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REVIEW PAPER

Breeding for water-saving and drought-resistance rice (WDR) in China

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Abstract

Rice is the staple food and rice production consumes about 50% of the fresh water resources in China. In addition, drought is one of the most important constraints in rice resulting in large yield losses and limiting the average yield increase of the country. There is an urgent need to enhance water-saving (W) capacity or drought resistance (DR) of rice. WDR varieties can be developed through introgressing the water-saving and drought resistance capacity mainly from the traditional upland to the commercialized paddy rice cultivars. The breeding target is a high yield potential under irrigation, an acceptable grain quality, and water consumption reduced by about 50% compared with paddy rice. In a water-limited environment, a higher level of drought resistance and reduced yield loss by drought stress are required. In recent years, the field drought-resistance screening facility was established and the evaluation standard was developed. Some DR rice varieties were identified and used in both molecular mapping and breeding programmes. Several WDR varieties were developed and released to farmers. This article describes our initial achievement towards this goal and provides some details on the rationale and the specific steps and methods used.

Key words: Breeding, drought resistance, gene/QTLs, hybrids, rice variety, water-saving, WUE.

Introduction

China is facing two major challenges: food security and water shortage, particularly in the rainfed areas of China where a significant proportion of the population is still in absolute poverty. Rice is the staple food for most Chinese people and is regarded as a strategic commodity in China. Historically, it has played an important role in ensuring food security (Luo and Zhang, 2001). Breeding semi-dwarf rice in the 1960s and the use of heterosis in the mid-1970s increased rice production by more than 2-fold, respectively. However, since the end of the last century, the average yield of rice production in China has been hovering around the 6.0 t ha^{-1} (http://zzys.agri.gov.cn) and there has not been a substantive breakthrough in average rice yield in recent years.

To enhance the yield potential of Chinese rice further, the 'super rice' breeding programme was initiated in the mid-1990s in order to break the yield plateau. Several super rice cultivars with a yield potential of over 10 t ha^{-1} in demonstration tests were developed. For example, China's first three-line subspecies hybrid rice Xieyou 413, which has been developed by the author, achieved a yield of up to 11.9 t ha^{-1} in Anji County, Zhejiang Province (Ying and Luo, 1996). However, this high yield potential could not be achieved in the large-scale production in the farmer's fields. The main reason is the quality of China's paddy fields which cannot meet the growing conditions required for current super hybrid rice. Drought is one of the most important limiting factors in more than 65% of paddy fields in China's (China State of the Environment, 2004), where super rice varieties cannot perform well under drought stress. In fact, since the 1990s China's average annual drought-affected area was up to 26.67 million hectares decreasing food production by 70–80 billion kg (Jing, 2007). Therefore, the development and production of drought-resistant rice varieties, to stabilize and improve the production levels in the low-middle-yielding fields, is needed.

On the other hand, the water resource per capita in China is only 2200 m^3, one-quarter of the world's average (Liu, 2006), while rice is the major water consumer in the country. Agriculture uses 392 billion m^3, which is 70.4% of the total water consumption, of which about 70% is used for rice production alone (Zhang, 2007). From the food safety viewpoint, China's grain output in 2020 should reach 6 billon tons, which will increase agricultural water use by 1200 m^3. However, over the next 30 years, China's agricultural water use can only maintain a zero growth or negative growth (Liu, 2006). Water shortage has become the bottleneck of China's food security. The development of water-saving rice varieties to decrease water consumption in rice production is inevitably a major goal in agriculture research.

Thus, to achieve long-term food security and sustainable development in China, 'Water-saving or drought-resistance' (WDR) rice varieties are urgently needed. This article aims to clarify the definition of WDR, examine the related achievements in drought resistance research, and provide a perspective of the strategies, the germplasm resources, breeding approach and progress toward the development of WDR.

Definition of WDR

Water saving

The plant's water-saving ability mainly refers to the effective use of water resource in the process of growth and development of plant. Broadly speaking, it relates to improving the effective use of rainfall (Zhang, 2003) and increasing crop water use efficiency (WUE).

