

Identification of drought stress tolerance in wild species germplasm of rice based on leaf and root morphology

Kumari Neelam^{1*}, Gurpreet K. Sahi¹, Kishor Kumar¹ and Kuldeep Singh^{1,2}

¹School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana-141004, India and

²ICAR – National Bureau of Plant Genetic Resources, New Delhi, 110073, India

Received 23 March 2017; Accepted 22 September 2017 – First published online 30 October 2017

Abstract

Drought is the major abiotic constraint to the rice production in the rain-fed areas across Asia and sub-Saharan Africa. Wild species of *Oryza* offer a wide spectrum of adaptive traits and can serve as potential donors of biotic and abiotic stress tolerance. At the Punjab Agricultural University, we are maintaining an active collection of 1630 accessions of wild species germplasm (AA, CC, BBCC and CCDD) of rice. These accessions were screened to assess genetic variation for drought tolerance under field conditions. Severe water stress was imposed at the late vegetative stage by withholding water initially for 25 d and then extended further to 35 d during *kharif* season in the years 2013–14 and 2015–16. The tolerance score for drought stress was based on the extent of leaf rolling and leaf drying. Based on the 2 years' data, seven accessions from *Oryza rufipogon*, four from *Oryza longistaminata* and one each from *Oryza officinalis* and *Oryza latifolia* were found tolerant to drought stress. These selected accessions were further phenotyped for root morphology. The average root length among the selected accessions ranges between 36 and 80 cm and the number of primary roots vary from 30 to 87 cm. The *O. rufipogon* accession IRGC 106433, *O. longistaminata* accession IRGC 92656A, *O. officinalis* accession IRGC 101152 and *O. latifolia* accession IRGC 80769 showed approximately 2–2.5 times longer root length and number than the *indica* rice elite cultivar PR121. The results indicated potentiality of selected wild species germplasm for conferring drought tolerance to the elite cultivars.

Keywords: abiotic stress, drought tolerance, leaf rolling, root morphology, wild species germplasm of rice

Introduction

Drought is one of the most devastating abiotic stress of rice under rain-fed ecosystem, reducing crop yield up to 50%. Approximately, 34 million hectares (Mha) of rain-fed lowland and 8 Mha of upland rice in Asia suffers from drought stress of varying intensities almost every year with 13.6 Mha area affected in India alone (Wopereis *et al.*, 1996; Singh *et al.*, 2016a, b). Developing rice cultivars with an inherent capacity to tolerate drought stress is one of the most

promising ways for having sustainable yield under rain-fed environment. Drought tolerance is a complex trait governed by many physiological and biochemical properties of plants. Genotypes that have deep, coarse roots with a high ability of branching and penetration, higher root-to-shoot ratio, elasticity in leaf rolling, early stomatal closure and cuticular resistance are reported as component traits of drought avoidance (Blum, 1988; Samson *et al.*, 2002; Wang and Yamauchi, 2006). Leaf rolling is one of the drought avoidance mechanism to prevent water deficits during drought stress. Loresto and Chang (1981) have also suggested leaf rolling as a criterion for scoring drought tolerance in tall and semi-dwarf rice cultivars. Severity of leaf

*Corresponding author. E-mail: kneelam@pau.edu

rolling as well as leaf drying increased with duration of drought stress. Leaf rolling during stress reduces the leaf surface exposure to sunlight energy and decrease transpiration leading to the closure of stomata, so that gaseous exchange and CO₂ entry into cells are reduced and photosynthesis is decreased. Many quantitative trait loci (QTLs) were mapped for secondary traits (Nguyen *et al.*, 2004; Ding *et al.*, 2011; Uga *et al.*, 2013) from the cultivated gene pool but none from wild species germplasm of rice. Wild species of rice constitute valuable resources for genes/alleles and QTLs for resistance to biotic and abiotic stresses and for enhancing the productivity of modern cultivars (Brar and Khush, 1997; Brar and Singh, 2011; Singh *et al.*, 2016a, b). Only a few reports on the screening or utilization of wild species of rice in improving drought tolerance are available (Liu *et al.*, 2004; Zhang *et al.*, 2006; Feng *et al.*, 2012). Keeping in view the above-said need of exploring new resources, the objectives of the present study is to assess genetic variation in component traits of drought among wild species germplasm of *Oryza* under water deficit.

