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Ascertain the Effect of PEG and Exogenous ABA on Rice Growth at Germination Stage and Their Contribution to Selecting Drought Tolerant Genotypes

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Abstract: In this study, the effect of exogenous ABA on radicle and plumule growth at germination stage was investigated, compared with PEG osmotic stress culture condition and the consistency between genotypic variation in exogenous ABA and PEG sensitivity at germination stage and in water deficit treatment at reproductive stage in the field was evaluated. Fifteen rice (*Oryza sativa* L.) cultivars with different drought resistant ability were studied, 7.8×10^{-6} mol L^{-1} ABA and 30% PEG1500 (-1.1 MPa) were used to culture the germinated seeds for 5 days and the length, fresh and dry weight of radicle and plumule were measured and compared with normal condition (distilled water). In the field water stress experiment, drip irrigation was served for all plots every other day and water was discontinued for 14 days when the flag leaf reached stage 0 for the main stems in the plot. In this study, both PEG and ABA applied could inhibit the growth of plumule and radicle and significant difference existed among genotypes in the reduction rate of plumule and radicle. While for most of the tested genotypes, the response to ABA was similar to PEG and water stress in the field as well, which indicated the genotypes variation and ranking for the treatment with ABA based on the reduction rate of radicle and plumule growth parameters and with PEG based on reduction rate of plumule growth were rather similar to the same genotypes in field water deficit experiment based on the yield reduction rate. So exogenous ABA at germination stage could be and even more effective compared with PEG to quantify the drought resistance among genotypes in rice.

Key words: *Oryza sativa* L., polyethylene glycol, abscisic acid, plumule, radicle

INTRODUCTION

Drought stress or insufficient water supply is one of most important limiting factors in world rice production. The improvement of drought resistance for the high yielding rice cultivars for upland or insufficient water supply area is a priority goal of rice breeders (Fukai and Cooper, 1996; Price *et al.*, 1997). In recent year, polyethylene glycol (PEG) has been widely used to induce water stress and drought tolerant cultivars have been identified in many crops (Cherian and Reddy, 2003; Badiane *et al.*, 2004) by developing strategies based on the use polyethylene glycol (PEG). The plant hormone abscisic acid (ABA) has long been known to play a critical role in stress responses (Giraudat *et al.*, 1994; Himmelbach *et al.*, 2003) and osmotic stresses induce increased levels of ABA (Zeevaart and Creelman, 1998), moreover, exogenous ABA also was found to defect germination in *arabidopsi* (Sumin *et al.*, 2004) and to affect seedling growth in subterranean clover (Xu *et al.*, 2002). In present study, we want to investigate the effect of exogenous ABA on radicle and plumule growth at germination stage in rice, compared with PEG osmotic stress and to evaluate the consistency between genotypic variation in exogenous ABA sensitivity at germination stage and in water deficit treatment at reproductive stage in the field, so to know their contribution to selecting tolerant genotypes in rice.

MATERIALS AND METHODS

Materials: Fifteen rice (*Oryza sativa* L.) cultivars with different drought resistant ability (based on the former field research) were used for study : AUS196, BALA, CT6510, DEHULA, HD1.4, IR55419, IR55435, IR64, IRAT104, KALINGAIII, N22, SALUMPIKIT, SATHI34-31, UPLRI5, VANDANA. Some characteristics of these genotypes are outlined in Table 1.

Methods

Experiment 1 (Field experiment): Experiments were conducted in International Rice Research Institute (IRRI),

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Table 1: Characteristics and origins of the fifteen rice genotypes studied in this experiment

Genotypes	Group	Origin	Type	RR yield in the field (%)
CT6510-24-1-2	1	SA	I	15
IR55419-04	1	SEA	I	32
IR55435-05	1	SEA	I	26
HD1.4	6	SA	I	35
IRAT104	6	AF	I	34
IR64	1	SEA	I	32
Aus196	2	EI	T	61
BALA	1	EI	I	54
DEHULA	2	EI	T	77
KALINGA111	1	EI	I	74
N-22	2	EI	T	75
SALUMPIKIT	1	SEA	T	69
SATHI34-36	1	EI	T	66
UPLRI-5	1	SEA	I	82
VANDANA	1	EI	I	67

Isozyme groups, 1: Indica subspecies, 2: Aus subspecies, 6: Japonica subspecies. Origin codes: AFR, Africa; SEA, Southeast Asia; EI, Eastern India; EA: East Asia, SA: South America; Type: T = Traditional variety; I = Improved genotypes

Los Banos, Laguna, Philippines. Fifteen rice (*Oryza sativa* L.) cultivars were sown in well-fertilized soil, with plot size 3×4 m rows, 25 cm spacing; 3×1 m. Seeding rate was at 60 kg ha^{-1} and three replications in each water level was arranged. Basal fertilizer was complete 40 kg N-P-K and top dressing was with 30 kg N ha^{-1} at maximum tillering stage. All plots were drip irrigated every other day and water discontinued for 14 days when the flag leaf reached stage 0 for the main stems in the plot. This should correspond to about 7 days before the first panicle, or about 14 days before 50% anthesis.

