



COMBINING ABILITY STUDIES USING LINE \times TESTER ANALYSIS FOR GERMINATION TRAITS IN HYBRID RICE UNDER STRESS CONDITIONS

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SUMMARY

The hybrid rice breeding for tolerance to drought is one of the important ways to reduce yield loss due to drought stress. Therefore, an experiment was conducted to estimate the combining ability and to determine the gene action for drought tolerance at germination stage. The genetic materials were thirty F₁ hybrid rice genotypes which were developed from six cytoplasmic male sterility (CMS) lines and five restorer testers. The germinating seed of parents and F₁ were subjected to drought stress simulation in 25% PEG 6000 for 7 days. The germination traits i.e. germination percentage, a length of seminal root, shoot, and seedling length, a dry weight of seminal root, a dry weight of shoot, and vigor seed index were measured. General and specific combining the ability of germination traits and genetic parameters were analyzed using line \times tester analysis. The general combining ability was significantly different among five drought tolerant traits. The specific combining ability of these traits was significantly different for all traits. Among the cytoplasmic male sterility (CMS) lines, IR 58025A was a good combiner for relative percentage of germination, while IR 80154A was a good combiner for relative seminal root and seedling length. Among the tester, PK 90 had been a good combiner for germination percentage and index vigor. Hybrids IR 80154A/PK 90, GMJ 13A/R 3, GMJ 14A/R 3, and GMJ 15A/PK 90 had the significantly and positive specific combining ability for all traits. The analysis revealed that the traits i.e. germination percentage, a length of seminal root, shoot, seedling length, and vigor seed index indicated dominance of non-additive genetic variance.

Key words: Line \times tester, combining ability, genetic parameters, hybrid rice, drought, germination

Key findings: This study revealed the potential traits i.e. germination percentage, a length of seminal root, shoot, seedling length, and vigor seed index for breeding of hybrid rice tolerance to drought.

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the important food crops in Asia and it is a staple food for more than half of the world's population. The average Indonesian consumption is about 139

kilograms of rice a year and the country's population will expect increase with a rate of 1.5% per year. Abiotic stresses, especially drought stress is a serious problem that disturbs improvement of crop production. It is one of the major causes of crop loss, which reduces

average yield for rice production by more than 50% (Bouman *et al.*, 2007).

Stages of germination and seedling growth is a critical growth stage affected by drought stress (Ahmad *et al.*, 2009). Interference at this stage can govern the grain yield. Rice could expose into different drought tolerance at the different growth stage. Several researchers who focus more on germination stage (Lestari and Mariska, 2006; Jiang and Lafitte, 2007; Ballo *et al.*, 2012) were reported that index of seed vigor and shoots length are the most sensitive traits to drought stress, followed by the length of root and coleoptile length. The rate of seed germination and final germination percentage decrease dramatically due to osmotic stress (Meutia *et al.*, 2010; Afa *et al.*, 2013).

The selection of drought tolerance at an early seedling stage is frequently accomplished using simulated drought induced by chemicals like polyethylene glycol (PEG 6000). PEG 6000 can be used to induce dehydration by decreasing the water potential of the nutrient solution (Mirbahar *et al.*, 2013). Govindaraj *et al.* (2010) revealed that molecules of PEG impermeable to cell membranes and can induce uniform water stress without causing direct physiological damage. Therefore, the method of simulated drought stress of polyethylene glycol (PEG-6000) hypertonic solution that was inert, non-ionic, simple, reproducible, short in test, and suitable for the early identification of drought resistance of large quantities of lines (Badiane *et al.*, 2004; Verslues *et al.*, 2006; Jiang and Lafitte, 2007; Boopathi *et al.*, 2013).

The combining ability was one of the main strategies in rice heterosis utilization. The traits such as yield (Chakraborty *et al.*, 2009; Dar *et al.*, 2014), grain quality (Adilakshmi and Upendra, 2014), and drought tolerance (Muthuramu *et al.*, 2010) were observed. However, there are a few reports available on combining ability of drought tolerance in germination traits. Researchers indicated that the relative importance of general combining ability (GCA) and specific combining ability (SCA) varied from different traits. The greater magnitude of variance due to GCA than SCA for any traits indicated a predominant role of additive gene action (Dorosti and Monajjem, 2014). Sometimes both additive and non-

additive gene action are involved in the inheritance of traits (Ghosh *et al.*, 2012). Understanding such relationships and their genetic basis would be useful in the development of effective breeding strategies to improve hybrid rice genotypes for drought tolerant. Therefore, a research program was planned to study the combining ability of parental lines, testers, and their F₁ hybrids rice and genetic parameters of germination traits under non-stress and drought stress conditions.

