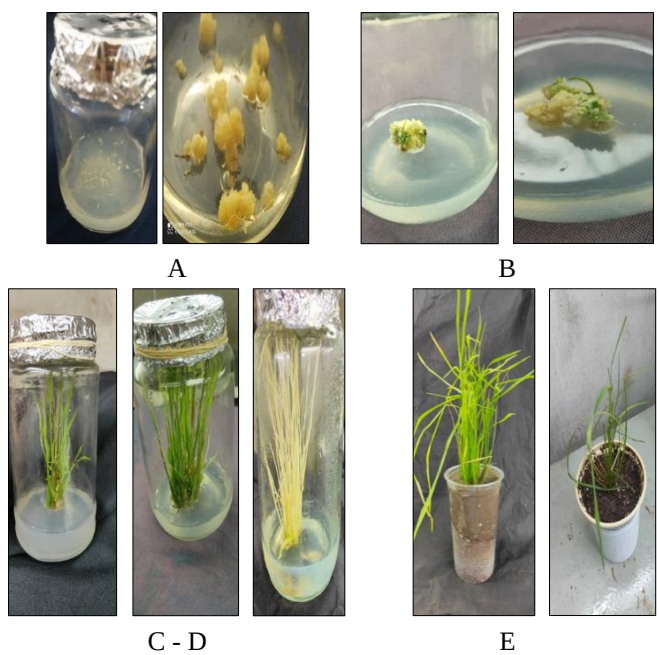

Figure (1): *In vitro* induction of haploid rice plants through anther culture grown on control (PEG-free medium) and their subsequent transfer to pots. A) Formation of callus in the cultured anthers. B) Callus differentiation into multiple shoots. C-D) Green and albino plantlets emerging from cultured anthers. E) plant transplanted from the jar to small pot with mixture of soil, sand and compost in greenhouse.

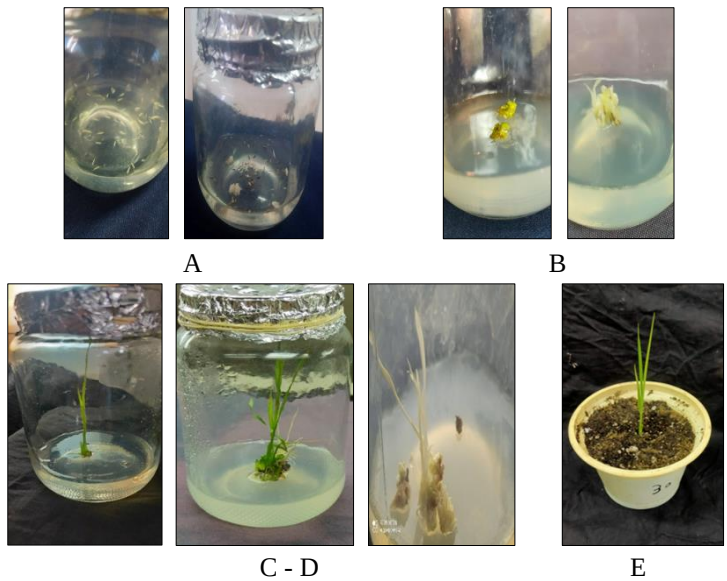

Figure (2): *In vitro* induction of haploid rice plants through anther culture grown on medium containing 30 g/l PEG and their subsequent transfer to pots. A) Formation of callus in the cultured anthers. B) Callus differentiation into multiple shoots. C-D) Green and albino plantlets emerging from cultured anthers. E) plant transplanted from the jar to small pot with mixture of soil, sand and compost in greenhouse.

The callus derived from anthers was subcultured on MS medium. When the calli were placed onto MS medium, some of calli differentiated into embryoids (Figure 1). However, many of calli did not differentiate and some of calli differentiated into shoots. Plant regeneration of the callus was dependent on the genotype and the culture medium employed. Effect of genotypes and PEG concentrations on the frequency of plant regeneration was presented in Table (2) and Figures (1 and 2). Results showed that the three crosses ($L_1 \times T_2$, $L_1 \times T_3$ and $L_3 \times T_1$) produced the highest mean values of plant regeneration (9.50%, 8.00% and 7.92%, respectively), while the IRRI 163 (T_5) produced the lowest one (3.17%). The control medium (PEG-free medium) gave better response to plant regeneration as compared to the other media containing 10, 20 and 30 g/L of PEG. The percentage of plant regeneration from the control medium was 7.12 %, while the medium containing 30 g/l of PEG gave the lowest one across all genotypes (3.76%). (Table 2, and Figures 1 and 2). Results presented in Table (2) indicated strong interaction between genotypes and concentrations of PEG. The highest response to regenerated plants frequency was recorded for the cross ($L_1 \times T_2$), when its cultures were grown on the control while the lowest response was recorded for the Sakha 107 (L_4) and cross

($L_3 \times T_4$) on the medium containing 30 g/l of PEG (2.33%). These results demonstrated the efficiency of the regeneration medium used. Effect of genotype on plant regeneration from callus cultures have been reported previously (Mon *et al*., 2020; Sharma *et al*., 2018; Tripathy *et al*., 2019).

Table (2): Effect of polyethylene glycol 6000 (PEG) concentrations on anther culture response of 24 F_1 rice crosses and their respective parents.

