抗逆基因组学—从实验到大田

徐建龙

(1) Hei Leung. Stressed genomics — bringing relief to rice fields. Current Opinion in Plant Biology, 2008, 11:201–208 IF=10.33

(2) J. R. Witcombe, P. A. Hollington, C. J. Howarth, S. Reader and K. A. Steele. Breeding for abiotic stresses for sustainable agriculture. Philosophical Transactions of the Royal Society, 2008, 363:703-716 IF=5.117

(3) Jose M Pardo. Biotechnology of water and salinity stress tolerance. Current Opinion in Biotechnology, 2010, 21:185–196 IF=7.82

Outline

(1)Importance and challenges in dealing with abotic stresses

(2) Identification of QTLs for abiotic stress tolerance

(3) Gene aggregates and expression domains

(4) Effects of over-expression of transcriptional factors on abiotic stress tolerance

(5) MAS for abiotic stress tolerance

(6) Our breeding strategies

- **Importance and challenges in dealing with abotic stresses**

Environmental factors are the primary cause of crop failure, causing average yield losses of more than 60% for major crops worldwide.

Most frequently and adversely abiotic stresses are drought and salinity.

Values of RWC around 85–95% are found in well-hydrated tissues. Soil moisture less than 60% will make crops drought and a RWC lower than the critical mark of 50% typically results in plant death.

Drought stress in farmer's field

Salinity is a major growing threat to rice production secondary only to drought. Rice plants are sensitive to salt, particularly at the seedling stage. EC 5-6 dSm^{-1} (0.3%) can cause significant yield loss in susceptible rice lines.

Salinity stress and water deficit are intimately related. Salts dissolved in the soil solution reduce the water potential causing 'physiological drought'. Similarly, due to shortage of irrigation water, salinity has become increasingly severe as salt is moving up to soil surfaces, which aggregates salt injury to crops.

Molecular responses to water and salt stress are largely identical except for the ionic component.

Rice plants suffered from severe salt stress

Drought tolerance (DT)

- **Escaping:** accelerate or delay flowering
- **Avoidance:** high water use efficiency, leaf rolling, wax leaf surface and thicker roots
- **Tolerance:** rapid osmotic adjustment, dehydration tolerance, partitioning and mobilization of stem reserve
- **Recovery:** restoring ability after water recovery

Salt tolerance (ST)

- **Salt exclusion:** take up less salt by selective absorption
- **Salt translocation:** translocate less Na^+ to the shoot
- **Salt compartmentation:** transport excess salt from younger to older leaves
- **Tissue tolerance:** compartmentalize excess salt in vacuoles within the leaves
- **Salt dilution:** dilute by fast growth rate and high water content in the shoot

Summary of common characters of drought and salinity stresses

Stress	Occurred stage	Effect on plants	Characteristics
Drought/ Salinty	Early stage (germination, seedling, tillering)	Poor crop establishment; reduced panicle number and panicle size	◆Tolerance may vary considerably at different developmental stages. ◆Stresses at reproductive stage depress grain yield much more than at the vegetative growth stage ◆High environmental variation and G x E variation
	Reproductive stage (panicle initiation, flowering, and grain filling)	Low spikelet sterility and poor grain filling, yield penalty	