Material and methods

Materials

A set of 1630 wild species germplasm of *Oryza* belonging to *O. sativa* and *O. officinalis* complex were screened for their vegetative stage drought tolerance (online Supplementary Table S1). These germplasm accessions were originally procured either from the International Rice Research Institute (IRRI), Philippines, or from the National Rice Research Institute (NRRI), Cuttack, and being actively maintained at the Punjab Agricultural University (PAU), Ludhiana.

Methods

Screening for drought tolerance at vegetative stage

(a) Phenotyping for leaf rolling and leaf drying

The experiment was conducted in the field area of the School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana (30°54' and 75°48'E) during the *kharif* crop season (May to November), 2013–2014 and 2015–16. The soil is clayey loam with soil pH ranging from 7.8 to 8.5. The seeds of all wild genotypes were sown on raised beds. Twenty-five days old seedlings were transplanted in the field along with the susceptible check PR121. The plot size consisted of a single row per accession with six plants each. The plant-to-plant distance was 30 cm with 60 cm row-to-row. After crop season was over, the plants were

left in the field as ratoon. In the next year, severe water stress was imposed at vegetative stage by withholding water initially for 25 d and then extended further to 35 d during the month of May to June. The drought condition was also ensured with the climate, temperature profile recorded above 40° continuously from 15 to 35 d during the drought period. At the termination of the experiment, soil moisture content (%) was recorded at four randomly chosen sites in the field. Soil samples were collected with screw augor from different depth and soil moisture content was determined in soil samples taken from consecutive depths of 0.15 cm down to a depth of 60.9 cm (A, B, C and D) in the field. To determine soil moisture content, soil samples were taken into moisture box. Initial weight of moisture box (empty) and with fresh soil was recorded separately. Then, the moisture boxes with fresh soil samples were kept into oven at 60° till the constant weight is attained. The calculations were done as per given formula: % moisture on dry weight basis = [(B–A)–(C–A)]/(C–A) × 100, where, A = weight of empty moisture box (in gm), B = weight of moisture box with fresh soil (gm), C = weight of moisture box with dry soil (gm), B–A = weight of fresh soil, C–A = weight of dry soil, [(B–A)–(C–A)] = (B–C) = weight of water in the soil.

Data on leaf rolling and leaf drying were recorded after 25 and 35 d of water stress (online Supplementary Table S2) between 12 and 2 PM using the modified protocol of Datta *et al.* (1988). The genotypes that showed a score range of 2–3 after 25 d of water stress were considered as susceptible and highly susceptible, respectively. The plants with no leaf rolling and leaf drying were scored as zero.

(b) Root phenotyping

In order to study root morphology, selected drought-tolerant accessions along with drought-susceptible *indica* cultivar Punjab Rice 121 (PR121) were grown in basket mesh kept above water-filled buckets under glass house. The experiment was set up in two replications. Root phenotyping was done using plastic mesh baskets (width × depth: 18 × 8 cm) method. The baskets were filled with soil in a green house (average air temperature 35°C, average humidity, 50–60% and natural light) and were kept on the top of a bucket (width × depth: 18 × 18 cm) filled with water. Sufficient water level was maintained for creating an anaerobic condition. The observations on root length and number of primary roots were taken after 3 months of experimental setup and were analysed using one-way analysis of variance followed by Dunnett's multiple comparison test using GraphPad Prism version 7.00, GraphPad Software, La Jolla California USA, (www.graphpad.com).

