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Assessment of drought resistance among wild rice accessions using a protocol based on single-tiller propagation and PVC-tube cultivation

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Abstract

To assess the variation in drought resistance among wild rice accessions, a protocol was developed based on single-tiller propagation and PVC-tube cultivation. Severe water stress was applied at late vegetative growth stage. The responses of eight accessions of *Oryza rufipogon* and one accesstion of *O. officinalis* were evaluated by measuring the morphological and physiological traits, including leaf rolling score, leaf water potential, free proline content, chlorophyll content, above-ground biomass, maximum root depth, maximum root length, root weight, deep root percentage and root/shoot ratio. Wild rice accessions showed differential abilities to tolerate and recover from the water deficit. The accession of *O. officinalis* had much stronger drought tolerance than all *O. rufipogon* accessions. Two *O. rufipogon* accessions (Or03, Or08) from Hainan (as a tropical island) had the best performance under drought stress, while two other accessions of (Or15, Or33) from Guangdong and Jiangxi provinces were sensitive to water deficit. The results revealed the preliminary evidences that *O. rufipogon* accessions from tropical area have stronger drought resistance than accessions from sub-tropical areas.

Keywords: biomass; drought resistance; root system; vegetative stage; wild rice.

Abbreviations: T1, T2-drought stress for 20 days, 28 days; T3-one month after restored irrigation; SWC-soil water content (SWC1 and SWC2 mean the measurements at T1 and T2, similarly for other traits); FPC-free proline content; LWP-leaf water potential; LCC-leaf chlorophyll content; LRS-leaf rolling score; RD -maximum root depth; RL-maximum root length; SRV-shallow root volume; DRV -deep root volume; SRW-shallow root weight; DRW-deep root weight; RW-root weight as the sum of SRW and DRW; AGB-above-ground biomass per tube.

Introduction

Drought is a major abiotic stress that causes severe yield loss in rice as a staple food crop. Improvement of drought resistant rice varieties has become an urgent task under the background of global crisis of water resource. Genetic resources played very important roles in crop genetic improvement, especially in targeting the resistance to biotic or abiotic stresses. Germplasm from wild relatives has widely been used as donors of resistant genes to diseases or pests in many crops. Wild rice accessions has great contribution in rice breeding by providing resistance genes (e.g. *Xa21*, *BPH14*, *BPH15*) (Ronald et al., 1992; Song et al., 1995; Yang et al., 2004; Du et al., 2009; Hu et al., 2012). It was also reported that wild rice can carry positive alleles of QTLs influencing grain yield or quality (Xiao et al., 1998; Fu et al., 2010; Mallikarjuna Swamy et al., 2011). There are tremendous differences in growth habits (e.g. photoperiodic response, seed dormancy, and prostrate growth, etc.) between wild rice and cultivated rice, or among wild rice species (Oka, 1974; Vaughan, 1994; Cai and Morishima, 2006; Tan et al., 2008). Because of the high percentage of outcrossing, wild rice accessions and their original populations contain heterozygous genetic background (Barbier, 1989; Morishima and Barbier, 1990; Vaughan, 1994). The seed-derived descendants of wild rices are usually segregating. On the other hand, seed dormancy is a popular characteristic of wild rice accessions (Vaughan, 1994; Cai and Morishima, 2006). So, it becomes difficult to prepare seedlings in synchronized growth situation across different wild rice accessions. Vegetative reproduction has been widely used in preservation of wild rice germplasm by transplanting the tillers. Replicated individuals with consistent genotypes and approximate growth situation could be developed via 1-2 rounds of tiller transplanting. As the identification of resistant genotypes to drought or other abiotic stresses heavily depends on population size and growth stage of plant (Boonjung and Fukai, 1996) very few reports have been published on screening of drought resistance in wild rice species. In few cases, screening was conducted in the backcross populations derived from single accession of wild rice. Thanh et al. (2006) obtained 39 drought resistant BC_1F_2 lines by backcrossing *O. meridionalis* or *O. nivara* to two varieties of *O. sativa* (RD23 and CN1). Zhang et al. (2006) developed a population of 159 introgression lines using an elite indica variety Guichao No2 as the receptor and Dongxiang wild rice (*O. rufipogon*) as the donor. The breeding line,IL23, contained two QTLs of drought resistance from the wild rice accession. Natural populations of *O. rufipogon* had wide distribution in southern China. From Hainan island as the most southern area to Jiangxi and Hunan provinces as the most northern area, the climate changes from tropical type (rain/dry seasons) to sub-tropical type (four seasons), respectively. In this study, wild rice accessions from Hainan, Guangdong and Jiangxi were evaluated for drought resistance via PVC-tube cultivation of replicated plants from vegetative reproduction. The result should be helpful to understand the variation in drought resistance among wild rice germplasm.