MATERIALS AND METHODS

Cytoplasmic male sterility (CMS) as lines and five Restorer as testers crossed under lines x testers mating design at Field Station Indonesian Center for Rice Research (ICRR) from November 2014 – February 2015. Three CMS lines (IR58025A, IR80154A, and IR80156A) and 2 restorers (R3 and R32) were obtained from the International Rice Research Institute (IRRI). Other CMS (GMJ13A, GMJ14A, and GMJ15A) and 3 restorers (PK90, PK12, and BP11) were developed by Indonesian Agency for Agricultural Research and Development IAARD. The 30 F₁s and 11 parents were grown in a randomized complete block design (RCBD) with three replications and evaluated from February – March 2015 at glass house of Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD).

Rice seeds (*Oryza sativa* L.) were surface sterilized with distilled water several times and then soaked in distilled water for 72 hours. Before the germination experiment, the uniform seeds were selected, washed several times with distilled water and soaked for 72 hours. For each genotype, 100 selected soaked grains were placed in 9 cm diameter of the culture dish, then cultured under indoor natural lighting. The soaked seeds were treated with 25% of PEG (6000 MW, m/v) and distilled water as a control. According to Afa *et al.* (2012), the osmotic potential of 25% PEG 6000 is approximate -9.9 Bar. In the PEG treatment, 10 ml PEG solution was added to the culture dish for 7 days. The experiment was repeated 3 times. The germination standard was defined to

be the time when the root length reached ± 2 mm. In the 8th day, 20 germinated seeds were selected from each culture dish to measure the germination traits. The germination traits studied were: germination percentage (%), seminal root length (cm), shoot length (cm), seedling length (cm), dry weight of seminal root (g), and dry weight of shoot (g).

The data recorded were subjected to analysis of variance according to Steel and Torrie (1980) to determine significant differences between the treatments and genotypes. All the germination traits were converted into relative value after variance analysis i.e. Relative percentage of trait $x = (\text{Value of trait } x \text{ under drought stress} / \text{Value of trait } x \text{ in non-stress}) \times 100$. The general combining ability (GCA) of parental lines and specific combining ability (SCA) of F_1 hybrids germination traits were calculated using standard statistical and biometrical method for combining ability (Singh and Chaudary, 1979) based on a value of relative germination traits. Statistical analysis was conducted using Excel and PBTtools from IRRI.

RESULTS

Mean squares from analysis of variance showed significant effects for drought stress treatment using 25% PEG 6000 on rice genotypes and traits including germination percentage, seminal root length, shoot length, seedling length, dry weight of seminal root, and dry weight of shoot (Table 1). The interactions of treatment \times genotype for all the traits were also significant except dry weight of seminal root that implied variable response of genotypes over the drought stress condition for various traits.

Responses to drought at the germination traits

The germination traits were observed at drought stress treatment using PEG 6000 concentration 25%. PEG 6000 (25%) induced water stress adversely and affected germination growth of parental lines of rice. There were differences among the parental lines on their response to germination traits under water stress. The mean

performance of parents and F_1 hybrids for germination traits is presented in Table 2. For germination percentage and seminal root length traits, all the parents showed maximum declines due to water stress, except the lines i.e. IR80154A and IR80156A and the testers PK90 and R3. In F_1 hybrids, the crosses IR80154A/PK90 and GMJ15A/PK90 showed minimum reductions in normal and drought conditions for germination percentage and seminal root length traits. The parents and F_1 hybrids that have minimum reduction indicating their being relatively more tolerant as compared to other genotypes in the test.

The relative germination traits and combination abilities

Mean square of hybrids (crosses) were significantly different in all traits (Table 3). Although among parents, the differences were significant only in relative germination percentage, relative seminal root length, and vigor index. It were indicated that they are suitable for genetic studies. The significant mean squares of parents versus hybrids in all studied traits, except for relative shoot length indicates significant heterosis for these traits. The significant variance of line \times tester interaction indicated the importance of specific combining ability (SCA).

Contributions of lines, testers, interaction of line \times tester for all germination traits are presented in Table 4. Lines has the significant role in the breeding of hybrid rice, especially for the traits of relative germination percentage, relative seminal root length, relative shoot length, and relative seedling length. This result indicated the high maternal effect on these traits and improvement is possible in all the traits due to male sterile lines. Tester has an important role for traits of a relative dry weight of shoot and relative vigor index, indicated the dominant influence of R line on the traits. Result revealed that the general combining ability variance ($\sigma^2\text{GCA}$) was lower than $\sigma^2\text{SCA}$ for most traits. It indicated predominance of non-additive gene action than additive gene action in the inheritance of these traits. In case of dry weight of shoot, both additive and non-additive gene action were important for control due to an

Table 1. Mean squares for germination traits of parents and F₁ hybrids of rice under non-stress and drought stress conditions.