Challenge in rice breeding for abotic stresses

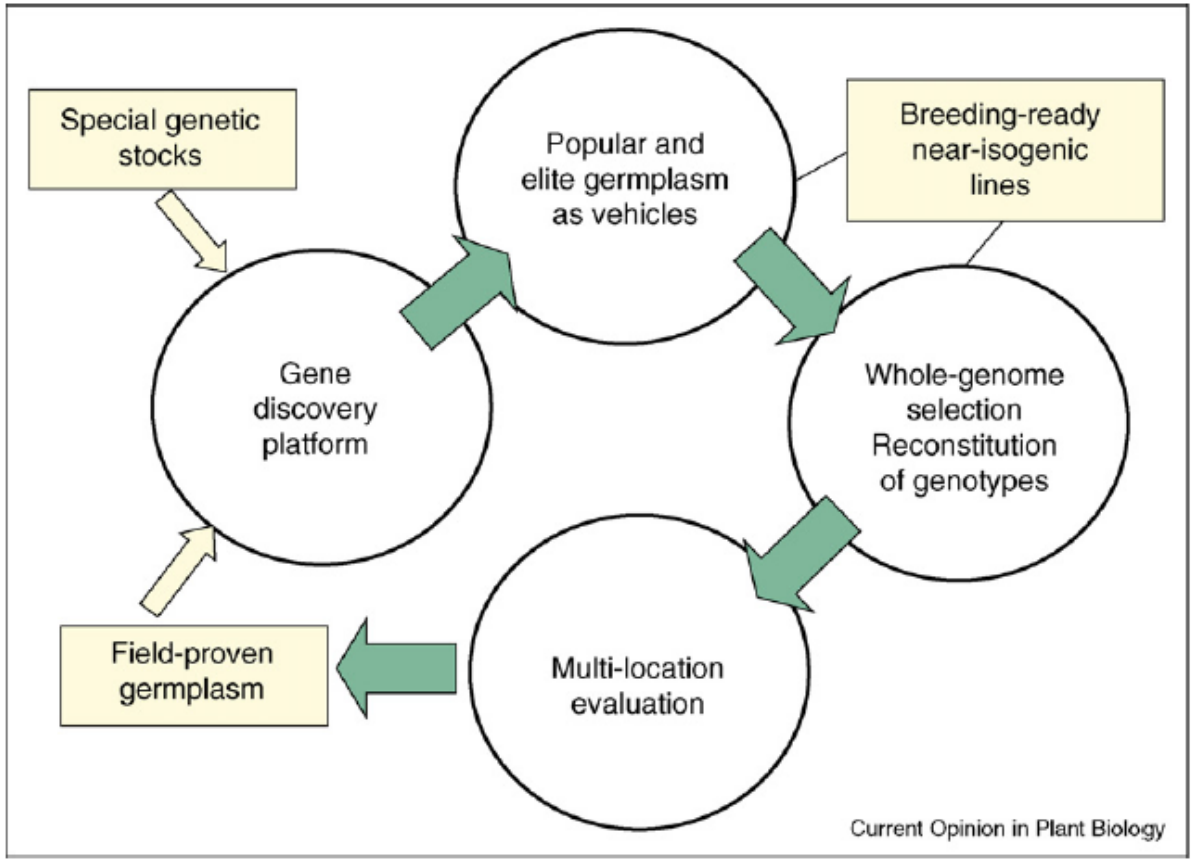
(1) Be complex both genetically and physiologically

(2) Narrow genetic variation in the gene pools

(3) Time-consuming and labor intensive to transfer stress tolerance from wild relatives into elite variety

(4) Linkage drag between stress tolerance and undesirable genes

(5) Strong environmental variation and G x E interaction



- **Good understanding of the gene or gene combinations underlying the traits**
- **Suitable germplasm as vehicles to ensure farmer adoption and consumer acceptance**
- **Robust and rapid methods to incorporate new genes in breeding programs**
- **Field environments for multiple-site testing and validation**

- **Identification of QTLs for abiotic stress tolerance**

Trait	N [1]	Env.[3]	Type	QTL #	Reference
Root traits	7	2	RIL	40	Li et al. 2005
Root traits	11	2	RIL	38	Yue et al. 2006
Root traits and shoot biomass	7	1	RIL	22	Kamoshita et al. 2002a
Shoot traits	3	2	DH	16	Hemamalini et al. 2000
Shoot traits	4	1	DH	8	Babu et al. 2003
Osmotic adjustment and DT	1	1	RIL	7	Lilly et al. 1996
Osmotic adjustment	1	1	DH	5	Zhang et al. 2001
Cellular membrane stability	1	1	DH	9	Tripathy et al. 2000
Leaf rolling/stomatal conductance	2	1	F_2	8	Price et al. 1997
Photosynthesis related traits	7	1	BC_2F_8	33	Zhao et al. 2008
Dehydration avoidance traits	3	2	RIL	17	Price et al. 2002
Leaf drying and rolling	2	2	RIL	10	Yue et al. 2006
Plant height and tillering	2	2	DH/NIL	3	Shen et al. 2001
Heading date, plant height	2	2	RIL	15	Lafitte et al. 2004
Heading date and plant height	2	1	DH	14	Babu et al. 2003
Heading date, plant height	2	5	DH	16	Lanceras et al. 2004
Grain yield	1	2	RIL	3	Lafitte et al. 2004
Yield components	6	2	RIL	48	Lafitte et al. 2004
Grain Yield	1	1	DH	5	Babu et al. 2003
Yield components	3	1	DH	12	Babu et al. 2003
Grain yield (DT)	1	5	DH	7	Lanceras et al. 2004
Biomass	1	2	RIL	4	Lafitte et al. 2004
Biomass	1	5	DH	8	Lanceras et al. 2004
Harvest index	1	5	DH	6	Lanceras et al. 2004
Harvest index	1	2	RIL	5	Lafitte et al. 2004