The water requirement of rice varies in different growth stages. There is sufficient evidence that drought stress at the seedling and adult stages has different effects on yield performance. Better synchronization of plant growth stages with the rainfall is considered as a good measure for water saving. The effective use of rainfall during a water-sensitive period can realize water saving. For example, there is usually much rainfall in late May and August in the Yangtze River region in China. The farmer can sow dry seed directly in the field and depend on the rainfall for germination. The variety should possess a very good root penetration ability in order to maximize soil moisture capture. In addition, the most sensitive stage, panicle initiation, should therefore be placed at the later August period because of the heavier rainfall at this season. In previous studies, drought escape was considered as a component of drought resistance, and it is also related to water-saving cultivation.

WUE is defined as the economic production per unit water consumption. It may or may not be related to drought resistance. WUE was widely used as a breeding target in water-saving agriculture (Condon *et al.*, 2004). The varieties with good drought resistance do not mean high water use efficiency. In turn, a high WUE variety is not necessarily a drought-resistant variety.

There is a continuing debate on the implications of WUE in crop breeding. It has been repeatedly indicated that genotypic variation in WUE was driven mainly by variation in water use rather than by variation in plant production (Blum, 2005). However, from the viewpoint of increasing WUE, improving the yield potential is more important than drought resistance. On the other hand, improved water saving can be achieved with less water use for the same productivity. Zou *et al.* (2006) investigated WUE and the productivity of a drought-resistant variety Zhonghan 3 and a drought-sensitive but high yield potential variety Shangyu 63 which has been widely planted in China for more than ten years. With the total amount of irrigation of 5250 m^3 ha^{-1}, Zhonghan 3 reached its highest yield value (3.10 t ha^{-1}) with a WUE of 0.59 kg m^{-3}, but Shanyou 63 had a significantly reduced yield to 3.45 t ha^{-1} with the WUE of 0.66 kg m^{-3}. In irrigating water of 7500 m^3 ha^{-1}, Zhonghan 3 showed little change in yield but significantly reduced its WUE. However, Shanyou 63 reached its highest value of 6.38 t ha^{-1} and its water use efficiency increased to 0.85 kg m^{-3}. The results indicated that improving yield potential is important for increasing water use efficiency.

Drought resistance (DR)

Different disciplines differ in their views of drought resistance. Plant physiologist proposed that the drought resistance of plants was the ability to survive or grow in water-stressed environment, but agronomists are more concerned with crop yields in drought conditions. Drought resistance can be defined as the crop survival ability and production capacity under drought conditions. Drought resistance of crops is complex, involving at least three important physiological areas. Firstly, crops under drought conditions need to maintain a high plant water status; secondly, crops, in the case of a low water status, need to maintain their physiological functions; thirdly, the crop can recover water status and function after drought stress (Blum, 1999). At present, a more consistent view is that the connotation of drought resistance consists of three aspects (Luo *et al.*, 2001; Zhang *et al.*, 2005).

(i) Dehydration avoidance (DA) refers to the plant's capacity to sustain high water status by water uptake or a reduction of water loss in dry conditions. DA is achieved through the development of a large and deep root system to capture the water from the soil as well as through the closure of stomata or a non-permeable leaf cuticle to reduce transpiration. In selection and phenotyping, the main criteria of DA include root morphological traits (such as root length, root diameter, and root volume, etc.) and physiological traits (such as stomatal conductance, leaf water potential, leaf relative water content, water loss rate, photosynthetic rate, and canopy temperature, etc).

(ii) Dehydration tolerance (DT) is defined as the relative capacity of plants to maintain function under low leaf water status. It refers to the active accumulation of osmotic adjustment in plant cells, thus increasing the capacity of osmotic adjustment to maintain a high turgor. It also includes capacity enhancement in the removal of harmful substances accumulated in plants and anti-oxidation, etc. The measure of this capacity includes several physiological traits such as osmotic adjustment, ABA content, proline content, soluble sugar content, peroxidase or superoxide dismutase activity, and chlorophyll content, etc.

(iii) Drought recovery (DR) refers to the recovery capability of plant after a period of severe drought which causes the complete session of growth, a complete loss of turgor, and leaf desiccation.

Though DA, DT, and DR possess various connotations, they are usually involved together in the plant function. DA is the major factor in drought-resistant performance, but the drought tolerance (dehydration tolerance) is seen as the second line of defence after dehydration avoidance (Blum, 2005).