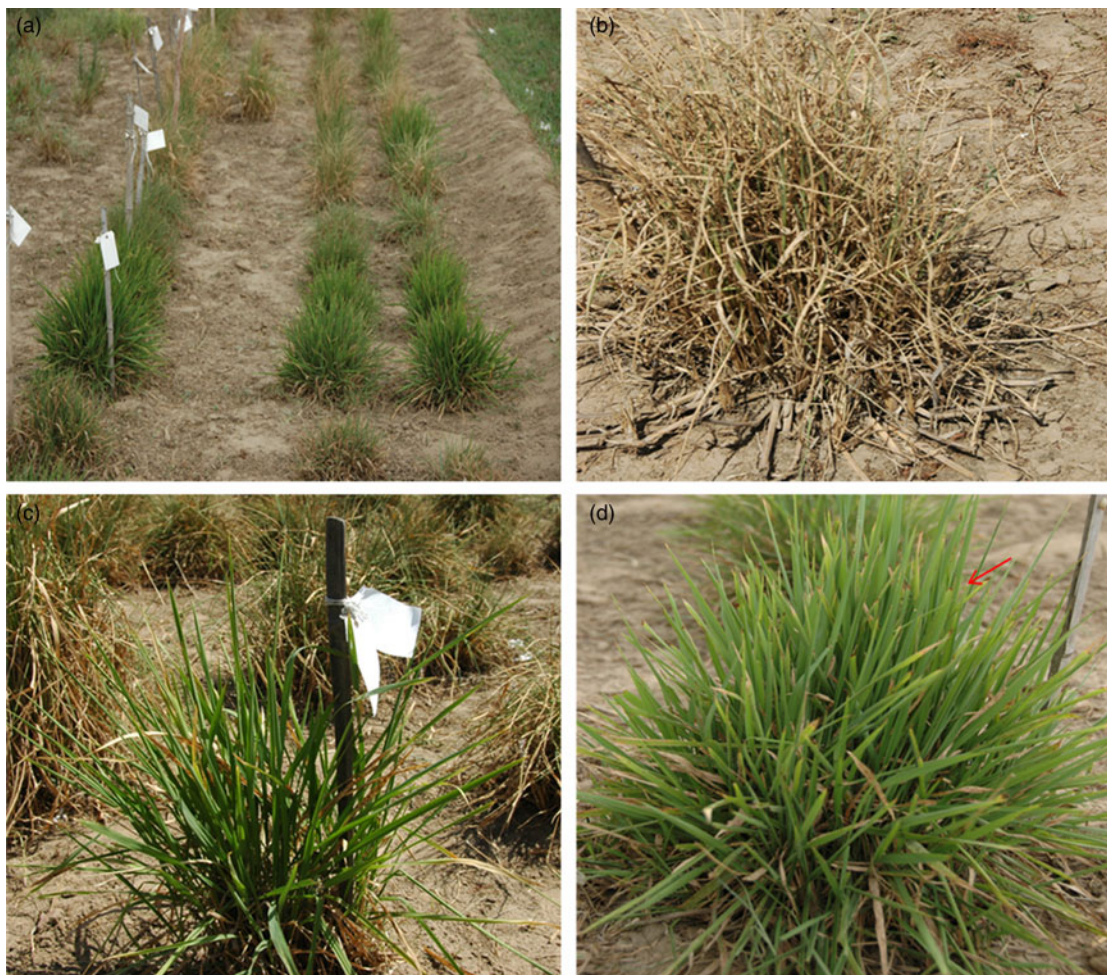


Fig. 1. Phenotypic evaluation of wild species germplasm of rice under field condition (a, b); the *Oryza rufipogon* accession IRGC 106433 with no leaf rolling and tip drying (c); the *O. rufipogon* accession IRGC 89012 with no leaf rolling, but slight tip burning after 35 d of water stress (d).

Results

The average moisture content of four randomly chosen sites and at a depth of 0.15, 15.3, 30.6 and 60.9 cm was found 3.52, 5.51, 8.02 and 8.66%, respectively, in the year 2013–2014 and these value corresponds to 2.58, 4.25, 7.62 and 8.36% in the year 2015–16. Soil moisture conditions coupled with high temperature (above 40°C) were sufficient to induce the severe water deficit conditions.