Germination traits	Replication (d.f. = 2)	Treatment (T) (d.f. = 1)	Genotypes (G) (d.f. = 40)	T x G (d.f. = 40)	Error (d.f. = 162)
Germination percentage	121.65*	120741.87**	509.48**	505.62**	96.24
Seminal root length	0.70*	1344.24**	2.53**	1.97**	0.49
Shoot length	0.701**	1841.39**	0.66**	0.60**	0.13
Seedling length	0.38	6329.69**	3.61**	2.89**	0.7
Dry weight of seminal root	0.005*	3.32**	0.002*	0.001	0.0019
Dry weight of shoot	0.014**	5.44**	0.002**	0.002**	0.0008

** , * = Significant at 1 and 5% probability levels respectively.

equal magnitude of non-additive genetic variance and total genetic variance.

General combining ability (GCA)

GCA is the average behaviour level of hybrids from crosses of one parent to other parent and it controlled by additive effects of genes which can be inherited to offspring. In this research, significant differences were observed on GCA of some traits and genotypes (Table 5). In relative germination percentage, the effects of GCA were positive for IR58025A, GMJ15A, PK90, R3 (Table 5). In relative seminal root length, all the cytoplasmic male sterility (CMS) lines had significant GCA effects. Three CMS lines i.e. IR80154A, IR80156A, and GMJ13A were found to be positive and high general combiner for seminal root length. The highest GCA value for relative seedling length was observed in IR80154A (CMS line). The highest GCA value for the relative dry weight of shoot and relative vigor index were found in R3 and PK90 as restorer lines respectively. However, some traits showed small value of GCA such as relative shoot length and relative dry weight. According Mohammadi *et al.* (2014), small combining ability effect indicates poor ability to transfer genetic superiority to hybrids.

Based on GCA value, it revealed that IR58025A (CMS line) was the best general combiner for percentage of germination, while IR80154A was the best general combiner for seminal root length and seedling length. Among the restorer (testers), PK90 was the best general combiner for germination percentage and index

vigor, while R3 was the best general combiner for dry weight of shoot (Table 5). Therefore, IR58025A, IR80154A, PK90, and R3 could be the best candidate parents lines to be used for improving drought tolerance at the germination stage.

Specific combining ability (SCA)

Specific combining ability is the average behaviour level of hybrids from two parents and controlled by non-additive genes, and represents dominant, over dominant and epistatic of genes action. Among 30 F₁ hybrids rice for relative germination percentage, cross GMJ14A x R3 appeared highest positive effect value of SCA, followed by IR80156A x R32 and GMJ13A x R3 (Table 6). The positive and significant value of SCA for relative seminal root length was estimated of GMJ14A x R3, IR80154A x PK90, and GMJ13A x R3. Combination of GMJ14A x R3 had the highest positive SCA values among the crosses for relative shoot length and seedling length, followed by crosses IR80154A x PK90 and GMJ13A x R3. The hybrids GMJ14A x R3, IR80154A x PK90, and GMJ13A x R3 were the good specific combiners with positive GCA effects for relative dry weight of shoot and relative vigor index. Four hybrids revealed positive and significant SCA for all germination traits i.e. IR80154A x PK90, GMJ13A x R3, GMJ14A x R3, GMJ15A x PK90, and GM15A x PK12. SCA can not be inherited, and is only reflected by the interactions of allele or non-allelic from the special parents (Yong *et al.*, 2010).

Table 2. Mean performance of parents and F₁ hybrids for germination traits under non-stress and drought stress conditions.