Water-saving and drought-resistant rice (WDR)

WDR is defined as a new type of rice variety which has both high yield potential and good quality as the current paddy rice, as well as the capacity of water-saving or drought resistance. WDR's drought resistance mainly refers to the capacity to maintain the higher water status to achieve normal metabolism in water-limited environments. That is, a high water status under drought, (dehydration avoidance) and dehydration tolerance (osmotic adjustment and anti-oxidation, etc).

The cultivated rice (*Oryza sative* L.) originated from the wild rice (*Oryza ruffipogon* L.) in the swamp areas with wet and dry alternation, adapted to both water and less-watered conditions. Long-term evolution led to two different ecological types according to the water requirements (Luo *et al.*, 2002). One is the paddy rice adapted to the aquatic environment; the other is the upland rice needing dry conditions to complete its growth and development. According to Ding (1957)'s classification of cultivated rice, rice and upland rice in botany and biology were not significantly different; the difference lies in their ecological adaptations. The upland rice has higher drought resistance, while paddy rice is much more sensitive to drought. Rice is the basic type, upland rice is a variant type adapted to the environmental change.

From a global perspective, breeding for paddy rice has been given prime attention in agricultural research. For half a century thousands of high yield and good grain quality rice cultivars were bred and used in the farmer's field. However, research on the upland rice has lagged behind. Upland rice cultivation has a long history in China, in the mountain area of Guangxi and Yunnan province; the local farmers are still in the habit of growing upland rice on the hillside. It is seeded directly in late March and depends on the rainfall around the Qingming festival (5 April) for seed germination. Harvest is in November. This kind of upland rice is low-yielding, but highly water-saving or drought resistant, with a water requirement of only one-third to one-quarter of that of paddy rice.

Clearly, WDR can be developed through an integrated water-saving and drought resistance capacity mainly from the traditional upland to the commercialized paddy rice cultivars. WDR should possess the following characteristics: in irrigation condition, its yield potential, and grain quality are basically the same as the current paddy rice with much less water consumption (saving about 50% water supply compared with the normal paddy rice). In water-limited environments, it shows a higher drought resistance to minimize yield loss.

Development of WDR

Water-saving and drought-resistant germplasm resources

From the middle of the last century, in the systematic collection and conservation of rice germplasm resources, China carried out an identification of the drought-resistant germplasm. The International Rice Research Institute performed large-scale germplasm screening for drought resistance. These works resulted in a number of drought-resistant accessions, which were not actually proven to be water-saving and their drought resistance was not verified

Table 1. The drought-resistant varieties with different origins

Several drought-resistant varieties identified based on the drought-resistance index (DRI) of yield in a drought-screening facility.

Varieties	Origin	Subspecies	Drought Index
Huhan3	China	Japonica	0.93
Zhonghan 210	China	Indica	1.04
Zhonghang 3	China	Indica	1.04
CICA4	Colombia	Indica	1.04
Jinhuangzhan	China	Indica	1.05
Yunlu 99	China	Indica	1.06
DINALAGA	Thailand	Japonica	1.06
LAC23	Liberia	Japonica	1.11
Mowanggunei	China	Japonica	1.14
IAC47	Nigeria	Japonica	1.15
Qingsizhan1	China	Indica	1.16
IR60080-46A	Philippines	Japonica	1.17
Tre Smeses	Brazil	Japonica	1.21
IR6115-1-1-1	Philippines	Indica	1.31
Huhan 15	China	Indica	1.31
IR75942-9	Philippines	Indica	1.31
KN361-1-8-6	Indonesia	Ibduca	1.32
IAC1	Brazil	Japonica	1.34
PR325	Puerto Rico	Indica	1.37
Handao3	China	Indica	1.39
IR45	Philippines	Indica	1.43
Ganlangu	China	Japonica	1.45
IAC1246	Brazil	Japonica	1.56
IR53236-275-1	Philippines	Indica	1.62
Maniangu	China	Indica	1.62
IRAT106	Africa	Japonica	1.64
AUS454	Bangladesh	Aus	1.88
Nephuong	Vietnam	Indica	1.89

physiologically. Due to funding and facility constraints as well as the limited understanding of DR, the DR germplasm identified earlier in China needs to be studied further for its value for breeding to be established.