Results

Change of soil water content and general responses of wild rice to drought stress

According to the readings from the portable instrument of ZL-90, soil water content (SWC) was much lower than CK in the drought-treated tubes and decreased continuously along the duration of drought stress, i.e. 10-20% at T1 to about or lower than 10% at T2 (Fig. 1). SWC also varied among tubes growing plants of different wild rice accessions, probably caused by the occasional differences in soil structure and the water consumption rate of plants. The correlation coefficients between SWC and different characters of plants are shown in Table 1. The SWC2 had significant correlations with all measurements of plants except DRW, while SWC1 was also correlated with most plant characters. It is obvious that the low water contents in treatment group had severe impact on the morphological and physiological performances of wild rice plants. Under drought stress at vegetative stage, wild rice plants had a series of changes on morphological or physiological criteria of above-ground plant parts, e.g. decreased LWP and increased FPC at T1 and T2 (Fig. 2), darker leaf color in most accessions and increased SPAD values for different proportions (Fig. 3a), higher LRS and desiccation of bottom leaves (Fig. 3b, Fig. 4) and decreased AGB except in Oo01 and Or34 (Fig. 3c). For root traits, RD and RL increased in a few accessions. The RW and RV declined extremely and the proportion of DRW in RW raised in all accessions (Fig. 5). The ratio of RW/AGB was inclined to decrease under drought, possibly against the common prediction. It was probably caused by the compensative growth during the period after restoring of irrigation. The correlation coefficients indicated the parallel or inverted changes under drought among morphological or physiological traits (Table 1). The LWP was highly correlated with eleven other traits (except RD and RL) at T2. The LRS had a similar situation as LWP, suggesting again both traits are effective to indicate the intensity of stress. It is noticeable that weight or volume of shallow root or deep root had positive impacts on other traits, e.g. negative correlations with FPC and LRS and positive correlations with LWP and AGB.

Varied responses to drought stress among wild rice accessions

Leaf water potential (LWP) and free proline content (FPC)

Among those wild rice accessions, the changes in LWP and FPC were comparable, i.e. temperate changes at T1 and extreme decrease or increase at T2 (Fig. 2). Wild rice accessions could be divided into three groups according to variation in LPW and FPC. In group 1, Oo01 had hardly any changes and Or08 had decreased LPW and increased FPC only at T2, but for much less extents than other accessions. In group 2, five accessions (Or01, Or03, Or05, Or06 and Or34) showed moderate changes in LWP and sharp increases of FPC at T2. Included in group 3, Or15 and Or33 had significant changes in both LWP and FPC at T1, decreased or increased drastically at T2 to reach the lowest LWP and highest FPC among all wild rice accessions.

Leaf chlorophyll content (LCC), leaf rolling score (LRS) and above-ground biomass (AGB)

Varied LCC was observed among wild rice accessions under non-stress condition. The accession of *O. officinalis* (Oo01) had the lowest value. Three *O. rufipogon* accessions (Or01, Or05 and Or06) had high LCC while the others fell in between. The LCC of stressed plants at T1 were higher than CK plants by different proportions. Briefly, there were larger LCC increasing in Oo01, Or08 and Or34 than in the others (Fig. 3a). After drought stress for 20 days (T1), four accessions (Or06, Or15, Or33 and Or34) had rolled leaves while this symptom start to appear in plants of Or01 and Or05. There isn’t typical leaf rolling in other wild rice accessions at T1 (Fig. 3b). When the stress continued for another week (T2), the accession of *O. officinalis* (Oo01) still had no leaf rolling while all accessions of *O. ruffipogon* had leaf rolling for varied levels (Fig. 3b, Fig. 4a). Three accessions (Or06, Or15 and Or33) appeared the worst (almost all leaves rolled, i.e. grade 4-5; Fig. 3b, Fig. 4b); three accessions (Or01, Or05 and Or34) had average scores of 3-4 and larger variance among tubes; two accessions (Or03 and Or08) had mild symptom (grade 2; Fig. 4a). Several genotypes of cultivated rice with different drought resistance were tested using similar protocol. Sharp differences in symptom of water deficit were shown in the picture that was taken on the same date as the wild rice accessions (Fig. 4c). Three lines, IRAT109 as a typical upland variety and two drought tolerant introgression lines (DT001 and DT002), maintained good growth status. Drought sensitive introgression line (DS001) is the most sensitive genotype to water deficit as it had severe leaf desiccation and growth inhibitation in comparison with the plants under normal condition (picture not shown). As the recurrent parent of those introgression lines, Zhong 413 (Z413) had high level of leaf rolling but less desiccation, similar to Hanyou 3 as a hybrid combination. From the comparison between wild rice accessions and cultivated rice genotypes, the variation in drought resistance among wild rice accessions is parallel to that among cultivated rice genotypes. The growth rates of wild rice plants had large variance among accessions under CK condition so that the final AGB had a wide range of 25g to 130g. Or15 was the highest; Or33 the second; Or06, Or01, Or05, Or03 and Or08 became lower in the descent order; Oo01 and Or34 had similar AGB as the lowest. Severe drought stress restrained the growth speed of wild rice plants so that the AGB of all accessions fell into a narrow range around 30g. The loss of AGB caused by drought varied among accessions, whereas it was very high in Or15 and Or33, very low in Oo01 and Or34, in comparison with CK condition. The five others had values between (Fig. 3c).

Root traits and root-to-shoot ratio

Limited by the capacity of PVC tubes, soil cylinders had lengths of about 85 cm and diameters less than 20 cm. Six *O. rufipogon* accessions (Or01, Or03, Or05, Or06, Or08 and Or15) had maximum root length (depth) of ≥80 cm under CK or drought condition, i.e. the root system reached the bottom of the tubes (Fig. 5a). *O. officinalis* accession (Oo01) had root length of 50 cm under CK and 80 cm under drought stress. The remaining two accessions (Or33 and Or34) had the root lengths from 70 cm under CK to about 80 cm under drought stress. Therefore, there was about 50% increase of root length (depth) after drought treatment in Oo01, less than 15% in Or33 and Or34, and little in other accessions. The maximum root length was significantly high in Oo01, Or03, Or06 and Or08; moderate in Or01, Or05 and Or34, and low in Or15 and Or33 under drought stress (Fig. 5b). Root dry weight and root volume had similar patterns of variance. According to RW, root biomass was largely different among wild rice accessions under CK condition (Fig. 5c). The Oo01 and Or34 had low RW while other accessions had much higher values, partially similar to the pattern of AGB. Drought stress also largely restrained root growth, resulting similar RW among accessions. In comparison with the RW under CK condition, RW of Oo01 and Or34

Table 1. The correlation coefficients among soil water contents and plant traits of wild rice accessions.

