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Bhavyasree RK
Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

A John Joel
Tamil Nadu Rice Research Institute, Aduthurai, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

P Jeyaprakash
Department of Plant Breeding and Genetics, Anbil Dharmalingam Agricultural College & Research Institute, Trichy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

M Raveendran
Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
Bhavyasree RK
Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

The effect of induced drought stress on seedling vigour and antioxidant enzymes in wild and cultivated *Oryza* species

Bhavyasree RK, A John Joel, P Jeyaprakash and M Raveendran

Abstract
The study was conducted by using two cultivated and four wild genotypes of the *Oryza* species. The genotypes were analyzed for the seed quality parameters and antioxidant enzyme activities during the polyethylene glycol (PEG) induced water stress and no stress conditions. All the *Oryza* species except *O. sativa* among the studied genotypes performed well during the induced water stress. *O. glumaepatula* and *O. barthii* showed better seed quality parameters in the stress condition. The reduction in the α-amylase activity was also found to be lower in the wild genotypes. There was a significant rise in the antioxidant enzymes in all the genotypes except *O. sativa.* So these wild *Oryza* genotypes along with *O. glaberrima* can be utilized for the improvement of drought tolerance in *O. sativa.*

Keywords: *Oryza,* crop wild relatives, drought, seedling, antioxidants

Introduction
Rice (*Oryza sativa* L.) is an important staple, feeding more than one third of the global population [1]. Even though several improvements have been made in the rice crop, due to the unidirectional selection for yield the variability is highly reduced [2]. The cultivated germplasm of rice presently accounts for only 10-20 percent of the variability [3]. With this reduced variability, the further improvement is limited. As the demand for the rice is increasing with the growing population, the further improvement is so essential.

The crop production is facing the biggest challenge of climate change in this era causing several stresses on the crop production. Several biotic and abiotic stresses are adversely affecting the crop production. So to sustain the food security of the globe intense crop improvement efforts are needed. With the limited variability in cultivated germplasm, it is impossible to reach the target level of production. So in this situation, the role of crop wild relatives, the treasure of variability, is inevitable. There are 24 species in *Oryza* genus among which only two are cultivated and rest of them are wild [4]. And the closely related species of the cultivated rice belong to the 'A' genome complex which consists of 8 species including the two cultivated rice species viz., *O. sativa* and *O. glaberrima* [5]. The species in this complex are easier to cross with the *O. sativa* producing fertile hybrids [6]. There are several desirable alleles in these wild species which can be used in the crop improvement programs to cope up with the changing climate. So elaborative studies are needed for the utilization of the crop wild relatives to introgress the tolerance to various biotic and abiotic stresses [5, 7].

The rice, being an aquatic crop, the water availability is so important for the survival and growth. So the water stress or drought will significantly impact all the metabolic processes of the crop. It affects the total expression of the yield by altering several physiological and biochemical processes [8]. Over 50 percent of the rice grown globally is affected by the water stress directly or indirectly [9]. Germination is a critical stage of growth and it is very sensitive to water stress and the initial vigour will affect the growth and eventually the yield of the plant [10, 11]. The water stress will severely affects the seed germination and seedling growth due to the oxidative damage caused by reactive oxygen species (ROS) produced during the stress [12]. The plant is laso having its own defense systems which includes the antioxidative enzymes to fight against ROS. These enzymes helps to overcome the oxidative stress caused during the water stress [13]. So the amount of the antioxidant enzymes can be a measure of tolerance to the drought stress in different genotypes. So in this background, the present investigation was carried out to access the changes in the seed quality parameters and the changes in the antioxidant enzymes during the induced drought stress in two cultivated and four wild *Oryza* species.