In recent years, drought resistance, as well as the major agronomic characteristics, physiological traits, and leaf anatomical and root traits of the drought-resistant varieties originated from Asian and Africa was re-examined, using a field screen facility (see discussion below). Several accessions showed a high level of drought resistance (Table 1). The results indicated that there are various drought-adaptive mechanisms of the genotypes correlated with drought resistance in terms of yield as measured by the drought resistance index (DRI) (Makara *et al.*, 2006). Of the morphological characters, some plants had stronger root systems; some possessed a thicker waxy layer on the leaves indicating good water retention; and some varieties showed deep bulliform cell retraction resulting in leaf-rolling when stress occurred, while others did not grow or roll the leaves under stress. One accession even shows a 'procedural death' feature where different tillers were killed in a sequential order as drought stress progressed (L-J Luo, unpublished results). Obviously, there is great diversity in drought-resistant mechanisms in the global rice collections.

In conclusion, there is a diversity of DR mechanisms in different originated DR varieties. It is necessary systematically to carry out in-depth research on the DR morphology and mechanisms of classification of these germplasms. The in-depth physiological, genetic, and molecular researches should be based on an investigation of the classification of drought-resistant mechanisms.

Screening methods for drought resistance

Much research has been conducted to investigate the genetic base and physiological mechanism of a crop's drought tolerance ability. Unfortunately, because of the extreme complexity of a plant's drought resistance and the lack of effective methodology, the progress on DR rice gene discovery and DR rice development have been very limited. Since the environment has a great influence on the expression of drought resistance, the accurate identification of drought resistance is the most critical. Blum (1999) noted that the lack of standard assays of drought resistance was a major obstruction to the proper assessment of genetic modification towards drought resistance. Currently, there is an agreement that, in order to identify drought resistance, the traits methods used should be under consensus regarding criteria, techniques, and facilities. The screening facilities must be able to simulate the actual field stress environment by effective moisture control. The test of drought resistance must be performed with whole plants and/or plant communities (Blum, 1999).

The pot method (Lilley *et al.*, 1996; Yadav *et al.*, 1997) is often used in DR researches. However, the indoor plant growth environment (greenhouse) is substantially different from field. Some field-based studies planted the experimental material in different field plots (eg, dry shed and paddy fields), however, it is difficult to achieve consensus on the environment including soil, light, temperature, and other non-water stress factors, thus reducing the reliability and accuracy of the experiment. Verslues *et al.* (2006) tried to clarify the methods in quantifying resistance to drought based mainly on the water status of plant cells in designing laboratory experiments, but from the breeding viewpoint, the situation in actual farmer's fields is very different from the laboratory.

In recent years, a field screen facility was established based on the 'line–source soil moisture gradient' (Fig. 1). The facility is based on a greenhouse with a gutter-connected arched roof designed to enable accurate management of soil water content necessary for drought screening. It realized different degrees of stress treatments on one genotype in one plot. Using this facility, the DT rice germplasm resources identified earlier in China were systematically re-screened and a number of highly drought-resistant accessions were identified (Liu *et al.*, 2006). By taking advantage of this facility, the agronomic characters and physiological trait of a mapping population were investigated over two years, which resulted in the identification of a number of DT-related gene/QTLs (Zou *et al.*, 2006, 2007; Liu *et al.*, 2008).

In the practice of breeding, water-stress environments can be designed according to the target area (Fischer *et al.*, 2005). Only in the arid environment was the drought resistance of rice fully expressed under field conditions. The promising lines with strong DR can be selected in early segregation generations under dry conditions. Starting from the end of the last century, conventional paddy rice cultivars were crossed with upland rice varieties and the selection for DT in the early generations was conducted in Hainan island during the dry season and in the mountain area in Zhejiang province, which is a successful site for breeding of a number of WDR varieties (discussion below). China Agricultural University also developed a series of drought-resistant rice varieties by crossing the traditional upland rice from Yunnan province with the improved japonica cultivars with early maturity from East Asia, through the, alternate use of both well-watered and drought-stress environments (Wang *et al.*, 2002).