Genotypes	PEG-stress levels (g/l)									
	Callus induction (%)					Plant regeneration (%)				
	Control	10	20	30	Comb.	Control	10	20	30	Comb.
Giza 177 (L_1)	40.00	32.00	24.33	19.67	29.00	8.00	7.33	5.00	4.67	6.25
Giza 178 (L_2)	23.33	20.33	17.67	16.67	19.50	4.33	4.33	3.67	3.67	4.00
Sakha 104 (L_3)	36.67	36.33	30.67	17.33	30.25	8.00	6.67	4.67	4.00	5.83
Sakha 107 (L_4)	30.00	27.67	21.67	14.67	23.50	6.67	5.67	3.67	2.33	4.58
IRRI-148 (T_1)	16.67	13.33	11.67	10.00	12.92	6.33	6.00	4.33	3.33	5.00
IRAT-112 (T_2)	21.67	17.00	15.00	14.00	16.92	5.67	5.33	5.33	3.00	4.83
IRGC (T_3)	23.33	20.00	20.00	19.67	20.75	4.33	3.67	3.33	3.33	3.67
N.22 (T_4)	32.00	29.00	19.33	14.00	23.58	6.33	3.67	3.33	3.00	4.08
IRRI-163 (T_5)	16.67	15.33	14.67	13.00	14.92	3.67	3.33	3.00	2.67	3.17
AZUCENA (T_6)	23.33	21.00	20.67	15.33	20.08	4.00	3.67	3.67	3.33	3.67
$L_1 \times T_1$	56.67	46.67	25.67	15.33	36.08	9.00	7.67	5.67	4.33	6.67
$L_1 \times T_2$	42.00	25.67	16.67	13.33	24.42	13.67	10.00	7.67	6.67	9.50
$L_1 \times T_3$	60.00	47.67	43.33	29.00	45.00	12.00	7.33	6.67	6.00	8.00
$L_1 \times T_4$	43.33	30.67	26.67	17.67	29.58	8.67	7.00	6.33	6.33	7.08
$L_1 \times T_5$	43.33	31.33	17.33	14.00	26.50	6.67	4.67	4.33	3.67	4.83
$L_1 \times T_6$	55.33	34.00	23.67	16.33	32.33	7.33	6.33	5.00	4.33	5.75
$L_2 \times T_1$	63.33	41.33	22.33	12.67	34.92	5.00	3.67	3.00	2.67	3.58
$L_2 \times T_2$	33.00	21.67	14.33	10.33	19.83	7.00	6.00	4.00	3.00	5.00
$L_2 \times T_3$	54.00	27.33	16.00	8.67	26.50	8.67	7.33	4.67	3.67	6.08
$L_2 \times T_4$	47.00	27.00	22.00	20.00	29.00	9.33	6.33	5.00	4.67	6.33
$L_2 \times T_5$	39.67	24.33	12.67	7.67	21.08	8.33	6.00	4.33	3.67	5.58
$L_2 \times T_6$	42.00	26.33	13.00	11.33	23.17	6.00	5.33	4.67	3.67	4.92
$L_3 \times T_1$	43.00	33.33	8.67	6.67	22.92	12.33	8.00	6.33	5.00	7.92
$L_3 \times T_2$	47.00	34.00	16.67	14.00	27.92	7.67	6.33	5.00	4.00	5.75
$L_3 \times T_3$	48.00	29.00	25.33	16.67	29.75	7.33	6.00	4.33	3.33	5.25
$L_3 \times T_4$	49.33	31.00	10.00	7.67	24.50	5.33	3.33	2.67	2.33	3.42
$L_3 \times T_5$	40.67	26.33	17.67	14.00	24.67	5.67	4.67	4.33	3.67	4.58
$L_3 \times T_6$	37.67	31.00	19.00	14.00	25.42	8.67	6.67	5.33	4.00	6.17
$L_4 \times T_1$	41.67	32.33	27.67	10.00	27.92	5.67	4.33	3.33	2.67	4.00
$L_4 \times T_2$	51.67	21.67	17.00	13.00	25.83	6.00	4.67	3.67	3.00	4.33
$L_4 \times T_3$	52.67	40.00	26.67	15.67	33.75	6.67	5.33	4.67	3.67	5.08
$L_4 \times T_4$	61.67	26.67	21.33	15.00	31.17	7.33	5.67	5.33	5.00	5.83
$L_4 \times T_5$	41.33	26.00	22.00	16.67	26.50	5.33	4.33	3.33	2.67	3.92
$L_4 \times T_6$	47.33	31.33	15.00	11.67	26.33	5.00	4.00	3.33	2.67	3.75
Mean	41.33	28.78	19.89	14.28	26.07	7.12	5.61	4.50	3.76	5.25
L.SD 0.05 PEG Levels (T) L.SD 0.01	-	-	-	-	0.06 0.08	-	-	-	-	0.06 0.08
L.SD 0.05 Genotypes (G) L.SD 0.01	0. 32 0.42	0.36 0.47	0.36 0.47	0.35 0.46	0.32 0.42	0.43 0.56	0.41 0.54	0.37 0.49	0.320.42	0.35 0.46
L.SD 0.05 Interaction (T×G) L.SD 0.01	-	-	-	-	0.35 0.46	-	-	-	-	0.38 0.50