Materials and methods

The experiment was conducted with six genotypes of *Oryza* species including Asian and African cultivated species (Table 1). The genotypes were raised in the glass of Department of rice, Tamil Nadu Agricultural University, Coimbatore during the period of May 2018- September 2018. The seeds. The germination test was conducted by using 400 seeds following ISTA [14] standards. Each genotype was raised in 20 percent of polyethylene glycol (PEG 6000) and in control (distilled water) using roll towel method. The germination test was conducted in the laboratory condition at a temperature of 25±2 ºC and 95±2% relative humidity (RH). Observations were taken at the end of 14th day and biochemical analysis were also done for the same. The parameters measured are as given below:

Table 1: The wild and cultivated *Oryza* species in the experiment

Sr. No.	Species	Variety/Accession no.
1	*O. sativa*	NSICRc 222
2.	*O. glaberrima*	CG14
3.	*O. rufipogon*	Acc. 106286
4.	*O. barthii*	Acc 100936
5.	*O. nivara*	Acc. 101508
6.	*O. meridonalis*	Acc. 105290

Germination percentage

The number of normal seedlings in the treatments were counted and their mean was expressed as percentage.

Root length (cm)

Ten seedlings were removed from each treatment without any damage. The root length was measured from the base of the seedling to the tip of the root and expressed in centimeters.

Shoot length (cm)

The shoot length of the ten seedlings used to measure the root length was measures and reported in centimeters.

Vigour index

The sum of root and shoot lengths were taken and vigour index was calculated according to the following formula and the mean values were expressed in whole numbers

Vigour index = Germination (%) x Total seedling length (cm)

α-Amylase activity

The seeds were pre-germinated in distilled water and PEG solution. 500 mg of seeds are taken and homogenized with 1.8 ml of cold 0.02M sodium phosphate buffer (pH 6.0). then the enzyme is extracted by centrifuging the samples at 20,000 rpm for 20 min. 0.1 ml of the supernatant was taken and one ml 0.067 per cent starch solution was added. To stop the reaction, one ml of iodine HCl solution was added and incubated for 10 minutes in the room temperature. The absorbance was measured at 620 nm. The activity was calculated and expressed as mg maltose min-1 [15].

$$\alpha - \text{ amylase enzyme activity } = \frac{\text{OD value}}{\text{Volume of sample pipetted out}} \times \frac{1000}{500}$$

Catalase (µmol H_2O_2 min^{-1} g^{-1} protein)

The leaves were taken from the 14 day old seedlings and 200 mg of sample was homogenized using 0.2 M potassium phosphate buffer. Then the sample was centrifuged and supernatant was used for the analysis of the enzymes. To 40 µl enzyme extract, 3 ml of H_2O_2-phosphate buffer was added and observed in 240nm. The decrease in absorbance was recorded after 1 min at 240 nm [16].

Peroxidase (U mg^1protein min^{-1})

The leaf sample was homogenized using Tris buffer (PH 6.0) and centrifuged at 10,000 rpm for 10 min at 5 °C to extract the enzyme. 0.4 ml of enzyme extract was taken in a test tube and to that 0.5 ml of 1% H_2O_2 and 0.5 ml of 0.5% pyrogallol was added and incubated it for 20 min at 25 °C. After 20 min, 0.5 ml of 5% H_2SO_4 was added. The OD value of the solution was measured in a UV-VIS spectrophotometer at 420 nm initially and after 10 min [17] and calculated as below:

$$\text{Peroxidase} = \frac{\text{Difference in OD value}}{10} \times \frac{1000}{500} \times 60$$

Superoxide dismutase (U mg^1protein min^{-1})

The enzyme extract was obtained by extraction using tris buffer. The 40 µl of the sample was mixed with 50 mM phosphate buffer (pH 7.8) 20 µl of 1 mM riboflavin. The reaction was initiated by illuminating the samples under a 15 W fluorescent tube for 10 minutes. The reaction mixture kept in the dark was used as blank. The absorbance of the samples was measured at 560 nm wavelength immediately after the reaction was stopped [18].

The data was analyzed by the procedures suggested by [19].

Results and discussion

There are several useful alleles for drought tolerance in wild species of rice [7, 20]. In this experiments also, it was found that the seed quality parameters were superior in wild genotypes in stress condition when compared to the cultivated (Table 2). Even though the germination percentage was low for the wild when compared to the cultivated in the non stress condition, the reduction of germination was more when the stress was imposed in the cultivated. This showed the susceptibility of cultivated accessions when compared to the wild [8]. This trend can also be observed in the parameters like root length, shoot length and vigour index showing a better adaptability of water stress in the wild than the cultivated.