Summary of QTLs affecting DT and its components in rice from 35 independent studies on 15 different rice populations

Summary

- **The number of loci affecting DT and each of its components are very large and widely distributed across the rice genome**
- **Very few QTLs are consistently detectable in any specific population/ environment**
- **Individual component traits each contributes little to DT**
- **Epistasis among QTLs affecting DT and its components has not been addressed adequately in most studies**

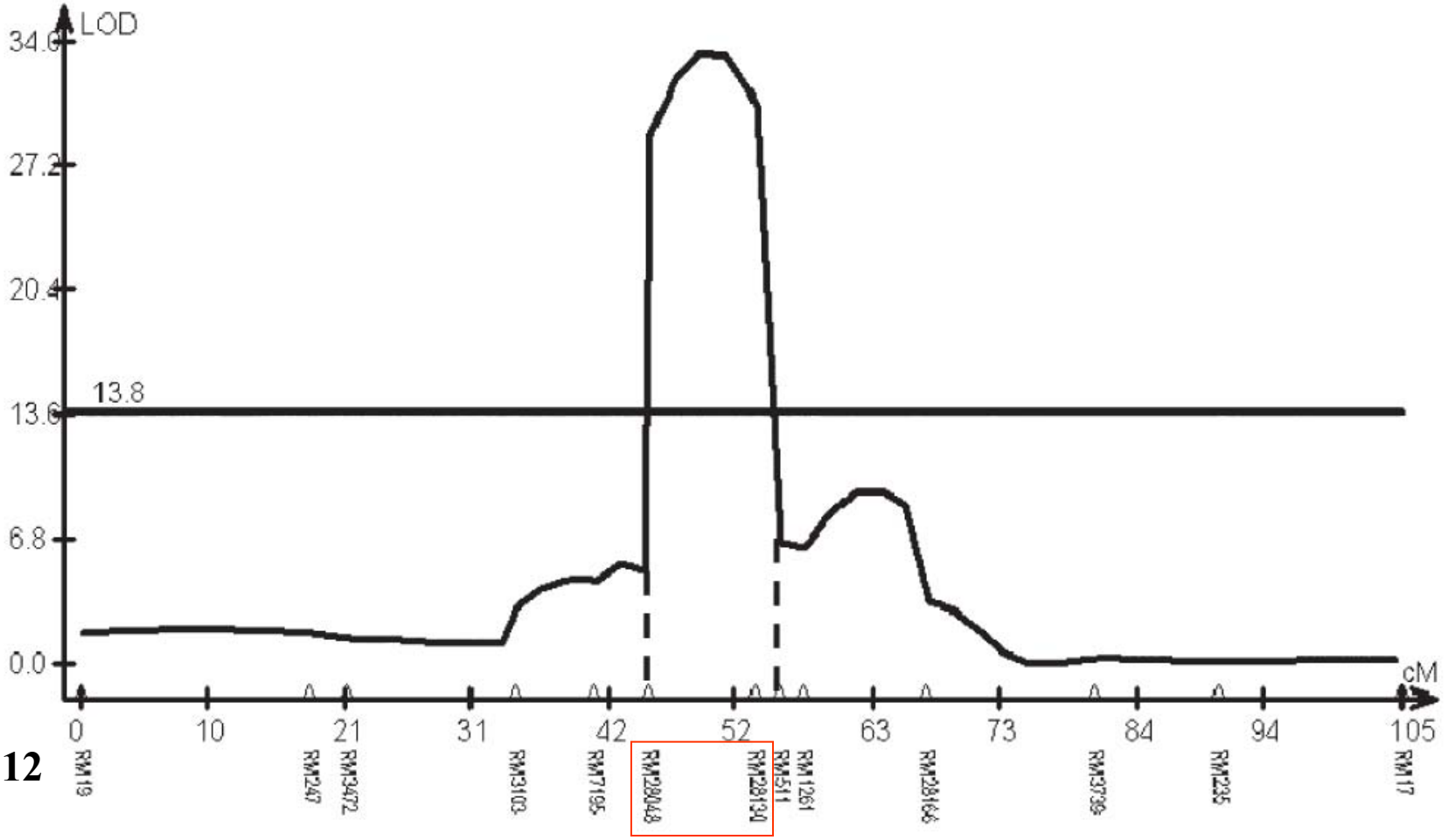
For a long time, genetic effects contributing to DT were considered too small and variable to detect consistently across genotypes and environments. This 'dogma' has recently been challenged by QTL analysis based on yield under stress in breeding materials.