Experiment 2 (germination stage): In a preliminary experiment, a series of different concentration of exogenous ABA and PEG (1500) were used to test the germinated seeds, according to the effect of exogenous ABA and PEG on most rice cultivars, we selected 7.8×10^{-6} mol L^{-1} ABA and 30% PEG 1500 (-1.1 MPa) for this germination study. Healthy appearing seeds of the same 15 genotypes used in the field were surfaced-sterilized for 10 min with 15% sodium hypochlorite (commercial bleach), rinsed three times with sterile water. After 24 h germinating, selected the good and even germinated seeds and planted on nylon mesh attached 12 holes of styron form which covers on the plastic tray(L×W×H; 30×28×6 cm). The medium was provided with (±)ABA 7.8×10^{-6} mol L^{-1} and 30% PEG 1500 (-1.1 MPa). Kept solution up to nylon mesh so that seeds were suspended slight above the solution. All the trays were kept in a dark incubator at 30±1.

Measurement of growth parameters: The length and weight of plumule and radicle with normal, stressed and ABA-treatment were was determined 5 days after incubation and seven seedlings from each variety and treatment were randomly selected for measurement. As a drought resistant index, reduction rate (%) = (1-(Vs/Vp)*100, Vs and Vp means performance value (such as grain yield, the length or weight of plumule and radicle of under stress and non-stress conditions, respectively)

Data analysis: All statistical analyses were performed with SAS (SAS Institute Inc., 1996). Results for ANOVA were considered significant at p<0.05 or p<0.01.

RESULTS

Genotypic responses to water stress in the field: In the field experiment, under water stress condition, genotypic difference was existed in drought tolerance. Among 15 tested varieties, grain yields were reduced slightly in CT6510-24-1-2, IR55435-05 by 15 and 26% of the control plots in stress trials, respectively. On the contrary, for varieties UPLRI-5, DEHULA, KALINGA111, N-22, the grain yields were seriously reduced and only reached 18, 23, 26 and 25% of the control, respectively (Table 1).

Exogenous ABA and PEG applied hampered the growth of plumule and radicle: At the germination stage of rice, both exogenous PEG and ABA applied to the culture medium could affect the normal growth of plumule and radicle in different degree (Table 2). The length of plumule and radicle was strong affected by ABA with average reduction 87.83, 87.64%, respectively, while the dry weight of plumule and radicle was less reduced (68.96, 75.86%). Compared with the effect by ABA, PEG induced less serious reduction in growth parameters, especially in the length with reduction 64.53, 55.14 and 58.22, 54.36% in the dry weight in plumule and radicle, respectively, so exogenous ABA (7.8×10^{-6} mol L^{-1}) could induced more serious reduction of the growth parameters than 30% PEG 1500 (-1.1 MPa) used in this experiment.

Genotypic difference in sensitivity to added ABA and PEG: There indeed exist significant genotypic difference in response to exogenous ABA and PEG applied. Among all tested genotypes, the variation of reduction of the length of plume from 48.73 to 83.38% caused by PEG and from 70.37 to 97.4% 8 by ABA. In general, those drought tolerant genotypes showed more slightly reduction caused whether by PEG or ABA than other fairly-good drought tolerance genotypes, for example, the drought resistant genotype CT6510-24-1-2 was led to 55.25 and 80.16% reduction in the length of plumule by ABA and PEG, respectively, while 78.55 and 97.67% inhibited for KALINGA111, a drought sensitivity genotype.

Table 2: Genotypes differences on reduction rate of radicle and plumule growth performance affected by PEG and ABA for 5 days at germinating stage