Parents/F ₁ hybrids rice	Germination percentage (%)		Seminal root length (cm)		Shoot length (cm)		Seedling length (cm)		Dry weight of seminal root (g)		Dry weight of shoot (g)	
	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
Parents												
IR58025A	100	62	5.79	1.70	4.62	0.41	10.41	2.12	0.23	0.05	0.23	0.03
IR80154A	100	77	6.43	2.07	5.78	0.46	12.21	2.53	0.30	0.05	0.37	0.03
IR80156A	100	83	4.37	1.87	4.96	0.46	9.33	2.33	0.22	0.05	0.27	0.03
GMJ13A	100	42	6.25	1.57	5.09	0.38	11.34	1.94	0.27	0.03	0.28	0.01
GMJ14A	100	47	6.26	1.55	4.86	0.38	11.11	1.93	0.31	0.03	0.26	0.01
GMJ15A	100	58	5.41	1.39	5.96	0.42	11.37	1.80	0.33	0.05	0.35	0.02
PK90	100	87	5.53	2.96	6.17	0.51	11.70	3.47	0.33	0.05	0.40	0.02
R3	100	78	7.76	3.59	6.20	0.48	13.96	4.07	0.32	0.09	0.32	0.03
PK12	93	52	4.69	2.48	6.28	0.39	10.97	2.87	0.32	0.05	0.36	0.02
R32	100	43	7.07	1.49	5.22	0.41	12.30	1.90	0.28	0.04	0.31	0.02
BP11	100	43	6.64	1.53	5.74	0.42	12.39	1.95	0.29	0.08	0.33	0.03
CV (%)	3.50	30.38	15.49	34.04	8.90	10.71	10.12	28.99	14.68	57.24	10.75	41.46
LSD 5%	5.93	31.59	1.59	1.17	0.84	0.08	1.99	1.21	0.07	0.05	0.06	0.02
F ₁ hybrids rice												
IR58025A/PK90	100	77	5.39	1.60	5.71	0.46	11.09	2.06	0.26	0.04	0.31	0.02
IR58025A/R3	100	65	7.06	1.35	5.29	0.41	12.35	1.76	0.22	0.03	0.26	0.02
IR58025A/PK12	100	55	5.16	0.73	6.29	0.38	11.45	1.11	0.25	0.02	0.29	0.01
IR58025A/R32	100	70	6.34	1.54	5.35	0.44	11.69	1.98	0.26	0.04	0.30	0.02
IR58025A/BP11	100	65	7.51	1.40	5.64	0.43	13.15	1.83	0.25	0.03	0.29	0.02
IR80154A/PK90	100	83	5.28	1.85	6.45	0.47	11.74	2.32	0.23	0.05	0.36	0.02
IR80154A/R3	100	55	7.29	2.81	6.02	0.51	13.31	3.32	0.30	0.06	0.35	0.03
IR80154A/PK12	100	62	5.00	1.51	5.90	0.45	10.90	1.96	0.29	0.03	0.34	0.02
IR80154A/R32	100	17	6.41	2.06	6.22	0.42	12.63	2.48	0.29	0.03	0.33	0.02
IR80154A/BP11	100	62	8.08	1.97	6.38	0.44	14.46	2.42	0.29	0.03	0.37	0.02
IR80156A/PK90	100	53	6.22	1.68	6.77	0.53	12.99	2.22	0.31	0.05	0.37	0.02
IR80156A/R3	100	27	7.42	1.91	6.47	0.46	13.89	2.37	0.29	0.06	0.37	0.02
IR80156A/PK12	100	38	6.24	1.61	6.42	0.57	12.65	2.18	0.26	0.04	0.35	0.02
IR80156A/R32	100	48	5.63	1.49	5.74	0.31	11.37	1.80	0.27	0.03	0.30	0.02

NS = Non-stress, DS = Drought stress condition

(cont'd.)

Table 2. Mean performance of parents and F₁ hybrids for germination traits under non-stress and drought stress conditions.

Parents/F ₁ hybrids rice	Germination percentage (%)		Seminal root length (cm)		Shoot length (cm)		Seedling length (cm)		Dry weight of seminal root (g)		Dry weight of shoot (g)	
	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
F ₁ hybrids rice												
IR80156A/BP11	100	28	4.45	2.22	6.00	0.63	10.45	2.86	0.28	0.05	0.32	0.02
GMJ13A/PK90	100	63	6.10	2.02	6.02	0.43	12.12	2.46	0.24	0.04	0.28	0.03
GMJ13A/R3	100	75	8.75	2.03	5.97	0.41	14.72	2.44	0.33	0.06	0.33	0.03
GMJ13A/PK12	100	40	8.64	1.92	6.51	0.51	15.14	2.43	0.27	0.04	0.30	0.02
GMJ13A/R32	100	28	6.66	2.71	5.86	0.42	12.51	3.13	0.25	0.04	0.29	0.01
GMJ13A/BP11	100	30	7.82	2.70	5.83	0.43	13.65	3.13	0.26	0.04	0.27	0.02
GMJ14A/PK90	100	40	6.58	0.91	6.05	0.36	12.63	1.27	0.25	0.02	0.30	0.02
GMJ14A/R3	100	80	6.02	1.68	5.77	0.45	11.78	2.13	0.26	0.05	0.32	0.03
GMJ14A/PK12	100	42	6.78	2.20	5.96	0.48	12.74	2.68	0.24	0.04	0.28	0.03
GMJ14A/R32	100	32	6.18	1.23	5.60	0.52	11.78	1.75	0.31	0.04	0.31	0.01
GMJ14A/BP11	100	60	5.93	1.54	4.82	0.40	10.74	1.94	0.25	0.03	0.31	0.02
GMJ15A/PK90	100	80	7.99	1.56	4.98	0.41	12.96	1.96	0.28	0.03	0.33	0.02
GMJ15A/R3	100	65	7.77	1.72	6.88	0.43	14.65	2.15	0.27	0.06	0.35	0.02
GMJ15A/PK12	100	70	6.84	1.11	8.02	0.40	14.86	1.51	0.29	0.05	0.36	0.02
GMJ15A/R32	100	60	6.63	1.49	6.69	0.43	13.31	1.91	0.32	0.04	0.33	0.02
GMJ15A/BP11	100	35	7.70	1.64	5.89	0.39	13.59	2.03	0.27	0.05	0.31	0.02
CV (%)	0	20.56	12.55	21.27	7.75	20.21	7.99	17.92	21.89	27.39	11.14	28.71
LSD 5%	0	17.98	1.37	0.60	0.77	0.15	1.66	0.64	0.09	0.02	0.06	0.01

NS = Non-stress, DS = Drought stress condition

Table 3. Analysis of variance for line x tester analysis for relative germination traits.