For nearly three decades, a large number of studies were performed, including the morphological responses of plants under drought (such as leaf-rolling), water status (such as leaf relative water content), agronomic traits (such as the seed setting), and physiological changes in the material (such as the ABA content) and canopy temperature. Blum (1988) proposed stem reserve utilization for grain-filling under drought stress as a form of drought tolerance mechanism. In our experiment, it was found that the panicle neck diameter and spikelet fertility were highly correlated with drought resistance by the stepwise regression and grey correlative analyses (Liu *et al.*, 2008).

The canopy temperature and panicle water potential were considered as effective criteria primarily to screen for drought resistance of germplasm resources. There was significant negative correlation between canopy temperature and seed setting. Our QTL mapping studies indicated that

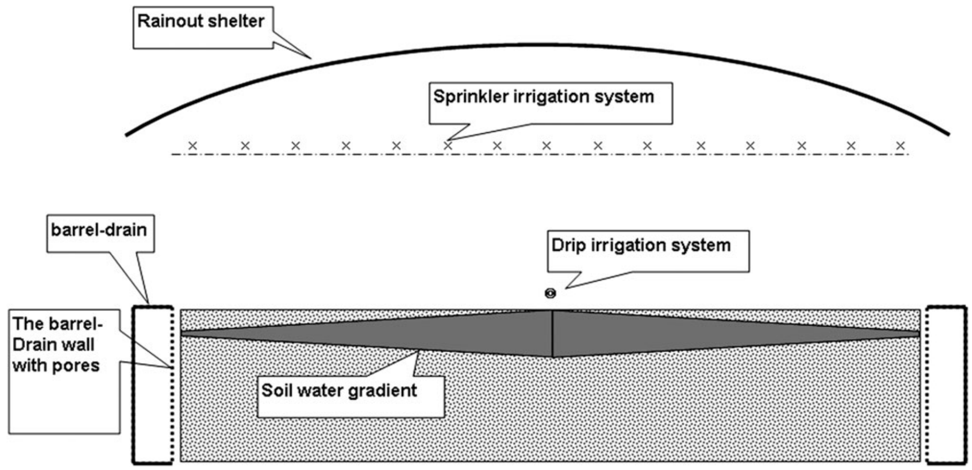

Fig. 1. A sketch map of the DR screening facility. The water stress was conducted at the reproductive stage. Sprinkler irrigation was ceased and the water in the barrel-drain was drained out until the water level was below the water table. Therefore, soil water led into the barrel-drain through pores in the barrel-drain wall. Drip irrigation was initiated to maintain the soil water in the middle of the field. As a result, the water gradient within the test field was established.

two QTL loci in chromosome 2 and 10 have effects on both canopy temperature and seed setting (Liu *et al.*, 2005). As it is easy to measure, the canopy temperature was used as an indicator in primary field screening for drought-resistant materials. In addition, panicle water potential (PWP) of rice plants in both normal (full water) and water stress showed the same daily changes as the leaf water potential (LWP). Both PWP and LWP could be used as indicators to represent the plant's water status under stress and to screen for drought-resistant genotypes. The PWP seemed to be more effective for distinguishing the upland rice varieties with different drought-resistant ability (Liu *et al.*, 2007).

However, the composite indicators, especially the yield-based resistance index, should be used in breeding programmes, together with yield under stress as the final breeding target. In fact, single morphological or physiological traits are not always consistent with the drought index. For example, Zhang *et al.* (2008) proposed five 'marker varieties' ('check varieties') representing the different levels of drought resistance (DRL) based on the drought resistance index (DRI) of yield. In general, the DRL was associated with the performance of most of drought-resistance-related traits but not always sensors of performance of a single trait, especially for the medium resistant varieties (DRL is 3 to 7). The single trait could not represent accurately the drought resistance of the variety. For example, seed setting was widely used as an index representing drought resistance, however, the sensitive marker cultivar, IR7790-18-1-2 showed a lower decrease than the medium resistant marker variety MONOLAYA after water stress (Table 2).

Integrating water-saving/drought-resistance and high yield potential

The general strategy for developing WDR is to combine water saving/drought resistance with high yield through conventional and molecular approaches. In fact, high yield potential is widely accepted as the first target trait in breeding programmes. As high yield is usually realized under non-stress conditions and intensive cultivation, most breeding activities are conducted in the experiment station with good irrigation and field fertility. This is totally different from the farmer's field in the rainfed areas. There are several conflicts between high yield characteristics and drought-resistance traits. For example, to maintain the high water status under the stress, smaller plants or reduced leaf area and limited tillering are components of dehydration avoidance. However, these traits are in contrast to a high yield potential (Blum 2005). Stomatal closure to conserve water status is in contrast to carbon assimilation and productivity (Zhang *et al.*, 2005). Therefore, in most cases, breeding for drought resistance may compromise high yield potential by way of the crossover interaction (Blum. 2005).