	Reduction of plumule length by (%)		Reduction of radicle length by (%)		Reduction of plumule fresh weight by (%)		Reduction of plumule dry weight by (%)		Reduction of radicle fresh weight by (%)		Reduction of radicle dry weight by (%)	
Genotypes	PEG	ABA	PEG	ABA	PEG	ABA	PEG	ABA	PEG	ABA	PEG	ABA
CT6510-24-1-2	55.25	80.16	50.44	79.60	63.75	51.38	41.33	37.78	71.11	63.33	37.82	62.82
IR55419-04	60.47	84.40	53.76	79.67	67.50	67.38	63.47	76.53	74.47	72.23	56.25	67.05
IR55435-05	52.88	88.87	56.61	85.62	63.73	68.81	35.38	67.92	69.96	70.25	50.29	71.10
HD1.4	57.04	74.30	48.72	76.28	60.89	39.30	51.71	54.45	71.24	67.35	56.34	69.01
IRAT104	64.44	70.37	76.31	72.17	66.17	48.48	60.77	45.34	76.76	55.07	69.80	75.25
IR64	48.73	80.80	40.37	69.85	60.89	54.83	37.63	49.48	63.00	40.33	30.40	61.60
AUS196	56.95	78.18	50.32	73.59	62.94	59.77	41.55	54.73	60.76	57.92	44.91	65.28
BALA	61.31	95.50	42.83	87.66	62.94	83.12	52.27	89.07	67.21	74.93	36.10	81.46
DEHULA	74.45	97.48	54.63	98.82	80.35	97.49	65.72	97.87	81.37	98.77	42.95	93.29
KALINGA111	78.55	97.67	49.82	99.27	79.89	91.18	70.64	72.02	64.76	98.86	71.25	75.00
N-22	57.20	93.74	29.79	98.26	61.43	92.36	70.91	79.80	57.35	96.73	86.64	85.88
SALUMPIKIT	83.38	96.09	71.42	98.77	82.41	94.33	79.10	77.53	85.68	96.23	63.16	80.12
SATHI34-36	78.64	97.66	70.93	99.38	79.39	96.36	58.89	72.50	80.43	99.27	66.00	84.00
UPLRI-5	64.14	85.59	69.17	97.93	64.35	91.12	81.54	81.54	70.89	80.17	70.00	81.88
VANDANA	74.39	96.63	61.93	97.80	75.56	92.89	62.33	77.85	70.11	90.44	33.53	84.12
Mean	64.52	87.83	55.14	87.64	68.81	75.26	58.22	68.96	71.01	77.46	54.36	75.86
LSD 0.05	8.23	5.74	13.27	4.70	7.80	5.66	11.64	23.91	10.28	6.33	18.40	23.91

Table 3: Correlationship between the reduction of plumule and radicle caused by ABA and that by PEG, the reduction rate of grain yield with water deficit in the field as well

	Reduction by ABA					
Parameters	Plumule length	Radicle length	Plumule FWG	Radicle FWG	Plumule DWG	Radicle DWG
Reduction by PEG	0.634*	0.175	0.674**	0.317	0.584*	0.314
Reduction of yield in the field	0.53*	0.67**	0.73**	0.62*	0.65**	0.67**

*p<0.05; **p<0.01

Consistency between genotypic variation in sensitivity to exogenous ABA and PEG and water stress in the field: Inside of the variation, the ranking of all tested genotypes was not always consistent, depending on the measured parameters. However, similar genotypic variation and ranking could be obtained based on the reduction rate of grain yield under water stress in the field and on the plumule and radicle reduction by PEG or ABA except radicle reduction rate by PEG and significant correlationship was also found between them (Table 3).

DISCUSSION

Polyethylene glycol (PEG) has been used to induce water stress in hydroponics culture and was found effective way for screening drought tolerance at early growth stage (Morgan, 1990; Bhupinder *et al*., 1998; Jing and Chang, 2003). In this research, both PEG (-1.1 Mpa) and ABA inhibited the growth of plumule and radicle in rice, as reported by Xu *et al*. (2002) and Sharp *et al*. (1994), which could be due to reduced mobilization of starch for the growth (Satvir *et al*., 1998) and also osmotic stress could affect cell elongation by cell division and cell enlargement (Cleland, 1981; Taiz, 1984). The significant relationship between the effect by PEG and by ABA on the plumule indicated that ABA might directly or indirectly affect the growth of plumule under osmotic stress condition.

Under PEG treatment condition, the reduction rate of plumule length and dry weight was significantly related with the reduction rate of grain yield in the field water stress condition, so the reduction rate of plumule growth under PEG (-1.1 Mpa) was good and reliable way to identify the drought tolerance ability in rice. Zhang *et al*. (2005) also reported that the osmotic stress coefficient (stress/control) of plumule length could be one of good drought tolerance evaluation index. However, Sheoran and Saini (1996) and Wang *et al*. (1997) reported that it should be the length of plumule not the reduction rate of plumule length that could be good indices for drought resistant identification. Above all, in this study, good consistency between genotypic variation in exogenous ABA sensitivity based on the reduction rate of both radicle and pulume growth (including length, fresh and dry weight) and in water deficit treatment at reproductive stage in the field. So besides PEG, exogenous ABA (7.8×10^{-6} mol L^{-1}) was also could be suggested to help us to screen and group drought tolerant cultivars in rice at germinating stage. In order to establish an effective screen system for drought tolerance, more rice cultivars will be needed for test in the next step.

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