Source of variation	Degree of freedom	Relative percentage germination (%)	Relative seminal root length (cm)	Relative shoot length (cm)	Relative seedling length (cm)	Relative dry weight of shoot (g)	Relative vigor index (%)
Replication	2	385.01	455.56**	0.94	126.96**	25.98*	17.43
Genotypes	40	1004.68**	298.40**	3.47*	70.37**	11.51*	72.57**
Parents	10	850.09*	393.21*	2.42	78.40	19.91	128.05**
P v H	1	1810.25**	1857.04**	4.56*	457.93**	38.23*	600.38**
F ₁ hybrids	29	1030.21**	211.96**	3.79*	54.23**	7.69*	35.24**
Lines	5	1472.94**	445.09**	3.93	111.93**	7.07	25.85**
Testers	4	1746.99**	93.65	1.27	34.40*	17.29**	65.16**
Lines x Testers	20	776.17**	177.34**	4.26*	43.78**	5.90	31.60**
Error	80	18.38	85.94	2.13	20.22	6.90	13.74

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

Table 4. Proportional contribution of lines, testers and their interactions to total variance in a set of line × tester crosses.

Traits	Line	Tester	Interaction L x T	Variance estimates and their ratio		
				σ^2 GCA	σ^2 SCA	σ^2 GCA/ σ^2 SCA
Relative germination percentage (%)	24.65	23.39	51.96	49.33	192.60	0.26
Relative seminal root length (cm)	36.21	6.09	57.70	8.29	30.47	0.27
Relative shoot length (cm)	17.89	4.62	77.49	0.03	0.71	0.05
Relative seedling length (cm)	35.58	8.75	55.67	2.29	7.85	0.29
Relative dry weight of shoot (g)	15.85	31.02	53.12	0.35	0.33	1.08
Relative vigor index (%)	12.65	25.50	61.85	1.16	5.96	0.19

DISCUSSION

Water stress induced drought is one of the most significant abiotic factors that limited seed germination, seedling growth, plant growth and grain yield (Jiang and Lafitte, 2007; Ji *et al.*, 2012). Rice is a sensitive crop to water stress and the variation in yield losses which depends on the duration of water stress and crop growth stage. Several methods have been developed to screen drought tolerant in a crop plant. Drought tolerance screening under field condition contains many resources (land, men power) and the environmental influences that affect phenotypic expression of the genotype. The in vitro screening method proves to be an ideal method to screen a large set of germplasm with less effort, accurately and the growth pattern differences are due to genotypes with least environmental influences (Khan *et al.*, 2013). The germination of seed under simulated

drought conditions offers possibilities for revealing seed weaknesses and predicting differences among seed in field emergence. One of the methods to identify promising genotypes is screening of seeds and seedlings to drought stress under simulated conditions using polyethylene glycol in solution cultures (Govindaraj *et al.*, 2010).

The use of PEG for the experimental control of external water potential has been verified to be effective method for studying the effect of water stress on seed germination and seedling growth characters (Afa *et al.*, 2012; Almaghrabi, 2012; Ballo *et al.*, 2012) and simple cost-effective method to screen large set of germplasm within very less time period and accurately (Govindaraj *et al.*, 2010). On the basis of the present investigation, imply that this technique can be adopted for rice as well other crops for early drought screening for fast track drought breeding program.

Table 5. Estimates of general combining ability (GCA) effects of parents (lines and testers) for relative germination traits.

Genotypes	Relative germination percentage	Relative seminal root length	Relative shoot length	Relative seedling length	Relative dry weight of shoot	Relative vigor index
Lines = Cytoplasmic Male Sterile						
IR58025A	12.74**	-5.48**	0.08	-2.66*	-0.4	0.8
IR80154A	2.07	5.62**	-0.02	2.52*	-0.22	1.9
IR80156A	-14.6**	4.68**	0.52	1.71	-0.41	-1.96
GMJ13A	-5.68**	4.07**	-0.14	2.81*	0.91	0.23
GMJ14A	-2.93	-2.36*	0.45	-0.88	0.81	-0.48
GMJ15A	8.4**	-6.53**	-0.9	-3.51*	-0.7	-0.49
SE (gi) lines	2.39	0.96	0.38	1.16	0.68	3.64
SE (gi-gi)	5.73	0.92	0.14	1.35	0.46	13.23
Testers = Restorer						
PK90	12.51**	-0.25	-0.02	-0.53	0.18	2.46*
R3	7.51**	-0.48	-0.08	0.27	1.17*	1.57
PK12	-2	-3.3	-0.14	-1.98	0.13	-1.44
R32	-11.1**	1.17	-0.22	0.47	-1.56*	-1.82
BP11	-6.93**	2.87	0.46	1.77	0.08	-0.76
SE (gi) testers	3.32	2.18	0.34	1.06	0.62	0.87
SE (gi-gi)	4.69	3.09	0.49	1.5	0.88	1.24