	SWC1	SWC2	FPC1	FPC2	LWP1	LWP2	LCC	LRS	RD	RL	SRV	DRV	SRW	DRW
SWC2	0.899**													
FPC1	-0.387**	-0.371**												
FPC2	-0.474**	-0.439**	0.858**											
LWP1	0.215	0.294*	-0.332*	-0.359**										
LWP2	0.710**	0.689**	-0.649**	-0.726**	0.367**									
LCC	-0.669**	-0.678**	0.150	0.208	-0.327*	-0.367**								
LRS	-0.707**	-0.642**	0.549**	0.779**	-0.321*	-0.868**	0.379**							
RD	-0.428**	-0.360**	0.122	0.084	0.070	-0.114	0.559**	0.130						
RL	-0.373**	-0.360**	0.028	-0.052	-0.064	-0.081	0.468**	0.035	0.709**					
SRV	0.463**	0.663**	-0.362**	-0.424**	0.576**	0.584**	-0.418**	-0.530**	-0.014	-0.143				
DRV	0.273	0.357**	-0.275*	-0.350*	0.247	0.482**	0.015	-0.401**	0.404**	0.362**	0.614**			
SRW	0.469**	0.609**	-0.326*	-0.385**	0.488**	0.569**	-0.326*	-0.521**	0.049	-0.183	0.927**	0.577**		
DRW	0.170	0.222	-0.241	-0.311*	0.204	0.398**	0.161	-0.311*	0.442**	0.327*	0.514**	0.872**	0.597**	
AGB	0.266	0.396**	-0.271	-0.303*	0.570**	0.434**	-0.224	-0.374**	0.184	-0.109	0.798**	0.416**	0.903**	0.495**

* and ** represent the significant levels of $P \leq 0.05$ and $P \leq 0.01$, respectively.

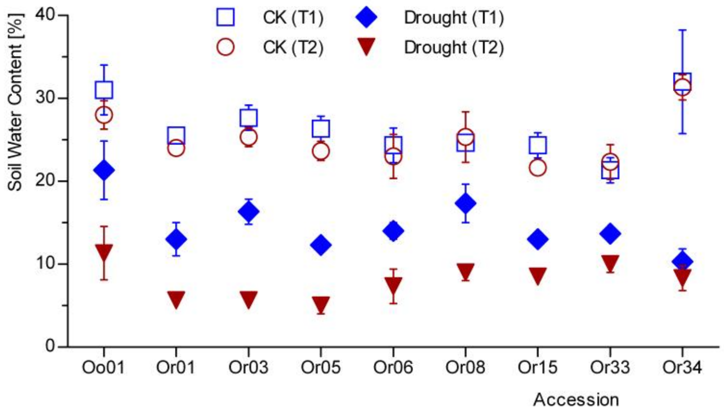

Fig 1. The soil water contents in the PVC tubes growing plants of different wild rice accessions under normal condition and after droght stress for 20 days (T1) and 28 days (T2) (Error bars represent standard deviations).

changed slightly under drought while other accessions had sharp decreases. Four accessions (Oo01, Or05, Or06 and Or08) maintained relatively higher root weight under drought stress. Shallow root weight (SRW) varied widely under CK among wild rice accessions, declined sharply, and lost the differences among accessions under drought treatment (Fig. 5d). Deep root weight (DRW) showed large variance among accessions under CK, declined for smaller proportion, maintained the variance among accessions under drought stress (Fig. 5e). In other words, the variance of DRW among accessions under drought was still parallel to the tendency under CK. The percentage of DRW in RW varied from 20% to 40% under CK, but increased to the range of 40% to 60% under drought. Five accessions (Oo01, Or03, Or05, Or06 and Or08) had deep roots for more than 50% in weight (Fig. 5f). The root-to-shoot ratio (RW/AGB) had variance among wild rice accessions, i.e. high in Oo01, Or03, Or05, Or06 and Or08; low in Or01, Or15, Or33 and Or34. Drought stress reduced the ratios in several accessions. However, in comparison with other traits, RW/AGB ratios showed smaller differences either among accessions or between treatments (Fig. 5g).

Overall evaluation of drought resistance among wild rice accessions

According to each of the observed traits, the wild rice accessions could be ranked for drought resistance. The resulted orders showed a common tendency but varied partially using different traits. Following the method in evaluation of genotypes using multiple parameters (Zeng et al., 2002; El-Hendawy et al., 2005), cluster analysis was conducted using data of all traits to obtain a general estimation of drought resistance of wild rice accessions (Fig. 6). Four accessions (Or01, Or03, Or08 and Oo01) exhibited strongest drought resistance and very similar performances. Or05 and Or34 were two accessions having strong drought resistance and little difference from the above four accessions. There is a larger decrease of drought resistance for Or06. Two remaining accessions (Or15 and Or33) are highly sensitive to drought stress, forming a clade with very large distance to all other accessions.