Bagheri and Jelodar (2008), and El-Hennawy *et al*. (2011) reported that plant regeneration from callus was found to be highly heritable. Genetic control of plant regeneration has been observed in several plant species and exploited in some cases to improve plant materials for use in tissue culture research including anther

culture (Ali *et al*., 2021; Dewia *et al*., 2009; He *et al*., 2006). Results in Table (2) showed that plant regeneration ability of all the tested genotypes was decreased significantly with increasing concentrations of PEG in selective media. The tested genotypes differed in their ability to regenerate plants after exposure to PEG concentrations (10, 20 and 30 g/l of PEG). The three crosses ($L_1 \times T_2$, $L_1 \times T_3$ and $L_3 \times T_1$) were most able to do this and were therefore the most tolerant genotypes with parent that exhibited very good tolerance, whereas the cross ($L_3 \times T_4$) had a high sensitivity response, especially at the highest stress level of 30 g/l PEG (Table 2). The typical decrease in plant regeneration of crop plants in response to water stress is due to water shortage in the cells, which leads to a decrease in cell turgor and eventually cell growth. Addition of PEG-6000 in culture media lowers water potential of the medium that affect cell division leading to reduced callus growth and consequently influences regeneration (Ehsanpour and Razavizadeh 2005; Kacem *et al.,* 2017; Sakthivelu *et al.,* 2008). Germana (2011) reported that the exploitation of haploid and DHs as a powerful breeding tool requires the availability of reliable tissue culture protocols that can overcome several methodology problems, such as low frequencies of embryo induction, albinism, plant regeneration, plant survival and the genotype-dependent response, in order to improve the regeneration efficiency in a wider range of genotypes. He also reported that there is no single standard condition or protocol for inducing pollen-derived plant formation. In the present investigation, the presence of significant variation in callus induction and plant regeneration due to genotypes, concentrations of PEG and their interactions were observed. The frequencies of green plantlets achieved of ten parents and their crosses studied under the four PEG concentrations are presented in Table (3). Results showed that the two crosses ($L_1 \times T_2$ and $L_3 \times T_1$) produced the highest mean values of green plantlets (7.75 % and 7.00% respectively) compared to the parental Giza 177 (L_1) and Sakha 104 (L_3), which gave 4.67%, while the N.22 (T_4) produced the lowest one (1.25%). The control (PEG-free medium) gave better response to green plantlets as compared to the other media containing 10, 20 and 30 g/l of PEG. The percentage of green plantlets from the control was 5.43%. In contrast, the medium containing 30 g/l of PEG gave the lowest one (2.76%) across all genotypes (Table 3). The interaction between concentrations of PEG and genotypes was highly significant (Table 3). The highest frequency of green plantlets in the control was recorded for the cross (L_1 x T_2). On the contrary, the lowest number of green plantlets per 100 cultured anthers was observed for the N.22 (T_4) under the highest stress level 30 PEG. Similar results were obtained by El-Hennawy *et al.* (2011), Vennapusa *et al.* (2015), and Mon *et al.,* (2020), who

found genotypic effect on green plant regeneration. As pointed out in different investigations, androgenic response in rice was a heritable trait and can be transferred into agriculturally desirable material by crossing (Mon *et al.,* 2020). Moreover, previous studies also showed that anther culture capacity in rice was highly heritable and was controlled by nuclear genes (Kwon *et al.,* 2002; Yamagishi *et al.,* 1998). These results suggested that it might be possible to overcome the limitation through genetic recombination and transferring genes controlling anther culturability from the high response into poorly responsive lines. Many researchers revealed that callus induction and green-plant regeneration are independently inherited, and the two traits are not correlative (He *et al*., 1998; Zhu, 1992). The occurrence of albino plantlets, which is usually uncounted in most similar studies, composes a major problem for the application of anther culture technique in rice breeding programs. Results in Table (3) showed that the percentage of albino plantlets ranged from 0.83% for $L_2 \times T_1$ to 3.50% for the variety Giza 177 (L_1) among the genotypes across the four different concentrations of PEG (Table 3). The response of albino plantlets varied according to different concentrations used, indicating that the control (PEG-free medium) gave the highest mean value of albino plantlets (2.66%). On the other hand, the medium containing 30 g/l of PEG gave the lowest one (1.18%) across all genotypes. Furthermore, two genotypes ($L_1 \times T_4$ and $L_1 \times T_6$) gave the highest percentage of albino plantlets (5.00% and 4.67%, respectively) in control, while three genotypes ($L_2 \times T_1$, $L_2 \times T_6$ and $L_3 \times T_1$) gave the lowest one (0.33%) under the stress level of 30 g/l PEG (Table 3). Similar results were found by Hassawi *et al*. (2005), who observed genotypic effect on albino plant regeneration. Andersen *et al*. (1988), and Sharma *et al*. (2018) stated that the formation of albino plantlets was genetically and environmentally controlled. In addition to the effect of genotype and the duration of maintaining calli in culture, the development stage of microspore at inoculation time as well as the chloroplast DNA deletions may affect occurrence of albino plantlets (Liang *et al*., 1990). Sun *et al*. (1979), and Mohiuddin *et al.* (2014) reported that the basic cause of albinism in rice is impairment of DNA (probably due to presence of chemicals added to the media) in plastids or nuclei or in both. It also identified the absence of ribosomes in ill-developed plastids as another cause of albinism in rice. Biswas *et al*. (2002) stated that probably due to the interference of PEG in proplastid biosynthesis during morphogenesis. The results of the present study are in accordance with previous reports of Zamani *et al.* (2003), El-Hennawy *et al.* (2011), and Mon *et al.* (2020), who indicated that the genotype played an important role in anther culture. Furthermore, the three crosses ($L_1 \times T_2$,

$L_3 \times T_1$ and $L_1 \times T_3$) with the highest response in anther culture had parent that exhibited very good response under the four stress levels.