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

The survival rate of genotypes at germination stage has been used as one of the selection criteria to identify the donor parents (Swain *et al.*, 2014). In this study, wide variation was observed among the parental lines for the germination traits (Table 2). Under drought stress, germination and other traits decreased due to a shortage of water required for early processes of germination. In the previous study, reduction water potential was significantly decreased germination index, root length, shoot length, seedling length, seed vigor, a fresh and dry weight of shoot and root (Khan *et al.*, 2013).

The relative value of germination traits allowed genotype response to drought condition. The higher relative value indicated the higher ability of a genotype to adapt to drought stress (Ghebremariam *et al.*, 2013). In general, the tolerant genotypes having a good response to drought stress showed a higher relative percentage of germination, seminal root length, and vigor index than sensitive genotypes. The same result was described by Almaghrabi (2012) and Radhouane (2007) in their study on sorghum and millet. Information on the genetic mechanism of the desired traits supports breeders in making decisions on the parental

lines to be used and selected the effective breeding methods. In this study, the lines, testers, and F₁ hybrids were significant for almost all the germination traits in the stress treatments.

Contributions of maternal and paternal interaction (line x tester) showed high value for all germination traits. Interaction proportion of line x tester to the total variance is greater than the proportion of each line or tester, indicated that the variance in the hybrid population is caused by high GCA (Sarker *et al.*, 2002; Hassan *et al.*, 2014). The ratio of GCA/SCA variance was more than 1.0 for a relative dry weight of shoot. It was shown that both additive effects and non-additive effects influenced this trait. The GCA/SCA ratio was below 1.0 for relative germination percentage, relative seminal root length, relative shoot length, relative seedling length, and relative vigor index suggesting incomplete dominance and the involvement of non-additive gene action for this trait (Mohammadi *et al.*, 2014). Subhani and Chowdhry (2000) mentioned that additive genetic effects were more profound for germination traits than non-additive effects, however, it was contrasted with our results; the

Table 6. Estimates of specific combining ability (SCA) effects of crosses for relative germination traits.

F1 hybrids	Relative germination percentage (%)	Relative seminal root length (cm)	Relative shoot length (cm)	Relative seedling length (cm)	Relative dry weight of shoot (g)	Relative vigor index (%)
IR58025A/PK90	-2.18	8.45	0.48	4.29	0.9	1.84
IR58025A/R3	-8.85**	-1.81	0.34	-0.74	0.36	-2.31
IR58025A/PK12	-9.34**	-3.56	-1.26	-2.86	-0.79	-3.07
IR58025A/R32	14.76**	2.11	0.9	1.78	-0.23	3.73
IR58025A/BP11	5.6*	-5.19	-0.46	-2.48	-0.24	-0.2
IR80154A/PK90	15.15**	27.92**	27.69**	28.2**	27.62**	25.21**
IR80154A/R3	-8.18**	-0.18	-0.59	-0.94	-1.24	-2.23
IR80154A/PK12	8**	9.3	6.14**	7.98**	6.26**	7.44**
IR80154A/R32	-27.9**	-40.17**	-38.78**	-39.47**	-38.59**	-37.18**
IR80154A/BP11	12.93**	3.13	5.54**	4.23	5.94**	6.76**
IR80156A/PK90	1.82	14.58**	14.36**	14.86**	14.29**	11.88**
IR80156A/R3	-19.85**	-11.85**	-12.26**	-12.61**	-12.9**	-13.9**
IR80156A/PK12	1.33	2.64	-0.53	1.32	-0.4	0.78
IR80156A/R32	20.43**	8.17	9.55**	8.86**	9.74**	11.16**
IR80156A/BP11	-3.74	-13.54*	-11.12**	-12.44**	-10.72**	-9.91**
GMJ13A/PK90	2.9	15.66**	15.43**	15.94**	15.37**	12.96**
GMJ13A/R3	19.57**	27.56**	27.15**	26.8**	26.51**	25.51**
GMJ13A/PK12	-2.98	-1.68	-4.84**	-3	-4.72**	-3.54
GMJ13A/R32	-8.49**	-20.76**	-19.37**	-20.06**	-19.18**	-17.76**
GMJ13A/BP11	-10.99**	-20.79**	-18.38**	-19.69**	-17.98**	-17.16**
GMJ14A/PK90	-23.18**	-10.42*	-10.64**	-10.14**	-10.71**	-13.12**
GMJ14A/R3	21.82**	29.82**	29.41**	29.06**	28.76**	27.77**
GMJ14A/PK12	-7**	-5.7	-8.86**	-7.02**	-8.74**	-7.56**
GMJ14A/R32	-7.9**	-20.17**	-18.78**	-19.47**	-18.59**	-17.18**
GMJ14A/BP11	16.26**	6.46	8.88**	7.56**	9.28**	10.09**
GMJ15A/PK90	5.49*	18.25**	18.02**	18.53**	17.96**	15.54**
GMJ15A/R3	-4.51	3.48	3.08**	2.73	2.43	1.43
GMJ15A/PK12	10**	11.3*	8.14**	9.98**	8.26**	9.44**
GMJ15A/R32	9.1*	-3.17	-1.78**	-2.47	-1.59	-0.18
GMJ15A/BP11	-20.07*	-29.87**	-27.46**	-28.77**	-27.06**	-26.24**