Discussion

The practicable screening protocol for drought resistance among wild rice accessions

The primary criteria for evaluation of drought resistance of cultivated rice varieties is the grain yield under drought stress (Atlin, 2003). Drought stress could be applied at vegetative or reproductive stage of rice plants. It was found that the results of evaluation at two stages could be largely different, suggesting possible differences in drought tolerance mechanisms (Lilley and Fukai, 1993; Boonjung and Fukai, 1996). The ability of rice plant to overcome the drought stress during the initiation of panicles appeared to be more important for the final grain yield than the drought stress during early stages. But the drought resistance at early growth stage is a precondition of final grain yield by keeping the seedlings survive or in well growth situation under drought at this period. Most wild rice species are perennial and sensitive to photoperiod (Barbier, 1989; Vaughan, 1994), causing the yield inconsistency and long (different) period for heading. The yield potential of wild rice plants is very poor and seldom regarded as an important criterion, *per se*. So, it is quite difficult and less meaningful to judge the drought resistance by measuring the grain yield of wild rice plants under drought. As outcrossing is popular in

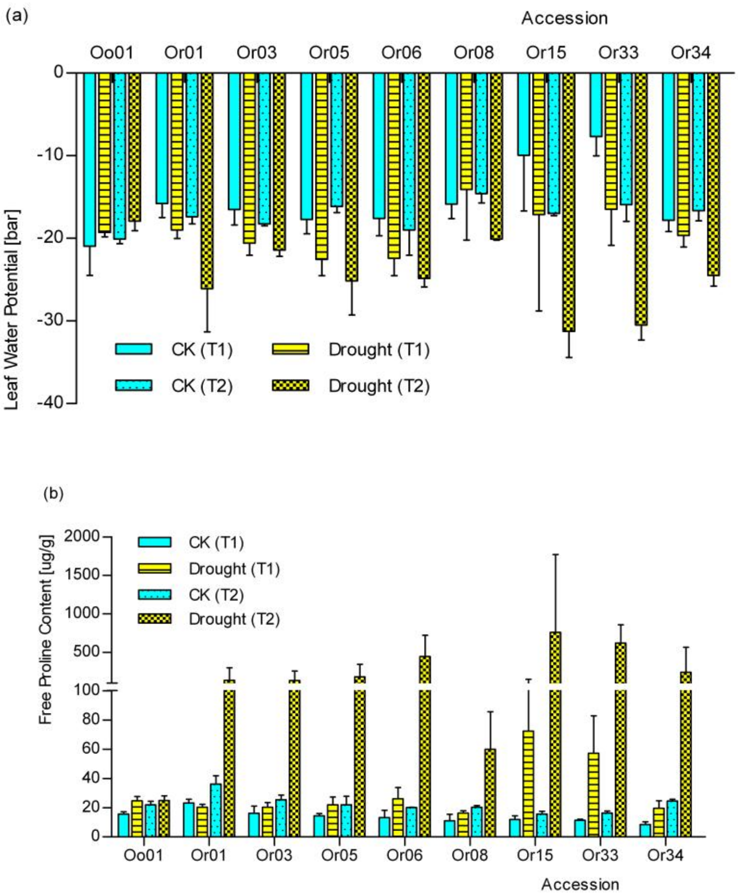

Fig 2. Changes of leaf water potential and free proline content after drought stress among wild rice accessions (Error bars represent standard deviations).

wild rice (Barbier, 1989; Vaughan, 1994), vegetative propagation in nurseries is widely used to preserve the germplasm for years around the world. This method can keep the original genetic components in comparison to sexual propagation. In this experiment, two-round tiller reproduction were used to generate replicated individuals within each wild rice accession that had well homogenized starting biomass and tiller numbers. Based on PVC-tube cultivation, water deficit with proper severity was applied to wild rice plants. A series of morphological or physiological characters, including root traits, were measured as criteria of drought resistance. Comprehensive evaluation of drought resistance was effectively achieved using cluster analysis of multiple traits, following the method in previous reports (Zeng et al., 2002; El-Hendawy et al., 2005). The variance in drought resistance among wild rice accessions could be well revealed. Therefore, this protocol is practicable to screen the wild rice germplasm for drought resistance at vegetative stage.

Possible differentiation of drought resistance in O. rufipogon related to habitat climate

The accessions of *O. rufippogon* in this study were originally collected from Hainan, Guangdong and Jiangxi provinces in China, geologically orientated from south to north. Hainan is an island located in the tropical zone, having annual alteration of rain season and dry season. It has less than 400 mm average rainfall during the period from November to April each year.