Out of 1630 wild species germplasm screened, only 13 were found tolerant after 35 d of severe water stress (Table 1; online Supplementary Table S3; Fig. 1). Out of 1369 accessions from *O. sativa* complex under study, only 11 accessions (seven from *Oryza rufipogon* and four accessions from *Oryza longistaminata*) were found tolerant to drought with a leaf-rolling score of zero. Among these 11 accessions of *O. sativa* complex, slight tip drying was observed in three accessions of *O. rufipogon* namely, IRGC 81802, IRGC 89006 and IRGC 89012. Among 126

accessions of *O. longistaminata* under study, only four accessions (three accessions from Mali and one accession from Ethiopia) showed tolerance to water stress. In case of *O. officinalis* complex, out of total 261 accessions, two accessions, one each from *O. officinalis* (accession no. IRGC 101152 from Brunei) and *Oryza latifolia* (accession no. IRGC 80769 from France) showed the tolerant reaction.

The mean number of roots and root length recorded for drought-susceptible *indica* rice PR121 was 42.5 and 31.5 cm, respectively (Table 1; Fig. 2, online Supplementary Fig. S1). Among *O. rufipogon* accessions, IRGC 106433 showed nearly two times higher root number (81.0 ± 1.4) than PR121, followed by CR 100375 (74.0 ± 1.4). Three of the accessions though had a comparable number of mean root number, but had root length almost 2–2.5 times higher than PR121. Sufficient variation was observed for root number (30–79) and root length (36 cm–68.5 cm) among selected *O. longistaminata* accessions. The *O. officinalis* acc. IRGC 101152 has the highest mean number of

Table 1. Leaf and root morphology of identified drought-tolerant accession along with their countries of origin

Sr. no.	Wild species	Origin	Leaf morphology				Root morphology	
			Leaf tip drying		Leaf rolling		Mean root no.	Mean root length (cm)
			25 DAS*	35 DAS	25 DAS	35 DAS		
1	<i>Oryza sativa</i> cv. PR121	India	9	9	9	9	42.5 ^a ± 6.4	31.5 ^a ± 2.1
2	<i>Oryza rufipogon</i> (#CR 100375)	India	0	0	0	0	74.0 ^{cd} ± 1.4	63.0 ^c ± 1.4
3	<i>O. rufipogon</i> (IRGC 81802)	Indonesia	0	1	0	0	46.0 ^b ± 5.7	62.5 ^c ± 3.5
4	<i>O. rufipogon</i> (IRGC 89006)	Cambodia	0	1	0	0	66.0 ^c ± 5.7	79.5 ^d ± 6.4
5	<i>O. rufipogon</i> (IRGC 89012)	Cambodia	0	1	0	0	35.0 ^a ± 7.1	71.0 ^c ± 1.4
6	<i>O. rufipogon</i> (IRGC 89230)	Cambodia	0	0	0	0	51.0 ^b ± 1.4	78.0 ^d ± 2.8
7	<i>O. rufipogon</i> (IRGC 106422)	Vietnam	0	0	0	0	35.0 ^a ± 7.1	78.5 ^d ± 4.9
8	<i>O. rufipogon</i> (IRGC 106433)	Vietnam	0	0	0	0	81.0 ^d ± 1.4	66.0 ^c ± 5.7
9	<i>Oryza longistaminata</i> (IRGC 105200)	Ethiopia	0	0	0	0	30.5 ^a ± 0.7	36.0 ^a ± 8.5
10	<i>O. longistaminata</i> (IRGC 83826A)	Mali	0	0	0	0	28.5 ^a ± 2.1	41.0 ^a ± 8.5
11	<i>O. longistaminata</i> (IRGC 92619A)	Mali	0	0	0	0	60.0 ^b ± 7.1	68.5 ^c ± 4.9
12	<i>O. longistaminata</i> (IRGC 92656A)	Mali	0	0	0	0	79.0 ^d ± 1.4	49.0 ^b ± 9.9
13	<i>Oryza officinalis</i> (IRGC 101152)	Brunei	0	0	0	0	87.5 ^d ± 3.4	64.0 ^c ± 2.8
14	<i>O. latifolia</i> (IRGC 80769)	France	0	0	0	0	75.0 ^{cd} ± 7.1	79.0 ^d ± 4.2

*DAS=days after imposing stress. #Code IRGC represents accessions from the International Rice Genetic Consortium, Philippines; CR represents accessions from the National Rice Research Institute, Cuttack, India. Superscripts (a–d) represent significant differences in the means of wild species germplasm accessions for root number and length.