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

SCA variances were greater than GCA for germination traits under stress condition. The relative dry weight of shoot was controlled by both additive effects and non-additive effects. The relative germination percentage, relative seminal root length, relative shoot length, relative seedling length, and relative vigor index were controlled by non-additive effects. Among the lines, IR80154A was good combiner for relative seminal root and seedling length. Among the tester, PK 90 and R3 had been good combiner for germination percentage, dry weight of shoot, and index vigor.

These results showed that GCA was important in selection and reliable evaluation for

drought tolerance. SCA represents dominance and epistatic interaction, which can be related to heterosis. Effects of general and specific combining ability are an important indicator of the potential of parental lines to generate superior breeding populations.

CONCLUSION

The high GCA effects of the parents in PEG conditions revealed that IR80154A, PK90, and R3 were good general combiners for most of the germination traits and potential parents to be used in breeding programs for the development

of drought tolerant of hybrid rice. The F1 hybrids IR80154A x PK90, GMJ13A x R3, GMJ14A x R3, GMJ15A x PK90, and GMJ15A x PK12 proved to be good specific combiners for most of the traits, thus offering potential parents for hybrid crop development in drought conditions. The germination percentage, a length of seminal root, shoot, seedling length, and vigor seed index controlled by non-additive gene action.

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REFERENCES

- Afa L, Purwoko BS, Junaedi A, Haridjaja O, Dewi IS (2012). Detection of drought tolerance of hybrid rice using polyethylene glycol (PEG-6000). *Agrivigor* 11(2): 292-299. (in Indonesian language).
- Afa L, Purwoko BS, Junaedi A, Haridjaja O, Dewi IS (2013). Early detection of hybrid rice tolerance to drought using PEG 6000. *Indonesian J. Agron.* 41(1): 9-15. (in Indonesian language).
- Adilakshmi D, Upendra A (2014). Combining ability analysis for quality and nutritional traits in rice. *Int. J. Farm Sci.* 4(2): 15-23.
- Ahmad S, Ahmad R, Ashraf MY, Ashraf M, Waraich EA (2009). Sunflower (*Helianthus annuus* L.) response to drought stress at germination and seedling growth stages. *Pak. J. Bot.* 41: 647-654.
- Almaghrabi OA (2012). Impact of drought stress on germination and seedling growth parameters of some wheat cultivars. *Life Sci. J.* 9(1): 590-598.
- Subhani GM, Chowdhry MA (2000). Genetic studies in bread wheat under irrigated and drought stress conditions. *Pak. J. Biol. Sci.* 3(11): 1793-1798.
- Badiane FA, Diouf D, Sane D, Diouf O, Goudiaby V, Diallo N (2004). Screening cowpea [*Vigna unguiculata* (L.) Walp.] varieties by inducing water deficit and RAPD analyses. *Afr. J. Biotechnol.* 3: 174-178.
- Ballo M, Ai NS, Mantiri FR, Pandiangan D (2012). Morphological response of some rice (*Oryza sativa* L.) cultivars to water deficit at the seedling stage. *Bioslogos* 2(2): 88-95. (in Indonesian language).
- Bouman BAM, Humphreys E, Tuong TP, Barker R (2007). Rice and water. *Adv. Agron.* 92: 187-237.
- Boopathi NM, Swapnashri G, Kavitha P, Sathish S, Nithya R, Ratnam W, Kumar A (2013). Evaluation and bulked segregant analysis of major yield QTL qtl 12.1 introgressed into indigenous elite line for low water availability under water stress. *Rice Sci.* 20(1): 25-30.
- Chakraborty R, Chakraborty S, Dutta BK, Paul SB (2009). Combining ability analysis for yield and yield components in bold grained rice (*Oryza sativa* L.) of Assam. *Acta Agron.* 58(1): 9-13.
- Dar SH, Rather AG, Ahanger MA, Sofi NR, Talib S (2014). Gene action and combining ability studies for yield and component traits in rice (*Oryza sativa* L.), A review: *J. Plant Pest Sci.* 1(3): 110-127.
- Dorosti H, Monajjem S (2014). Gene action and combining ability for grain yield and yield related traits in rice (*Oryza sativa* L.). *The J. Agric. Sci.* 9(3): 100-108.
- Ghebremariam KM, Yan L, Zhengcai Z, Wang Q (2013). Effect of drought stress on physiological growth parameters of tomato inbred lines at germination stage. *European Sci.* 9(33): 25-33.
- Ghosh SC, Chandrakar PK, Rastogi NK, Sharma D, Sarawgi AK (2012). Combining ability analysis using CMS breeding system for developing hybrids in rice (*Oryza sativa*). *Bangladesh J. Agric. Res.* 37(4): 583-592.
- Govindaraj M, Shanmugasundaram P, Sumathi P, Muthiah AR (2010). Simple, rapid and cost-effective screening method for drought resistant breeding in Pearl Millet. *Electr. J. Plant Breed.* 1(4): 590-599.
- Hasan MJ, Kulsum MU, Rahman MM (2014). Combining ability of different yield related characters in rice. *SAARC J. Agric.* 12(2): 143-153.
- Jiang W, Lafitte R (2007). Ascertain the effect of PEG and exogenous ABA on rice growth at germination stage and their contribution to selecting drought tolerant genotypes. *Asian J. Plant Sci.* 6(4): 684-687.
- Ji K, Wang Y, Sun W, Lou Q, Mei H, Shen S, Chen H (2012). Drought-responsive mechanisms in rice genotypes with contrasting drought tolerance during reproductive stage. *J. Plant Physiol.* 169: 336-344.