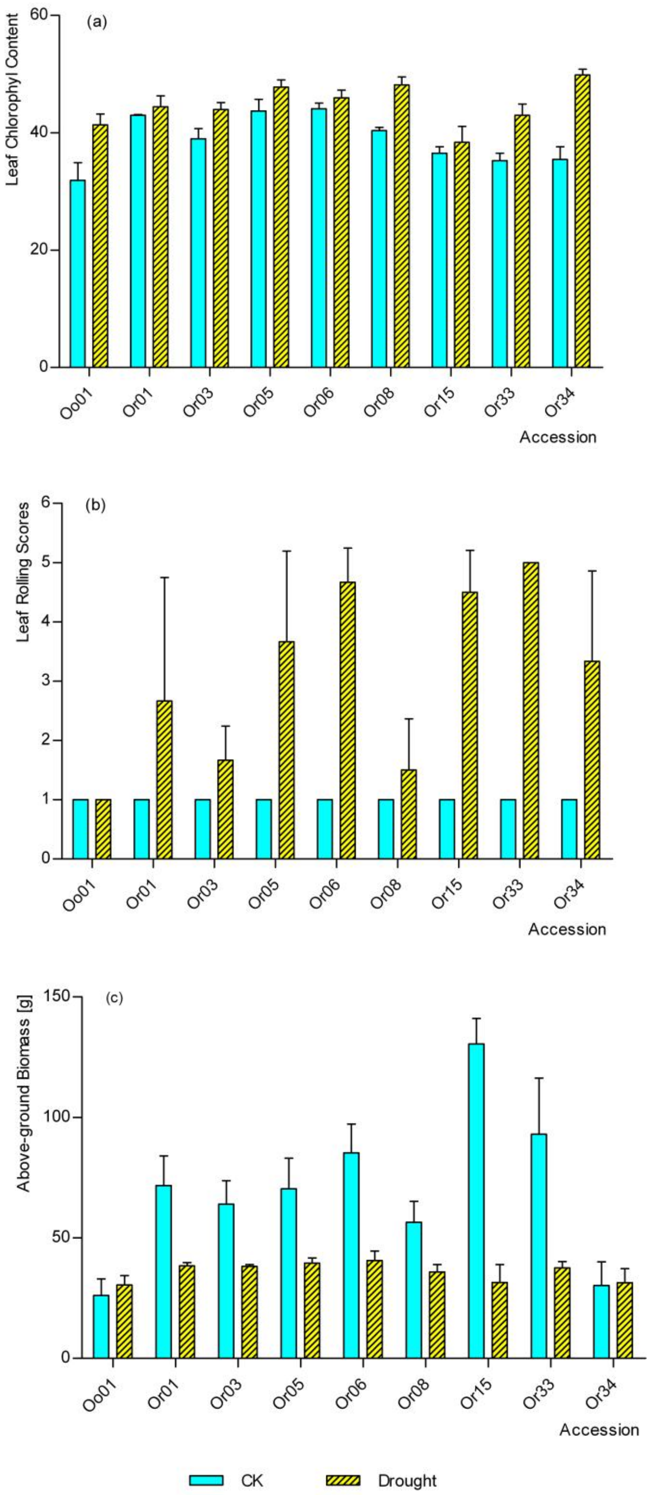

Fig 3. The influence of drought stress on leaf chlorophyll content, leaf rolling score and above-ground biomass per tube of wild rice accessions (Error bars represent standard deviations).

Drought frequently happens during March and April along with less rainfall and high temperature. Water deficit could easily develop in the habitats of wild rice populations in this area. Guangdong and Jiangxi located in the intermediate zone between tropical and sub-tropical climates, having abundant precipitation and alteration of four seasons. The occurrence of drought together with high temperature in wild rice habitats might have less frequency in this area. In this experiment, four accessions from Hainan (Or03, Or05, Or06 and Or08) showed good performance under drought stress. Among four accessions from Guangdong (Or01 and Or15) and Jiangxi (Or33 and Or34), two accessions (Or15 and Or33) were highly sensitive to drought stress while another (Or34) grew weakly under both normal and drought conditions. Therefore, we suggest that the wild rice accessions collected from Hainan Island have higher drought resistance than those from Guangdong and Jiangxi provinces.

Possible utilization of wild rice accessions in breeding for drought resistance

As an external example to *O. rufipogon,* one accession of *O. officinalis* was included in this experiment. The plants of this accession (Oo01) grew very well under both CK and drought stress with emerging panicles and having normal seed-setting as well (Fig. 4a). Compared to CK, there were no significant changes in LWP, FPC, LRS, AGB and RW, but we observed increase in LCC, RD, RL and DRW, under drought stress. In addition, we noticed that more roots distributed at shallow soil layer under normal condition (possibly in favor of absorption of nutrients and water after short rainfall), while doubled RD and DRW observed under drought condition. This kind of adjustment in rice plants is very helpful to obtain a high level of drought avoidance mechanism. Having CC genome and low biomass, the value of this accession in breeding of *O. sativa* is still unclear. Among *O. rufipogon* accessions, Or03 and Or08 could be recommended as wild rice germplasm in the breeding program targeting the drought resistance. Both accessions had better performance in LWP, FPC and LRS than other drought resistant accessions (Or01, Or05 and Or34). Despite the fact that Or15 and Or33 showed poor performance under drought stress, both accessions had highest biomass and shallow root criteria than other materials under normal condition. Therefore, they should be appropriate germplasm if considering the growth vigor and grain yield under well irrigated conditions. The upland rice varieties normally had less tillers, lower biomass and grain yield in comparison to lowland genotypes (Gupta and O'Toole, 1986). This tendency existed among wild rice accessions with different drought resistance according to the result of this experiment. Recently, water-saving and drought-resistance rice (WDR) was suggested as a new type of rice varieties by Luo (2010). Favorable characters from modern lowland varieties (like high yielding, resistance to multiple pests and diseases, etc.) and the drought resistance from upland varieties will be integrated in WDR breeding program. Large scale screening of rice germplasm, perhaps including the wild relatives, should probably be the starting step to check if the dilemma between high yielding and drought resistance could be broken. The wild rice accession with both drought resistance and high growing speed, if available, will be valuable in the breeding of cultivated rice varieties which can produce high biomass and grain yield under both irrigated and rainfed conditions.