Example 1:

A large-effect QTL for grain yield under reproductive-stage drought stress

Materials: a population of 436 random F_3 lines from a cross derived from a cross between Way Rarem, a drought-sensitive Indonesian upland rice cultivar, and Vandana, an Indian upland rice cultivar that is considered DT.

Bernier, et al., Crop Sci,2007, 47:507–518



Ch12

Figure 2. QTL likelihood curves of the LOD score of grain yield under stress for Chromosome 12. The SSR marker locations are listed on the Y axis. The black horizontal line indicates the significance threshold of LOD score 13.8 to detect putative QTLs. The vertical dotted lines indicate the position of *qtl12.1*.

This is the first report of a QTL with a large and repeatable effect on grain yield under severe drought conditions in a field experiment.

Table 4. (a) QTL identified under upland drought-stress conditions at flowering and grain-filling stages in F_3-derived lines from Vandana/Way Rarem: IRRI, dry season 2005 and 2006. (b) QTL identified under well-watered upland conditions over two consecutive dry seasons in F_3-derived lines from Vandana/Way Rarem: IRRI, dry season 2005 and 2006.

(a)						2005			2006			Combined		
Trait	Chr.	Interval	Marker closest to LOD peak	Position	QTL name	LOD	Add.†	R^2	LOD	Add.	R^2	LOD	Add.	R^2
				cM				%			%			%
Grain yield (kg ha^{-1})	12	*RM28048-RM511*‡	RM28130	49	*qtl12.1*	29.7	221	30	21.2	146	24	34	172	33
Biomass yield (kg ha^{-1})	3	RM523-RM545	RM545	25	*qtl3.1*				4.8	914	19			
	6	RM587-RM314	RM225	11	*qtl6.1*				5.2	−425	5	4.8	−233	2
	12	*RM28048-RM28166*	RM28130	51	*qtl12.1*	18	765	16	12.5	587	10	23	634	18
Harvest index	12	RM7195-RM28166	RM1261	61	*qtl12.1*	21.8	0.04	25				22	0.03	26
Days to 50% flowering	1	RM431-OSR23	OSR23	184	*qtl1.1*	5.4	5.6	15				5.1	4.7	13
	3	*RM7332-RM545*	RM523	14	*qtl3.1*	30	12.7	66	29.7	10.1	55	34	11.2	64
	5	RM122-RM5374	RM122	4	*qtl5.1*	5.1	−4.3	11				4.8	−3.6	9
	7	RM234-RM118	RM118	117	*qtl7.2*	5.6	6	18				5	4.7	14
	12	*RM3103-RM511*	RM28048	47	*qtl12.1*	7.9	−3.3	5	5.8	−2.9	6	7.3	−3.2	6
Plant height at maturity (cm)	1	RM212-RM431	RM315	152	*qtl1.1*	3.8	−1.8	5				5.1	−1.8	9
	12	*RM28048-RM28166*	RM1261	51	*qtl12.1*	9.1	2.2	8	4.9	0.7	1	9.8	1.4	5
Panicle number m^{-2}§	2	RM1367-RM250	RM3212	148	*qtl2.1*				4	−14	3			
	6	RM587-RM314	RM225	11	*qtl6.1*				4	−22	5			
	12	RM7195-RM28166	RM28130	51	*qtl12.1*				12.9	26	9			
Flowering delay¶	12	RM7195-RM28166	RM28130	51	*qtl12.1*							19	−2.4	16
Drought-response index	12	RM28048-RM511	RM28130	51	*qtl12.1*							39	0.89	37

The Way Rarem allele at this QTL is 172 kg ha^{-1}, explaining 33% of the total phenotypic variance for grain yield under stress, suggesting an epistatic interaction between this locus and other loci from the Vandana genetic background. This QTL appears to increase grain yield under stress by increasing the number of panicles, the biomass accumulation, and the harvest index while reducing flowering delay.