Table (3): Effect of polyethylene glycol 6000 (PEG) concentrations on anther culture response of 24 F_1 rice crosses and their respective parents.

Genotypes	PEG-stress levels (g/l)									
	Green plant regeneration (%)					Albino plant regeneration (%)				
	Control	10	20	30	Comb.	Control	10	20	30	Comb.
Giza 177 (L_1)	6.33	5.67	4.67	3.00	4.67	4.33	3.67	3.00	3.00	3.50
Giza 178 (L_2)	3.00	3.00	2.92	2.67	2.92	3.67	3.67	3.00	2.67	3.25
Sakha 104 (L_3)	6.33	5.33	4.83	3.33	4.67	3.33	2.67	2.67	2.00	2.67
Sakha 107 (L_4)	6.00	3.67	3.67	2.33	3.67	3.00	2.67	2.33	2.00	2.50
IRRI-148 (T_1)	5.67	5.00	3.67	2.00	3.67	2.33	2.00	1.67	1.00	1.75
IRAT-112 (T_2)	3.67	2.67	2.67	2.33	2.83	3.33	2.67	1.67	1.33	2.25
IRGC (T_3)	3.67	2.33	1.67	1.67	2.25	2.00	2.00	1.33	1.00	1.58
N.22 (T_4)	1.33	1.33	1.25	1.00	1.25	4.00	2.67	2.67	1.33	2.67
IRRI-163 (T_5)	3.00	2.67	2.17	1.33	2.17	2.33	1.67	1.67	1.00	1.67
AZUCENA (T_6)	2.67	2.33	2.00	2.00	2.17	2.67	2.33	1.33	1.00	1.83
$L_1 \times T_1$	8.00	6.00	4.33	3.67	5.50	3.67	1.67	1.33	1.00	1.92
$L_1 \times T_2$	12.00	7.33	6.33	5.33	7.75	3.00	2.67	2.33	0.67	2.17
$L_1 \times T_3$	7.67	6.00	6.00	4.33	5.83	3.67	3.33	1.33	0.67	2.25
$L_1 \times T_4$	6.67	6.33	5.00	4.33	5.00	5.00	3.33	2.67	2.67	3.42
$L_1 \times T_5$	7.67	5.67	2.67	2.33	4.58	2.67	2.67	2.00	2.33	2.42
$L_1 \times T_6$	5.67	4.67	4.00	3.67	4.50	4.67	2.67	2.00	1.67	2.75
$L_2 \times T_1$	3.67	3.00	2.67	2.00	2.83	1.33	1.33	0.33	0.33	0.83
$L_2 \times T_2$	5.00	3.33	3.00	2.33	3.42	2.00	1.00	1.00	0.67	1.17
$L_2 \times T_3$	6.67	5.33	4.00	3.00	4.75	3.33	1.67	1.33	1.33	1.92
$L_2 \times T_4$	7.00	5.00	4.33	3.33	4.92	2.33	1.33	1.00	1.00	1.42
$L_2 \times T_5$	6.33	5.00	3.33	2.00	4.17	2.00	1.00	1.00	1.00	1.25
$L_2 \times T_6$	5.00	3.67	3.67	3.33	3.92	1.67	1.67	1.00	0.33	1.17
$L_3 \times T_1$	11.00	7.00	5.33	4.67	7.00	1.67	1.33	1.00	0.33	1.08
$L_3 \times T_2$	6.00	4.67	4.00	3.00	4.42	3.00	2.67	1.00	1.00	1.92
$L_3 \times T_3$	6.67	4.67	3.67	2.67	4.42	2.00	1.33	0.67	0.67	1.17
$L_3 \times T_4$	3.33	2.67	2.33	1.67	2.50	1.33	1.00	0.67	0.67	0.92
$L_3 \times T_5$	4.00	3.33	3.00	2.67	3.33	1.67	1.33	1.00	1.00	1.25
$L_3 \times T_6$	6.33	5.00	4.00	3.33	4.67	3.67	1.67	1.33	0.67	1.83
$L_4 \times T_1$	4.33	3.00	2.67	1.67	2.92	1.33	1.33	2.33	1.00	1.50
$L_4 \times T_2$	4.67	3.33	3.00	2.33	3.33	1.33	1.33	1.00	0.67	1.08
$L_4 \times T_3$	5.00	3.67	3.33	2.67	3.67	1.67	1.67	1.33	1.00	1.42
$L_4 \times T_4$	5.67	5.00	4.00	3.00	4.42	3.00	2.00	1.33	1.33	1.92
$L_4 \times T_5$	3.67	3.67	2.67	2.00	3.00	1.67	1.33	0.67	0.67	1.08
$L_4 \times T_6$	3.33	3.33	3.00	3.00	3.17	1.67	1.33	1.00	1.00	1.25
Mean	5.43	4.22	3.38	2.76	3.95	2.66	2.02	1.53	1.18	1.85
L.SD 0.05 PEG Levels (T) L.SD 0.01	-	-	-	-	0.07 0.10	-	-	-	-	0.07 0.10
L.SD 0.05 Genotypes (G) L.SD 0.01	0.69 0.91	0.70 0.92	1.13 1.49	1.02 1.34	0.49 0.65	0.44 0.57	0.42 0.55	0.42 0.56	0.40 0.53	0.28 0.37
L.SD 0.05 Interaction (T×G) L.SD 0.01	-	-	-	-	0.23 0.31	-	-	-	-	0.23 0.31