Table 2: The seed quality parameters of the *Oryza* species during stress and non stress conditions

Genotypes	Germination		Root length		Shoot length		Vigour index	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
O. sativa	95±0.77^{c}	60±0.02^{c}	16.5±0.26^{d}	10.6±0.09^{d}	8.8±0.08^{d}	5.1±0.05^{d}	2408±2.29^{f}	940±4.80^{f}
O. glaberrima	93±0.74^{a}	88±0.15^{a}	18.8±0.11^{ab}	18.7±0.15^{ab}	8.5±0.08^{c}	8.0±0.04^{c}	2525±2.10^{b}	2345±8.13^{b}
O. rufipogon	85±0.87^{b}	82±0.22^{b}	17.0±0.21^{c}	16.6±0.18^{c}	9.2±0.07^{b}	9.0±0.11^{b}	2252±6.51^{e}	2091±2.72^{e}
O. nivara	92±0.63^{a}	89±0.85^{a}	16.6±0.05^{c}	16.6±0.19^{c}	9.2±0.15^{b}	8.9±0.08^{b}	2356±6.22^{c}	2247±8.63^{c}
O. barthii	91±0.46^{a}	88±0.63^{a}	19.5±0.13^{a}	18.5±0.23^{a}	9.5±0.15^{ab}	9.0±0.12^{ab}	2628±2.68^{a}	2472±4.77^{a}
O. glumaepatula	82±0.45^{c}	80±0.95^{c}	18.9±0.01^{b}	18.4±0.08^{b}	9.6±0.05^{a}	9.2±0.08^{a}	2322±4.48^{a}	2166±8.68^{a}

Data presented are means from four replicates with standard errors. Within each treatment, different letters at each column indicate significant differences by Duncan's multiple range test at P<0.05.