Materials and methods

Wild rice accessions and cultivated rice genotypes

Wild rice accessions are preserved in a nursery in Baihe Experimental Farm in Qingpu District of Shanghai City *in situ*. Nine accessions were used in this experiment as one accession of *O. officinalis* (Oo01); eight accessions of *O. rufipogon*, including four accessions from Hainan (Or03, Or05, Or06,

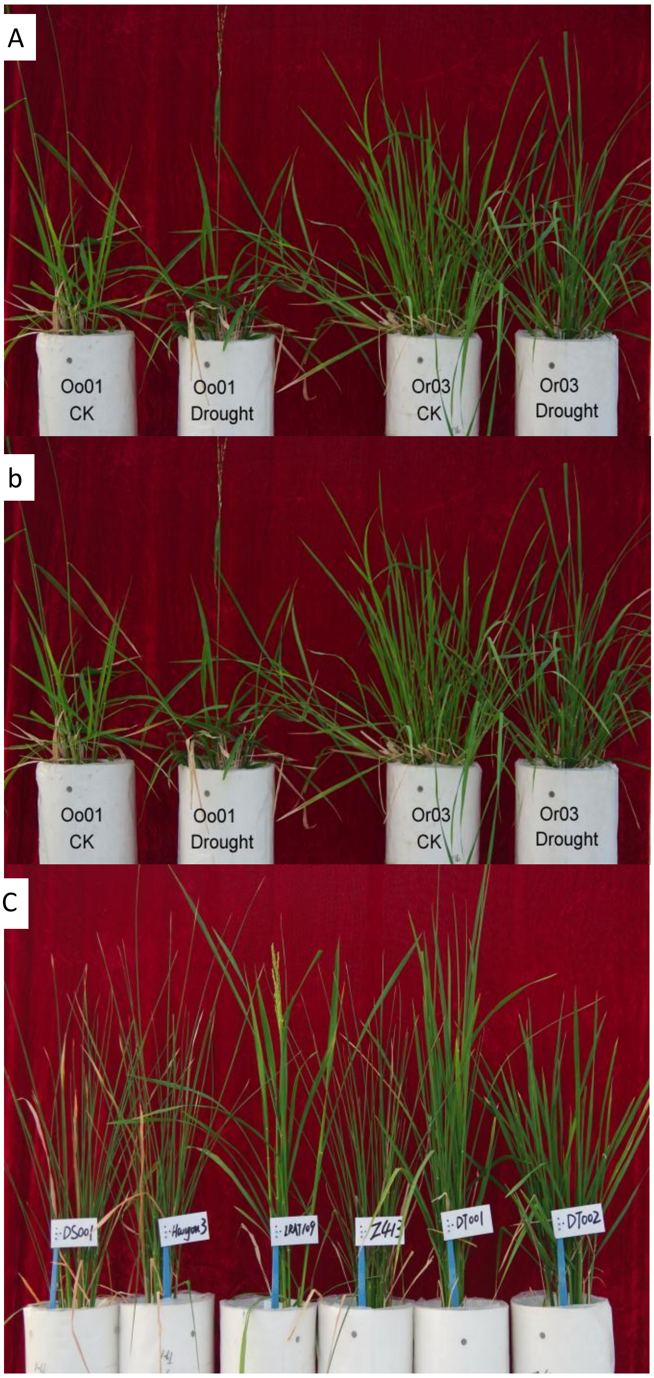

Fig 4. Performance of wild rice plants (a, b) and cultivated rice plants (c) under normal condition (CK) and drought stress (Drought) at T2 (DS001-drought sensitive introgression line; Hanyou3-hybrid combination; IRAT109-upland rice variety; Z413-Zhong413 as a paddy rice variety, the recurrent parent of DS001, DT001 and DT002; DT001 and DT002-drought tolerant introgression lines)

Or08), two accessions from Guangdong (Or01, Or15) and two accessions from Jiangxi (Or33, Or34). In the same place, six genotypes of cultivated rice were tested using similar protocol, including upland rice variety IRAT109, high yielding restorer line Zhong413 (Z413), three introgression lines (ILs) using Z413 as the recurrent parent (DS001 as a drought sensitive line; DT001 and DT002 as two drought tolerant lines), and a hybrid combination with moderate drought resistance (Hanyou 3). Grains were soaked in water for 24 hrs and sowed to nursery on May 30th. Seedlings were transplanted into PVC tubes one month later. In one (T7) of seven treatments, drought stress was initiated on 20th day after trantsplanting. Photoes were taken for both cultivated rice genotypes and wild rice accessions at the same day (Fig. 4)

PVC-tube cultivation and drought stress treatment

Each PVC tube had a diameter of 20 cm and a height of 100 cm, having two columns of small holes (1.4 cm in diameter) on the wall at heights of 5, 35, 65, 95 cm from the bottom. A plastic bag with same size as the tube was put inside. A mixture of dry soil and sand (2:1) with total weight of 38 Kg, with 25g fertilizer ($N:P_2O_5:K_2O$=15:15:15) were prepared by an electric blender and filled in each tube. Before and after the stress, the holes on tube wall were filled with rubber plugs to stop the leaking of water from the tube (Yue et al., 2006).

Single tiller was separated and transplanted in the field with space of 20 × 10cm. Two months later, twelve seedlings with similar size were selected in each accession. To avoid the influence from the primary difference in biomass and tiller numbers among those seedlings, six pairs of seedling were formed by putting together the maximum with the minimum, the second maximum with the second minimum, and so on. Each pair of seedlings was transplanted in a PVC tube after cutting roots and most part of the leaves. The seedlings in each tube have 8-12 tillers for *O. rufipogon* and about 5 tillers for *O. officinalis*. Three weeks after transplanting, two contrasting treatments were applied with three replicated tubes in each. The control tubes (CK) kept about 2 cm water layer by adding water at late afternoon every day. For the stress treatment (Drought), the rubber plugs were removed and the plastic bags were pierced to leak out the water from the tubes (T0 time). Rain was kept off by closing the roof during periods of rain. The treatment lasted for four weeks to allow the development of water stress, and ended by restoring water supply like the control tubes. Observation of morphological traits and measurement of physiological characters was conducted at three time points, i.e. T1 (20 days after T0), T2 (28 days after T0), and T3 (one month after restoring of water supply).