These results indicate that one high responding parent could be used to generate responding F_1 hybrids, although there is no guarantee of a high response in the hybrids because the inheritance of an anther culture response may be more complicated (Masojc *et al.*, 1993). Zamani *et al.* (2003) also reported that a well-responding parent could lead to the production of sufficient green plants for breeding purposes. In addition, the data obtained from this study indicate that

hybrids originating from one parent with very good or intermediate performance in anther culture would be of value for developing an *in vitro* system with a high production of green plants under the drought stress conditions.

3.2 General and specific combining abilities for callus induction and plant regeneration under drought stress treatments in rice

Information on the inheritance or combining ability of plant regeneration in anther culture is of great importance in an attempt to increase the efficiency of anther culture (Can and Yoshida 1999). Hou *et a1*. (1994) reported that by understanding the inheritance patterns of anther culture response, breeders could improve the procedures by crossing highly responsive with non-responsive genotypes and could predict the level of response of the hybrids or optimize the allocation of resources for doubled haploid production. The analysis of variance for the combining ability of different concentrations of polyethylene glycol (PEG) for all *in vitro* studied traits is presented in Table (4). Results revealed that variance due to general combining ability (GCA) was highly significant for callus induction under control, 10, 20 g/l PEG and the combined analysis. Furthermore, variance due to specific combining ability (SCA) was highly significant for all *in vitro* traits tested under the four stress levels and their combined analysis except albino plant regeneration under the stress levels of 10, 20 and 30 g/l PEG as well as combined data. These results indicate the importance of both additive and non-additive gene action in the expression of these traits.

Table (4): Mean squares of single (S) and combined (Comb.) analysis for general and specific combining abilities of F_1 rice crosses and their parents for the *in vitro* studied traits under drought stress levels (L).

Source of variation	d.f.		PEG - stress levels g/l									
	S	Comb.	Callus induction (%)					Plant regeneration (%)				
			Control	10	20	30	Comb.	Control	10	٢٠	30	Comb.
σ^2 GCA	9	9	0.047**	0.156**	0.127**	0.022	1.366**	0.005	0.003	0.002	0.003	0.146**
σ^2 SCA	23	23	5.026**	2.241**	3.117**	1.673**	1.579**	0.400**	0.153**	0.091**	0.075**	0.159**
σ^2 GCA × L	-	27	-	-	-	-	0.004	-	-	-	-	0.002
σ^2 SCA × L	-	69	-	-	-	-	1.913**	-	-	-	-	0.026*
Error	297	1188	0.014	0.017	0.017	0.016	0.016	0.024	0.022	0.018	0.013	0.020
σ^2 GCA /σ^2 SCA	-	-	0.009	0.070	0.041	0.013	0.865	0.013	0.019	0.021	0.040	0.915
(σ^2 GCA × L) / σ^2GCA	-	-	-	-	-	-	0.003	-	-	-	-	0.013
(σ^2SCA × L) / σ^2 SCA	-	-	-	-	-	-	1.211	-	-	-	-	0.165
Source of variation	d.f.		PEG - stress levels g/l									
	S	Comb.	Green plant regeneration (%)					Albino plant regeneration (%)				
			Control	10	20	30	Comb.	Control	10	20	30	Comb.
σ^2 GCA	9	9	0.005	0.002	0.001	0.002	0.085**	0.004	0.002	0.001	0.001	0.066**
σ^2 SCA	23	23	0.341**	0.102**	0.066**	0.044**	0.122**	0.034*	0.002	0.002	0.004	0.010
σ^2 GCA × L	-	27	-	-	-	-	0.001	-	-	-	-	0.003
σ^2 SCA × L	-	69	-	-	-	-	0.038**	-	-	-	-	0.001
Error	297	1188	0.019	0.025	0.021	0.018	0.021	0.019	0.018	0.020	0.017	0.019
σ^2 GCA /σ^2 SCA	-	-	0.015	0.019	0.015	0.045	0.694	0.118	0.975	0.500	0.250	6.535
(σ^2 GCA × L) / σ^2GCA	-	-	-	-	-	-	0.017	-	-	-	-	0.030
(σ^2SCA × L) / σ^2 SCA	-	-	-	-	-	-	0.311	-	-	-	-	0.050

* and ** denote significant at 0.05 and 0.01 levels of probability, respectively.

However, the ratio of GCA/SCA was found to be less than unity for all *in vitro* studied traits under the four stress levels, revealing the predominant role of dominance gene action in the inheritance of these traits. He *et al.* (2006), and Al-Ashkar (2014) also reported similar results for all *in vitro* traits. On the other hand, Yan *et al.* (1996), and Bagheri and jelodar (2008), found that general combining ability variances were greater than specific combining ability ones for callus formation in rice. The ratio for SCA × PEG stress levels / SCA was higher than GCA × PEG stress levels / GCA for all traits studied. These results indicated that non-additive genetic effects were much more influenced by the drought stress levels than additive genetic effects in these traits. Such results indicate that PEG stress treatments are considered as an effective factor for declaring GCA and SCA. Thus, the breeder should utilize the appropriate breeding method under each drought stress for developing desired rice genotypes. Similar results were obtained by Gholizadeh *et al*. (2018). Estimates of general combining ability effects for each parent under PEG stress treatments are presented in Table (5).