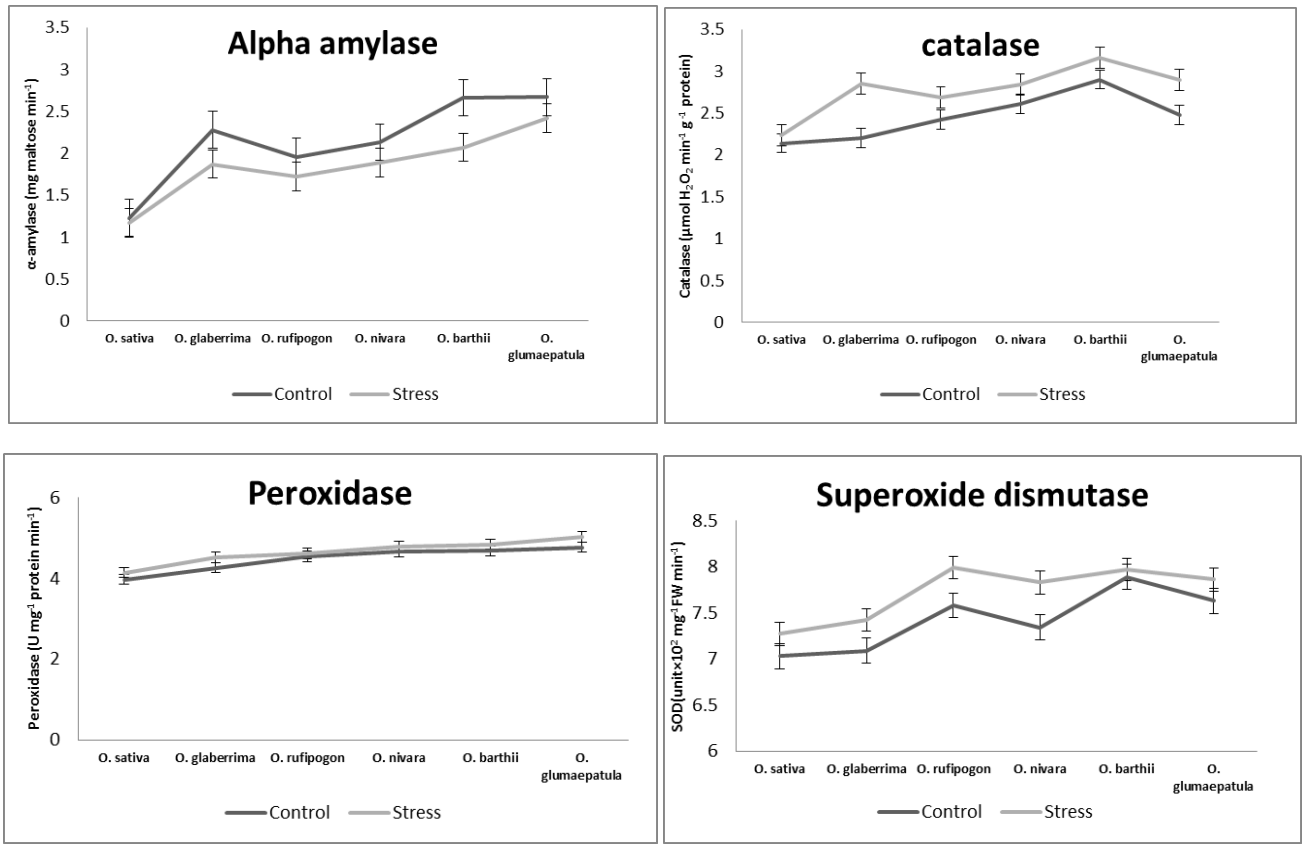

Fig 1: The biochemical changes during stress and non stress condition in different *Oryza* species

The germination percentage in the stress was minimum in *O. sativa* (60 percent) and was maximum in *O. nivara* (89 percent). In case of root length, shoot length and vigour index, the wild *Oryza* species and *O. glaberrima* showed greater values in both stress and non stress conditions. In stress condition, a minimum reduction was observed in these species. The maximum root length was observed in *O. barthii* (19.5 cm) followed by *O. glumaepatula* (18.9 cm) in non stress conditions. Minimum decrease in root length was observed in *O. nivara.* While in the case of shoot length, maximum shoot length was observed in *O. glumaepatula* in both stress (9.2 cm) and non stress (9.6 cm) conditions. The vigour index was maximum for *O. barthii* for both the conditions. The wild *Oryza* species are having the capacity to withstand several stresses especially the water stress as an adaptation to the extreme environments they grow [21]. Also for *O. glaberrima* is known for its drought tolerance capacity [22, 23].

The α-amylase activity is an indicator of biological processes in seed and is having inevitable role in germination[24]. During the induction of stress the activity of α-amylase was reduced in all the accessions and that could be the cause of reduced germination. The reduction was comparatively lesser in wild *Oryza* species. The highest α-amylase activity was observed in *O. glumaepatula* in both the stress and non stress conditions. The antioxidant enzymes are essential to neutralize the reactive oxygen species produced during the stress [25, 26]. And during stress the antioxidant enzyme activity will increase and the extend of increase will show how much the plant can survive in the stress [27]. In this experiment also, the level of antioxidant enzyme activity like catalase, peroxidase and superoxide dismutase increased in all genotypes during stress. The increase in these enzymes was negligible in *O. sativa* but higher in all other species. *O. glumaepatula* had the highest values for catalase (2.90 µmol H_2O_2 min^{-1} g $^{-1}$ protein) and peroxidase (5.03 U mg^{-1} protein min^{-1}) while *O.rufipogon* showed the maximum value of Superoxide dismutase (7.99 unit×10^2 mg^{-1}FW min^{-1}) during stress. These antioxidant enzymes will help the genotypes to survive better in the stress conditions.

Conclusion

The wild *Oryza* species are having several desirable genes for biotic and abiotic stresses. This was evident in this experiment showing the better seed quality parameters for wild genotypes when compared to the cultivated during stress. Among the cultivated species *O. glaberrima* performed better than *O. sativa*. The biochemical analysis also revealed the same results.

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