Measurements of morphological and physiological traits

Soil water content (SWC) at 60 cm depth was monitored by a portable instrument (ZL-90, model 9109-2; Xintai instrument, Shanghai, China) at T1 and T2. Free proline content (FPC) was measured in leaves at T1 and T2 according to the commonly used protocol (Bates et al., 1973). Leaf water potential (LWP) was measured using portable plant pressure chamber PMS1000 (PMS instrument, USA) at T1 and T2 by sampling the last full-extended leaves from three tillers for each tube. Leaf chlorophyll content (LCC) was measured in the middle of the last full-extended leaf blades using SPAD 502 (Konicaminolta, Japan) at T1. The average from three tillers in each tube was used for further analysis. Leaf rolling scores (LRS) was recorded as grade 1 to 5 at T2 (O'toole and Moya, 1978). The above-ground biomass and root traits were measured at T3. The plants, together with soil cylinders, were removed from the PVC tubes and the plastic bags. Maximum root depth (RD) was recorded by measure the distance of the deepest part of roots from soil surface. Soil was washed away to obtain the root system. The maximum root length (RL) was measured. Cutting at the base of shoot and 30cm beneath the base of shoot, each plant was divided into three parts, i.e. above-ground biomass, shallow roots and deep roots. Shallow root volume (SRV) and deep root volume (DRV) were measured by overflowing the water in measuring cylinder. Above-ground biomass per tube

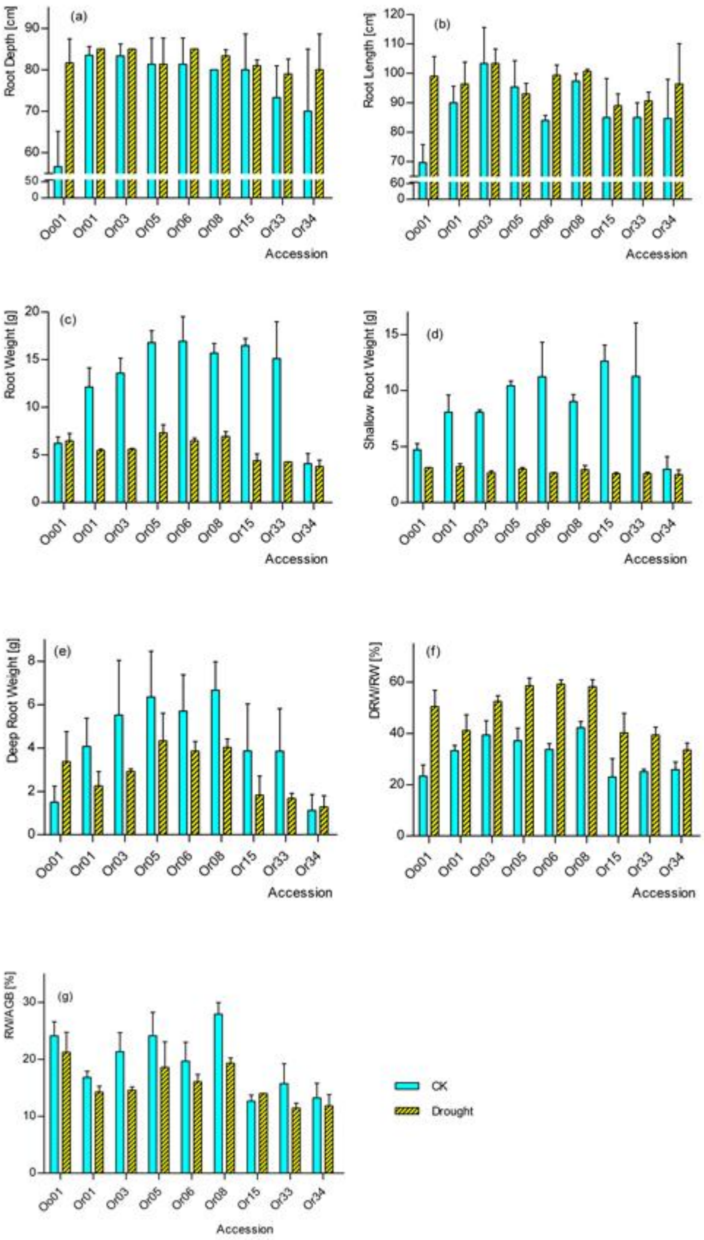

Fig 5. The influence of drought stress on root traits and root/shoot ratio of wild rice accessions (Root weight data are measurements per tube; Error bars represent standard deviations).

(AGB), shallow root weight per tube (SRW) and deep root weight per tube (DRW) was measured after drying in 80 ºC oven for two days.

Statistical analysis

The Pearson correlations among soil water contents and plant traits of wild rice accessions and ANOVA were calculated using SPSS for Windows v16.0. The charts of plant traits were prepared using the GraphPad PRISM v5.01 (http://www.graphpad.com). Cluster group rankings were used to estimate the drought resistance among wild rice accessions based on multiple characters, similar to the evaluation of salt tolerance among rice or wheat genotypes (Zeng et al., 2002; El-Hendawy et al., 2005). All the data were converted to relative values such as drought tolerance indices, which was defined as the means under drought stress divided by the means of the controls. Ward's minimum-variance cluster analysis was conducted on the means of the drought tolerance indexes for all traits using SAS Cluster procedure (SAS Institute, 2000). Dendrogram was used to present the clustering result with group numbers determined by maximum pseudo t^2 value.