Table (5): Estimates of general combining ability effects of ten rice parents tested for the *in vitro* studied traits under four drought stress treatments.

Parents	PEG - stress levels (g/l)							
	Callus induction (%)				plant regeneration (%)			
	Control	10	20	30	Control	10	20	30
Lines								
Giza 177 (L_1)	0.762**	1.467**	1.658**	1.142**	0.558**	0.388**	0.371**	0.383**
Giza 178 (L_2)	-0.321**	-0.933**	-0.992**	-0.608**	-0.092	-0.029	-0.129*	-0.117*
Sakha 104 (L_3)	-0.988**	-0.100*	-1.142**	-0.492**	0.042	-0.013	-0.013	-0.067
Sakha 107(L_4)	0.546**	0.433**	0.475**	-0.042	-0.508**	-0.346**	-0.229**	-0.200**
SE (gi)	0.047	0.053	0.053	0.052	0.063	0.060	0.054	0.047
SE (gi- gj)	0.067	0.075	0.075	0.074	0.090	0.085	0.076	0.067
Testers								
IRRI-148 (T_1)	1.079**	2.192**	0.317**	-0.792**	0.092	0.013	-0.038	-0.083
IRAT-112 (T_2)	-1.246**	-1.608**	-1.158**	-0.342**	0.267**	0.263**	0.113	0.067
IRGC (T_3)	1.829**	1.467**	2.342**	1.108**	0.292**	0.188*	0.113	0.067
N.22 (T_4)	0.829**	-0.683**	- 0.008	0.383**	-0.008	-0.087	0.037	0.192**
IRRI-163 (T_5)	-1.896**	-1.233**	-0.783**	-0.217**	-0.358**	-0.288**	-0.188**	-0.158**
AZUCENA (T_6)	-0.596**	-0.133*	-0.708**	-0.142*	-0.283**	-0.087	-0.038	-0.083
SE (gi)	0.058	0.065	0.065	0.064	0.078	0.073	0.067	0.058
SE (gi- gj)	0.082	0.091	0.092	0.090	0.110	0.104	0.094	0.082
Parents	PEG - stress levels (g/l)							
	Green plant regeneration (%)				Albino plant regeneration (%)			
	Control	10	20	30	Control	10	20	30
Lines								
Giza 177 (L_1)	0.567**	0.417**	0.288**	0.279**	0.392**	0.283**	0.200**	0.154**
Giza 178 (L_2)	-0.133*	-0.117	-0.079	-0.104	-0.108	-0.133*	-0.100	-0.063
Sakha 104 (L_3)	0.050	-0.017	-0.013	-0.004	-0.075	-0.067	-0.100	-0.079
Sakha 107(L_4)	-0.483**	-0.283**	-0.196**	-0.171**	-0.208**	-0.083	0.000	-0.013
SE (gi)	0.055	0.064	0.059	0.062	0.057	0.055	0.058	0.053
SE (gi- gj)	0.077	0.091	0.083	0.088	0.081	0.078	0.082	0.075
Testers								
IRRI-148 (T_1)	0.208**	0.042	-0.004	-0.004	-0.142*	-0.108	-0.008	-0.096
IRAT-112 (T_2)	0.258**	0.017	0.096	0.071	-0.042	0.042	0.017	-0.071
IRGC (T_3)	0.133*	0.092	0.146*	0.046	0.058	0.067	-0.033	-0.021
N.22 (T_4)	-0.117	0.042	0.021	0.021	0.133	0.042	0.042	0.129*
IRRI-163 (T_5)	-0.192**	-0.058	-0.229**	-0.229**	-0.142*	-0.058	-0.033	0.079
AZUCENA (T_6)	-0.292**	-0.133	-0.029	0.096	0.133	0.017	0.017	-0.021
SE (gi)	0.067	0.078	0.072	0.076	0.070	0.068	0.071	0.065
SE (gi- gj)	0.095	0.111	0.102	0.107	0.099	0.096	0.0101	0.092

* and ** denote significant at 0.05 and 0.01 levels of probability, respectively.