Is it possible to achieve both high yield potential and drought resistance in one phenotype? In theory, several traits, such as a strong root system, WUE, and osmotic adjustment, could be combined with the high yield phenotype. These depend largely on the genetic mechanism of drought resistance in the target environment (Blum, 2005). Yue *et al.* (2006) investigated the genetic base of drought tolerance (DT) and drought avoidance (DA) at the reproductive stage in rice using a recombinant inbred line population. Only a small portion of QTL for fitness and yield-related traits overlapped with QTL for root traits. There is also no correlation between DT and DA related traits, indicating that DT and DA had distinct genetic bases and could be recombined in the breeding. Blum (2005) proposed that an ABA-insensitive genotype might perhaps be an appropriate solution for expressing both drought resistance and high yield potential. In addition, osmotic adjustment has no negative association with low yield potential. Thus, selection for these traits in high-yielding materials resulting from high yield by DR parents might

Table 2. The drought-resistance performance of marker varieties under water stress

Drought resistance level (DRL) based on the drought resistance index (DRI) of yield represents the performance of drought-related single traits well. However, the single trait could not represent accurately the drought resistance of the variety, especially for the medium-resistant variety. For example, seed setting was widely used as the index representing drought resistance, however, the most sensitive marker cultivar, IR30385-18-1-2 showed a lower decrease than the medium-resistant marker variety MONOLAYA after water stress.

Marker varieties	Drought resistance level	Drought resistance index	Leaf rolling score	Leaf death score	Days of delayed heading (d)	Plant height reduced (%)	Leaf water potential reduced (%)	Seed setting rate decreased (%)	Effective panicles decreased (%)
IR55459-05	1 (High resistance)	1.48	1	0	1	4	17	19.20	–16.67
MARAVILHA	3 (Resistance)	0.96	1	0	6	3	24	39.20	–37.50
MONOLAYA	5 (Medium resistance)	0.81	2	1	10	12	8	56.20	–22.33
IR30358-084-1-1	7 (Medium sensitive)	0.56	4	2	27	12	22	37.90	33.33
IR7790-18-2	9 (Sensitive)	0.20	3	1	23	3	105	43.30	53.40

offer an opportunity for progress. It follows that success in recombining high yield potential with DR depends on the specific DR trait involved.

The effective and successful selection for yield and its related agronomic components under both stress and non-stress can be achieved by the genetic recombination of high-yield and drought-resistant traits. For example, a cross was made between a commercial paddy rice cultivar Qixiuzhuan and the DR variety Zhonghan 3 and the individual plants were selected, mainly based on its DR performance at the F_2 generation in a dry environment, The promising lines were selected mainly according to the agronomic performance at the F_3 generation in non-stress conditions. This alternative co-selection was carried out in the following generations. Finally, the new DR variety, Huhan 15, which possesses the high yield potential of Qixiuzhuan and the drought-resistance of Zhonghan 3 was released in 2006. The growing area of this DR cultivar with the water-saving cultivation reached more than 10 thousand hectares in 2009 in China.

Breeding for WDR

Molecular approach versus conventional breeding

With the development of modern molecular biology, the molecular genetics and functional genomics of DR is receiving increasing attention. Two approaches are widely used in DR-related gene identification. One is mapping based gene identification: usually, a mapping population is developed from a cross between a drought-resistant parent and a drought-sensitive parent and investigated on both phenotypes in the well-watered and drought-stress environments and genotype with molecular markers. The linkage analysis results in the identification of drought-related genes (QTL). Based on the primary mapping results, the fine mapping and map-based cloning approaches were applied to obtain the linked marker used in the marker assistant selection and the candidate genes. The second is the creation and screening of drought-resistant (or drought-sensitive) mutants. Based on DR performance and gene sequence changes, the candidate genes are identified and their functional verification is carried out in the laboratory. So far, there are a large number of reports regarding the DR-related gene/QTL and molecular linked markers. In addition, a large number of drought-related candidate genes and transcription factors were identified and reported to show some drought-resistant effects (http://www.plantstress.com/). However, as Pennisi (2008) argued, of the large number of published candidate DR genes revealed by genomics almost none had shown any impact in field performance. In fact, there is hardly any DT variety developed purely by genomics research to date.