Conclusion

A protocol was developed based on single-tiller propagation and PVC-tube cultivation to assess the variation in drought tolerance among wild rice accessions. Wild rice accessions showed varied abilities to deal with and recover from the water deficit. Wild rice accessions from tropical island, where it has low precipitation and high temperature in dry season, appeared to have stronger drought resistance than the accessions from sub-tropical area. The result indicated there was differentiation of drought resistance in wild rice, probably relating to the character of habitat environment.

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References

Atlin G (2003) Improving drought tolerance by selecting for yield. In: Fischer KS, Lafitte R, Fukai S, Atlin G, Hardy B (*eds*) Breeding rice for drought-prone environments. IRRI, Philippines

Barbier P (1989) Genetic variation and ecotypic differentiation in the wild rice species *Oryza rufipogon*. II. Influence of the mating system and life-history traits on the genetic structure of populations. Jpn J Genet 64: 273-285

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205-207

Boonjung H, Fukai S (1996) Effects of soil water deficit at different growth stages on rice growth and yield under upland conditions. 2. Phenology, biomass production and yield. Field Crop Res 48: 47-55

Cai HW, Morishima H (2002) QTL clusters reflect character associations in wild and cultivated rice. Theor Appl Genet 104: 1217-1228

Du B, Zhang WL, Liu BF, Hu J, Wei Z, Shi AY, He RF, Zhu LL, Chen RZ, Han B, He GC (2009) Identification and characterization of *Bph14*, a gene conferring resistance to brown planthopper in rice. Proc Natl Acad Sci USA 106: 22163-22168

El-Hendawy SE, Hu YC, Yakout GM, Awad AM, Hafiz SE, Schmidhalter U (2005) Evaluating salt tolerance of wheat genotypes using multiple parameters. Eur J Agron 22: 243-253

Fu Q, Zhang PJ, Tan LB, Zhu ZF, Ma D, Fu YC, Zhan XC, Cai HW, Sun CQ (2010) Analysis of QTLs for yield-related traits in Yuanjiang common wild rice (*Oryza rufipogon* Griff.). J Genet Genomics 37: 147-157

Gupta PC, O'Toole JC (1986) Upland rice a global perspective. Los Banos, Laguna, Philipines, IRRI

Hu J, Li X, Wu CJ, Yang CJ, Hua HX, Gao GJ, Xiao JH, He GC (2012) Pyramiding and evaluation of the brown planthopper resistance genes *Bph14* and *Bph15* in hybrid rice. Mol Breeding 29: 61-69

Lilley JM, Fukai S (1993) Effect of timing and severity of water deficit of four diverse rice cultivars I. Rooting pappern and soil water extraction. Field Crop Res 37: 205-213

Luo LJ (2010) Breeding for water-saving and drought-resistance rice (WDR) in China. J Exp Bot 61: 3509-3517

Mallikarjuna Swamy BP, Kaladhar K, Ramesha MS, Viraktamath BC, Sarla N (2011) Molecular mapping of QTLs for yield and related traits in *Oryza sativa* cv Swarna x *O. nivara* (IRGC81848) backcross population. Rice Sci 18: 178-186

Morishima H, Barbier P (1990) Mating system and genetic structure of natural populations in wild rice *Oryza rufipogon*. Plant Species Biol 5: 31-39

Oka HI (1974) Experimental studies on the origin of cultivated rice. Genetics 78: 475-486

O'Toole JC, Moya TB (1978) Genotypic variation in maintenance of leaf water potential in rice. Crop Sci 18: 873-876

Ronald PC, Beng A, Rodante T, Abenes L, Wu KS, McCouch SR, Tanksley SD (1992) Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*. Mol Gen Genet 236: 113-120

SAS Institute, 2000. SAS User's Guide, version 4.0.2. SAS Inst., Cary, NC, USA.

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald P (1995) A receptor kinase-like protein encoded by the rice disease re-sistance gene *Xa21*. Science 270: 1804-1806

Tan LB, Li XR, Liu FX, Sun XY, Li CG, Zhu ZF, Fu YC, Cai HW, Wang XK, Xie AX, Sun CQ (2008) Control of a key transition from prostrate to erect growth in rice domestication. Nat Genet 40: 1360-1364

Thanh PT, Sripichitt P, Chanprame S, Peyachoknagul S (2006) Transfer of drought resistant character from wild rice (*Oryza meridionalis* and *Oryza nivara*) to cultivated rice (*Oryza sativa* L.) by backcrossing and immature embryo culture. Kasetsart J (Nat Sci) 40: 582-594

Vaughan D (1994) The wild relatives of rice: a genetic resources handbook. IRRI, Los Banos, Philipines, 147pp.

Xiao JH, Li JM, Grandillo S, Ahn SN, Yuan LP, Tanksley SD, McCouch SR (1998) Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. Genetics 150: 899-909

Yang HY, You AQ, Yang ZF, Zhang FT, He RF, Zhu LL, He GC (2004) High-resolution genetic mapping at the *Bph15* locus for brown planthopper resistance in rice (*Oryza sativa* L.). Theor Appl Genet 110: 182-191

Yue B, Xue WY, Xiong LZ, Yu XQ, Luo LJ, Cui KH, Jin DM, Xing YZ, Zhang QF (2006) Genetic basis of drought resistance at reproductive stage in rice: separation of drought tolerance from drought avoidance, Genetics 172: 1213-1228

Zhang X, Zhou SX, Fu YC,Su Z, Wang XK, Sun CQ (2006) Identification of a drought tolerant introgression line derived from dongxiang common wild rice (*O. rufipogon Griff.*). Plant Mol Biol 62: 247-259

Zeng L, Shannon MC, Grieve CM (2002) Evaluation of salt tolerance in rice genotypes by multiple agronomic parameters. Euphytica 127: 235-245