- Khan MI, Shabbir G, Akram Z, Shah MKN, Ansar M, Cheema NM, Iqbal MCS (2013). Character association studies of seedling traits in different wheat genotypes under moisture stress conditions. *SABRAO J. Breed. Genet.* 45(3): 458-467.
- Radhouane L (2007). Response of Tunisian autochthonous pearl millet (*Pennisetum glaucum* L.) to drought stress induced by polyethylene glycol (PEG-6000). *Afr. J. Biotechnol.* 6: 1102-1105.
- Lestari EG, Mariska I (2006). Drought tolerance identification of Gajahmungkur, Towuti, and IR 64 rice somaclones using polyethylene glycol. *Bul. Agron.* 34(2): 71-78. (in Indonesian language).
- Meutia SA, Anwar A, Suliansyah I (2010). Tolerance test to drought stress on some genotypes of West Sumatra local paddy (*Oryza sativa* L.). *Jerami* 3(2): 71-81. (in Indonesian language).
- Mirbahar AA, Saeed R, Markhand GS (2013). Effect of polyethylene glycol-6000 on wheat (*Triticum aestivum* L.) seed germination. *Int. J. Biol. Biotechnol.* 10(3): 401-405.
- Muthuramu S, Jebaraj S, Ushakumari R, Gnanasekaran M (2010). Estimation of combining ability and heterosis for drought tolerance in different locations in rice (*Oryza sativa* L.). *Electr. J. Plant Breed.* 1(5): 1279-1285.
- Mohammadi R, Mendioro MS, Diaz GQ, Gregorio GB, Singh RK (2014). Genetic analysis of salt tolerance at seedling and reproductive stages in rice (*Oryza sativa*). *Plant Breed.* 133(5): 548-559.
- Sarker U, Biswas PS, Prasad B, Mian MAK (2002). Heterosis and genetic analysis in rice hybrids. *Pak. J. Biol. Sci.* 5(1): 1-5.
- Singh RK, Chaudhary BD (1979). *Biometrical Method in Quantitative Genetic Analysis*. Kalyani Publ. New Delhi.
- Steel RGD, Torrie JH (1980). *Principles and Procedures of Statistics, Second Edition*. New York: McGraw-Hill Book Co.
- Swain P, Anumalla M, Prusty S, Marndi BC, Rao GJN (2014). Characterization of some Indian native land race rice accessions for drought tolerance at seedling stage. *Aut. J. Crop Sci.* 8(3): 324-331.
- Verslues PE, Agarwal M, Agarwal KS, Zhu J, Zhu JK (2006). Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stress that affect plant water status. *Plant J.* 45(4): 523-539.
- Yong C, Min G, Ye C, ChongShun Z, XueKun Z, HanZhong W (2010). Combining ability and genetic effects of germination traits of *Brassica napus* L. under waterlogging stress condition. *Agric. Sci. China* 9(7): 951-957.