Fig. 2. Root phenotyping using plastic mesh baskets (1) After 1 month of vegetative growth: (a) rice cultivar PR121, (b) *Oryza rufipogon* accession IRGC 106433, (c) *Oryza officinalis* accession IRGC 101152, (2) after 3 months of vegetative growth, (d) rice cultivar PR121, (e) *O. rufipogon* accession IRGC 106433, (f) *Oryza latifolia* accession IR80769.

roots (87.5 ± 3.4) among all the selected drought-tolerant accessions from four different species.

Discussion

Drought is one of the most serious abiotic stress limiting rice productivity in the world and poses a serious threat to the

sustainability of rice yields in rain-fed agriculture. Developing water use efficient and drought-tolerant variety may help to combat this problem in the era of global climate change. Leaf morphology and associated traits have been suggested as one of the parameters for selecting drought-tolerant genotypes (Biswal and Kohli, 2013). The presence of genetic variability for leaf rolling and correlation between

leaf area index and drought have also been studied by various scientist, indicating the probable role of leaf traits as a measure of drought susceptibility or tolerance (Subashri et al., 2009; Farooq et al., 2009; Salunkhe et al., 2011; Cerqueira et al., 2013; Singh et al., 2013; Kumar et al., 2014; Sokoto and Muhammad 2014). Our results revealed, sufficient genetic variation in leaf and root morphology among selected wild species germplasm accessions. Some of the accessions had a higher number, whereas some of them had a higher root length, suggesting a different underlying mechanism of drought tolerance in them. We noticed the presence of more secondary root mass in the deeper soil zone in these selected accessions suggesting absorption of more water or moisture and thus helping them to withstand under water stress condition. The presence of drought resistance in *O. rufipogon* and *O. officinalis* accessions were also reported by Feng et al. (2012) by assessing morphological and physiological traits related to drought tolerance. He observed stronger drought resistance in *O. officinalis* accessions as in our case. Greater membrane stability, more stomatal conduction and elongation of the leaves along with higher root mass in deeper soil levels were also observed under water deficit among *O. rufipogon* and *O. longistaminata* accessions as compared with *O. sativa* by Liu et al. (2004). One of the probable explanations for the occurrence of novel alleles for drought tolerance in *O. longistaminata* and *O. latifolia* is their natural habitat, that is, *O. longistaminata* usually found in seasonally dry areas, whereas *O. latifolia* used to be found on hill slopes. Therefore, they might have developed some adaptive traits for their survival under adverse environment. In the context of occurrence of drought responsive traits in *O. rufipogon* accessions, the putative role of differentially expressed tissue-specific miRNA was explained by Zhang et al. (2017). He suggested that these differentially expressed miRNA might be involved in the regulation of the auxin pathways, flowering pathways, drought pathways and lateral root development and hence conferring resistance against water deficit. The transfer of drought tolerance from the identified *O. rufipogon* (IRGC 89006, IRGC 106433) and *O. longistaminata* (IRGC 92619A) accessions to elite rice cultivars PR121 and PR122 have already been initiated at the Punjab Agricultural University, which would definitely help in getting sustainable yield under water stress.

Supplementary material

The supplementary material for this article can be found at <https://doi.org/10.1017/S1479262117000284>.

Acknowledgement

The authors are thankful to the International Rice Research Institute, Philippines, Manila and National Rice Research

Institute, Cuttack, India for providing wild species germplasm of rice. The authors also acknowledge the School of climate change and agricultural meteorology, Punjab Agricultural University, Ludhiana for providing meteorological data.

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