Results showed that the IRGC (T_3) followed by Giza 177 (L_1) were the best combiners for callus induction under varying concentrations of PEG due to their highly significant positive GCA values, revealing the great value of such parents as promising progenitors for high callus induction. On the other hand, the five parents; IRRI 163 (T_5), IRAT 112 (T_2), Azucena (T_6), Sakha 104 (L_3) and Giza 178 (L_2) showed negative general combining ability effects for this trait under drought stress conditions. For plant regeneration, one parental genotype Giza 177 (L_1) was the best combiner for plant regeneration under varying concentrations of PEG due to their highly significant positive GCA values, revealing the great value of such parents as promising progenitors for plant regeneration. On the other hand, IRRI 163 (T_5), Sakha 107 (L_4) and Azucena (T_6) had poor general combiners as they showed negative GCA effects in the four stress treatments. For green plant regeneration, positive general combining ability effects were detected for Giza 177 (L_1) and IRGC (T_3) under control and some stress levels. On the other hand, Sakha 107 (L_4) and IRRI 163 (T_5) had poor general combiners as they showed negative GCA effects in the four stress treatments. For albino plant regeneration, positive general combining ability effects were detected for Giza 177 (L_1) under the four stress levels. On the other hand, Giza 178 (L_2) and Sakha 107 (L_4) had poor general combiners as they showed negative GCA effects under drought stress conditions. Specific combining ability effects (SCA) for each cross are shown in Table (6). Results indicated that 3 out of 24 crosses showed highly significant and positive specific combining ability effects for callus induction under the four stress levels (control, 10, 20 and 30 g/l PEG). They included three types of combinations; high × high, low × high and low × low general combining ability effects. The significant desirable SCA effects were obtained in 3 out of 24 F_1 crosses for plant regeneration under the four stress levels. The three crosses; $L_3 \times T_1$, $L_1 \times T_2$ and $L_4 \times T_4$ recorded the highest SCA effects for plant regeneration under the four stress levels. Therefore, these crosses could be of great value for a rice anther culture selection system in breeding programs. For green plant regeneration, highly significant and positive SCA effects were detected in the two crosses; $L_3 \times T_1$ and $L_1 \times T_2$ under drought stress conditions. In contrast, negative specific combining ability effects were observed in seven crosses for this trait under the four stress levels. It is worthy to note that it is not necessary that parents having high estimates of GCA effects would also give high estimates of SCA effects. In the cross ($L_4 \times T_3$) for callus induction under control and 20 g/l PEG and in the cross ($L_1 \times T_3$) for plant regeneration under the 10 g/l PEG, both parents involved had high GCA effects, but gave comparatively very low SCA effects. El-Hennawy *et al*. (2016) found that low SCA effects in such cases might be attributed to some

internal cancellation of favourable factors or to genetic similarity of the involved parents.

Table (6): Estimates of specific combining ability effects of 24 F_1 rice crosses tested for the *in vitro* studied traits under four drought stress treatments.

Crosses	PEG - stress levels (g/l)							
	Callus induction (%)				Plant regeneration (%)			
	Control	10	20	30	Control	10	20	30
$L_1 \times T_1$	0.888**	1.008**	-0.283*	0.108	-0.258	0.138	-0.046	-0.183
$L_1 \times T_2$	-1.188**	-1.492**	-1.508**	-0.942**	0.967**	0.588**	0.404**	0.367**
$L_1 \times T_3$	1.138**	2.033**	2.992**	2.308**	0.442**	-0.138	0.104	0.167
$L_1 \times T_4$	-2.863**	-0.917**	0.342**	-0.367**	-0.258	0.038	0.079	0.142
$L_1 \times T_5$	-0.137	-0.167	-1.683**	-0.867**	-0.508**	-0.463**	-0.296*	-0.308**
$L_1 \times T_6$	2.163**	-0.467**	0.142	-0.242	-0.383*	-0.163	-0.246	-0.183
$L_2 \times T_1$	3.971**	1.808**	1.367**	1.058**	-0.808**	-0.646**	-0.346**	-0.183
$L_2 \times T_2$	-2.804**	-0.292*	0.442**	-0.092	-0.383*	-0.196	-0.196	-0.233*
$L_2 \times T_3$	0.421**	-1.667**	-2.558**	-2.042**	0.092	0.279	0.004	-0.033
$L_2 \times T_4$	-0.679**	0.383**	1.592**	2.083**	0.592**	0.254	0.179	0.142
$L_2 \times T_5$	-0.154**	0.133	-0.433**	-1.017**	0.642**	0.354*	0.204	0.192
$L_2 \times T_6$	-0.754**	-0.367**	-0.408**	0.008	-0.133	-0.046	0.154	0.117
$L_3 \times T_1$	-1.463**	-1.425**	-2.583**	-0.858**	1.258**	0.638**	0.538**	0.467**
$L_3 \times T_2$	2.063**	2.575**	1.292**	0.892**	-0.317*	-0.113	-0.012	0.017
$L_3 \times T_3$	-0.713**	-2.000**	0.392**	0.242	-0.442**	-0.138	-0.213	-0.183
$L_3 \times T_4$	0.688**	0.750**	-1.858**	-1.733**	-0.742**	-0.663**	-0.638**	-0.608**
$L_3 \times T_5$	0.813**	-0.100	1.217**	0.767**	-0.292	-0.063	0.088	0.142
$L_3 \times T_6$	-1.388**	0.200	1.542**	0.692**	0.533**	0.338*	0.238	0.167
$L_4 \times T_1$	-3.396**	-1.392**	1.500**	-0.308*	-0.192	-0.129	-0.146	-0.100
$L_4 \times T_2$	1.929**	-0.792**	-0.225	0.142	-0.267	-0.279	-0.196	-0.150
$L_4 \times T_3$	-0.846**	1.633**	-0.825**	-0.508**	-0.092	-0.004	0.104	0.050
$L_4 \times T_4$	2.854**	-0.217	-0.075	0.017	0.408**	0.371*	0.379**	0.325**
$L_4 \times T_5$	-0.521**	0.133	0.900**	1.117**	0.158	0.171	0.004	-0.025
$L_4 \times T_6$	-0.021	0.633**	-1.275**	-0.458**	-0.017	-0.129	-0.146	-0.100
SE (sij)	0.116	0.132	0.125	0.128	0.155	0.147	0.133	0.116
SE (sij-sik)	0.164	0.186	0.177	0.181	0.220	0.208	0.189	0.164
Crosses	PEG - stress levels (g/l)							
	Green plant regeneration (%)				Albino plant regeneration (%)			
	Control	10	20	30	Control	10	20	30
$L_1 \times T_1$	-0.192	-0.042	-0.113	-0.079	0.108	-0.208	-0.175	-0.054
$L_1 \times T_2$	0.958**	0.383*	0.388**	0.346**	-0.192	-0.058	0.100	-0.179
$L_1 \times T_3$	-0.217	-0.092	0.238	0.071	-0.092	0.117	-0.150	-0.229
$L_1 \times T_4$	-0.267*	0.058	0.063	0.096	0.233	0.142	0.175	0.221
$L_1 \times T_5$	0.108	-0.042	-0.388**	-0.254	-0.192	0.042	0.050	0.171
$L_1 \times T_6$	-0.392**	-0.267	-0.188	-0.179	0.133	-0.033	0.000	0.071
$L_2 \times T_1$	-0.792**	-0.408**	-0.246	-0.196	-0.092	0.108	-0.175	-0.038
$L_2 \times T_2$	-0.442**	-0.283	-0.246	-0.171	0.008	-0.142	0.000	0.038
$L_2 \times T_3$	0.183	0.242	0.004	0.054	0.308*	0.033	0.150	0.188
$L_2 \times T_4$	0.533**	0.192	0.229	0.179	-0.067	-0.042	-0.025	-0.063
$L_2 \times T_5$	0.408**	0.292	0.179	0.029	0.108	-0.042	0.050	-0.013
$L_2 \times T_6$	0.108	-0.033	0.079	0.104	-0.267	0.083	0.000	-0.113
$L_3 \times T_1$	1.225**	0.692**	0.488**	0.504**	-0.025	0.042	0.025	-0.021
$L_3 \times T_2$	-0.325*	0.017	-0.013	-0.071	0.275*	0.292*	0.000	0.154
$L_3 \times T_3$	0.000	-0.058	-0.163	-0.146	-0.125	-0.133	-0.050	0.004
$L_3 \times T_4$	-0.750**	-0.608**	-0.538**	-0.421**	-0.400**	-0.208	-0.125	-0.146
$L_3 \times T_5$	-0.475**	-0.308	0.113	0.129	-0.025	-0.008	0.050	0.004
$L_3 \times T_6$	0.325*	0.267	0.113	0.004	0.300*	0.017	0.100	0.004
$L_4 \times T_1$	-0.242	-0.242	-0.129	-0.229	0.008	0.058	0.325*	0.113
$L_4 \times T_2$	-0.192	-0.117	-0.129	-0.104	-0.092	-0.092	-0.100	-0.013
$L_4 \times T_3$	0.033	-0.092	-0.079	0.021	-0.092	-0.017	0.050	0.038
$L_4 \times T_4$	0.483**	0.358*	0.246	0.146	0.233	0.108	-0.025	-0.013
$L_4 \times T_5$	-0.042	0.058	0.096	0.096	0.108	0.008	-0.150	-0.163
$L_4 \times T_6$	-0.042	0.033	-0.004	0.071	-0.167	-0.067	-0.100	0.038
SE (sij)	0.134	0.157	0.144	0.134	0.140	0.136	0.142	0.130
SE (sij-sik)	0.190	0.222	0.204	0.189	0.198	0.192	0.201	0.183