In the 1990s, by using the upland variety IRAT 109 as a drought-resistant parent, both molecular genetics and breeding studies were carried out. IRAT 109 was crossed with Zhenshan 97 (a popular paddy rice variety in China) to develop a mapping population. The molecular dissection on this population resulted in positioning a number of drought-related QTL (Zou *et al.*, 2006; Yue *et al.*, 2006) and cloning several candidate genes from the QTL region that showed some function in drought resistance. Although much work has been done in the breeding programme by using the QTL linkage markers, we have failed to deliver a new DT variety until now.

However, IRAT 109 has been successfully used in the conventional breeding programme. IRAT 109 as a DR donor was widely crossed with the paddy rice parent. The phenotype selection was carried out in the segregation generations in a water-stressed environment. In 2004, a DR variety Huhan 3 was registered at the national level and released to farmers. As Huhan 3 possesses ability to maintain the CMS sterility of Japonica, it was used to integrate its drought resistance to Hanfong B paddy rice, by three backcrosses. Selection was carried out in both stress and non-stress environments after the F_2 generation. This led to the release of Huhan 2B, in 2009 keeping the same yield potential as Hanfong B and the same drought resistance as Huhan 3 (Fig. 2).

Clearly, There is a big gap between 'molecular breeding' and conventional breeding. The main reason is due to the

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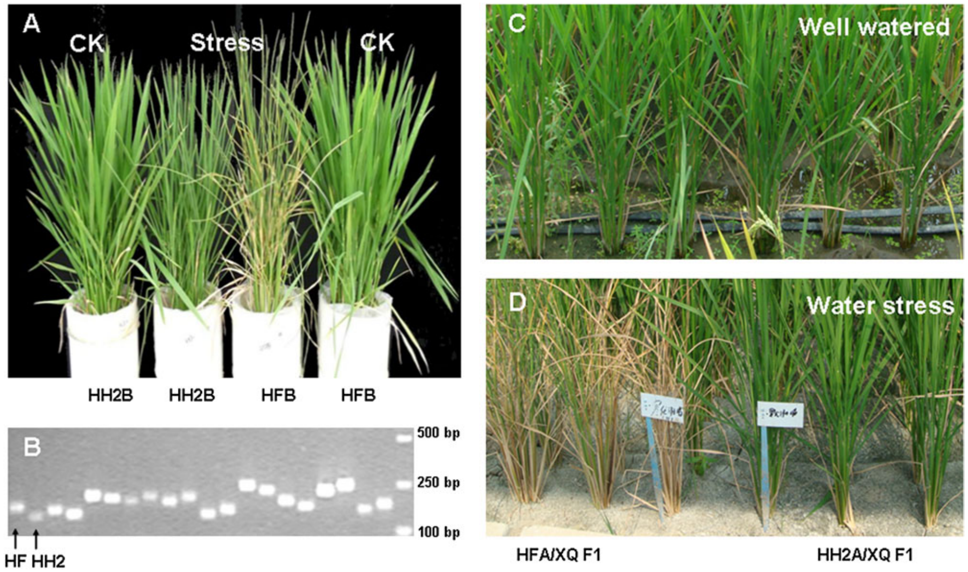

Fig. 2. The field performance of HH2B, its parent HFB, their hybrid F_1 under well-watered and water-stressed conditions (A, C, D), and the different band patterns between HH2B and HFB based on 3% agarose gel electrophoresis (B). HH2B shows higher DR than HFB after 15 d water stress (A). There is 5.36% genetic variation between HFB and HH2B based on 2000 molecular markers (B).The hybrid F_1 of HH2A (the CMS line of HH2B) and XQ shows higher DR than that of HFA (the CMS line of HFB) and XQ (L-J Luo, unpublished data).

extreme complexity of drought resistance. For example, more than 2000 molecular markers (SSR and indel markers) were used to investigate the genetic variation between Huhan 2B and Hangfong B. Based on 3% agarose gel electrophoresis, 1271 markers with identical band patterns and 72 markers with different band patterns were identified. There is only 5.36% variation between two varieties. The polymorphism markers are distributed among 11 of the 12 rice chromosomes. After comparisons with our early mapping study (Zou *et al.*, 2005, Yue *et al.*, 2006), a total of 21 candidate genes were found that were located in the QTL region, belonging to eight kinds (Figs 2, 3). The results implied that only one time hybridization following the selection in the stress environment could integrate more than 21 DR-related candidate genes.

In summary, although the molecular methods showed great success in improving quality traits controlled by the single gene with large effects on traits such as pest and disease resistance, it showed less success with the complex traits related to the many genes (QTLs) in the complex genetic network until now. Conventional selection in extreme environments is an effective approach to integrate several genes involved in the same genetic network.

WDR versus heterosis utilization

Hybrid rice has been successfully commercialized for over 30 years in China. While great effort was extended to improving the yield potential and grain quality as well as the biotic and abiotic stress resistance, less progress has been achieved in the water-saving or drought resistance of hybrid rice. The DR performance of hybrids depended mainly on the parental level of drought resistance. The F_1 generation usually showed a higher DR than the sensitive parent but lower DR compared with the DR parent.

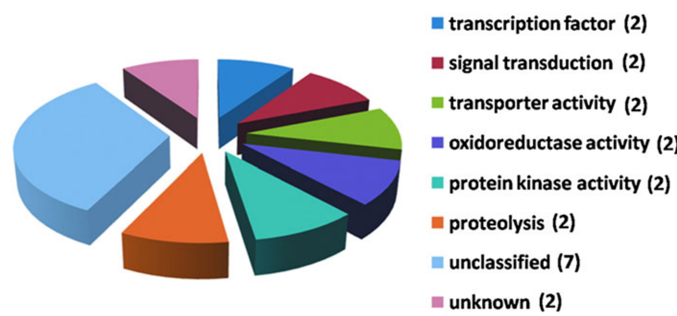

Fig. 3. Relative Function categorization of 21 genes expressed in Huhan 2B under water stress.

It is difficult directly to find the CMS female line from the upland rice germplasm. Thousands of accessions were screened and nothing was obtained in the last ten years. Therefore, introducing drought resistance from the upland maintainer to the current paddy CMS lines would be an effective approach in breeding the DR CMS line. In 1998, an upland accession was found to maintain the sterility of Zhanshan 97 A, a CMS line with the largest growing area in China. After several generations's backcross with Zhanshan 97 B, a DR CMS line was developed and registered as Huhan 1A in 2003. Then, Huhan 1A was used as the female parent to cross with several rice restorers in the following year. Two DR hybrid rice combination was released in 2005. These showed 50% of water use in production in the farmer's irrigated field and were drought-resistant as upland rice in the water-limited environments. Using the Huhan 2A described above, a new DT Japonica hybrid combination, Hanyou 8 was also developed in 2009. The hybrid F_1 shows the significant heterosis on yield potential and partial dominance for drought resistance (Fig. 2).

WDR in the future

Currently, the commercial WDR include two types, the conventional varieties, such as Zhonghan 3, Huhan 3, Huhan 15, Handao 297, Zhonghan 209, and hybrid combinations, such as Hanyou 2 and Hanyou 3. These varieties have performed better in terms of drought resistance or water-saving properties, and are particularly suitable for cultivation in low-middle-yielding fields. In high-yield paddy fields, their yield potential needs to be enhanced further, insect and pest resistance need to be further improved, and fertilizer use efficiency need to be further increased.

To improve WDR based on the 'Green Super Rice' concept (Zhang, 2007), there is a need for a gradual reduction in the application of pesticides, fertilizers, and water while still achieving continuous yield increases and quality improvement. The general strategies are to explore germplasm resources for drought resistance further. The more effective indicators (new secondary traits) have been identified and are used in drought-resistant screening. The DR core collection including different kinds of DR mechanisms were set up and the genes related to water-saving, drought avoidance, drought tolerance, and high water use efficiency were integrated into one variety. The other elite genes for insect and pest resistance, efficient use of N and P, high yield, and better quality were also incorporated.

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