* and ** denote significant at 0.05 and 0.01 levels of probability, respectively.

On the contrary, the cross ($L_3 \times T_2$) involving parents with very low GCA effects for callus induction and the cross ($L_2 \times T_5$) involving parents with very low GCA effects for plant regeneration, recorded high SCA effects for these traits under the control and10 g/l PEG, which might be due to high genetic diversity among the parents. Moreover, the parents having low GCA effects had a relatively high magnitude of non-additive gene effects and thus resulted in high SCA effects when crossed. Regarding albino plant regeneration, negative SCA effects were obtained in three out of 24 F_1 crosses under the four stress levels. On the contrary, the four crosses; $L_2 \times T_3$, $L_1 \times T_4$, $L_3 \times T_6$ and $L_4 \times T_1$ gave positive SCA effects for this trait under the drought stress levels. Silva (2010), and Tripathy (2018) reported that quantitative inheritance of anther culture response could complicate the selection process. Combining ability analysis provides a guideline to the breeder in selecting the suitable parents and desirable cross combinations to be used in the formulation of systematic breeding program for the improvement of quantitative characters (Dar *et al*., 2014; Kalhoro *et al*., 2015). Evaluating a good combination depends on both the values of GCA and SCA. A cross combination, which has high SCA value and good parental GCA effects, may be considered as a suitable cross combination. For instance, the cross combination $L_1 \times T_3$ is considered to be promosing cross for varietal improvement purpose, as they showed significant positive SCA effect for callus induction under the four stress levels and the cross combination $L_1 \times T_2$ is considered to be promosing cross for varietal improvement purpose, as they showed significant positive SCA effect for plant regeneration under the four stress levels (Table 6) and involved two good general combiner parents (Table 5). In such cases, it would be expected that diverse genes contributing to the better general combining ability effects of the parents are available in the crosses and in the segregating generations, these are likely to give transgressive segregates. Therefore, GCA and SCA effects should be taken into account generally, when developing the strategy of the selection of genotypes for obtaining cross combination with high callus induction and high green plant regeneration ability under drought stress conditions.

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