Example 2:

A large-effect QTL for grain yield under reproductive-stage drought stress

Materials: DH population from CT9993/IR62266. CT9993 is a deep-rooted upland-adapted tropical japonica genotype. IR62266 is a lowland indica type with shallow roots but moderate DT.

Kumar, et al., Field Crops Research,2007,103: 42–52

Table 2 Means and variances from the combined analysis over years: effect of chromosome 1 marker EM11_11 allele classes for traits measured under drought and well-watered conditions in a population of 111 RILs from CT9993/IR62266 in Raipur, 2000–2002

	Grain yield ($g\ m^{-2}$)		Shoot biomass at flowering ($g\ m^{-2}$)		Harvest index (%)		Days to flowering (days)	
	Stress	Non-stress	Stress	Non-stress	Stress	Non-stress	Stress	Non-stress
Means of homozygous classes								
CT9993 marker homozygotes	53.9	257	416	648	10.5	25.6	96.9	92.7
IR62266 marker homozygotes	40.7	239	363	651	8.7	24.1	99.6	93.8
Probability of greater F value	<0.002	ns	<0.0001	ns	<0.005	ns	ns	0.02
Variance components								
σ^2_G	243	2120	2772	3319	0.00039	0.00104	7.55	3.39
σ^2_{QTL}	78	75	1265	0	0.00014	0.00008	1.49	0.49
$\sigma^2_{Line(QTL)}$	165	2045	1507	3319	0.00025	0.00096	6.06	2.90
σ^2_{GY}	232	1779	940	53677	0.00067	0.00134	14.74	5.03
$\sigma^2_{Year*QTL}$	0	34	0	356	0	0	3.67	0
$\sigma^2_{Year*Line(QTL)}$	232	1745	940	5321	0.00067	0.00134	11.07	5.03
σ^2_E	427	2041	8783	23799	0.00141	0.00294	6.65	2.19
Proportion of genetic variance (%) explained by EM11_11	32	4	46	0	36	8	20	14

ns = nonsignificant at $p = 0.05$.

Marker EM11_11, which accounted for 32%, 42%, and 36% of the genetic variance for yield, shoot biomass at flowering, and harvest index under stress, respectively, and no significant effect on yield and days to flowering under non-stress conditions and days to flowering under stress. The QTL appears to be related to stress tolerance, rather than yield potential and avoidance of stress at flowering. Although CT9993 was not itself tolerant, its allele contributed to increased yield under stress.

Summary

(1) The genetic correlation between yield in stress and non-stress conditions was 0.8, indicating that direct selection for yield under drought stress can produce yield gains under stress without reducing yield potential.

(2) There was no secondary trait for which selection resulted in greater predicted response in yield under stress than direct selection for stress yield per se.

(3) These studies show that lines with superior DT can be identified by direct selection against yield under managed stress.

A large and small effect QTLs for ST at the seedling stage

Recent results from QTL mapping studies indicate that ST and its components in rice at the seedling stage are involved multiple QTLs, but single genes/QTLs with large effects on ST were reported in several cases.

Two major ST QTL, *SKC1* and *Saltol1*, from a japonica line Nona Bokra and indica line Pokkali, was identified, respectively. They are probably different alleles at the same locus. This gene *SKC1* turns out to be a protein in the HKT family that exclusively mediates Na^+ translocation between roots and shoots, thereby regulates K^+/Na^+ homeostasis in the shoots, resulting in improved ST in rice.

***Saltol1* gene has been transferred to rice varieties in Bangladesh, Vietnam, and India. It is necessary to demonstrate whether the salt-tolerant QTLs can indeed raise the performance of local varieties.**

- **Gene aggregates and expression domains**

Hurst et al.(2004) reviewed whole-genome expression studies in a range of taxa, and concluded that eukaryotic gene order is far from random and that gene expression patterns are often affected by chromosomal context. It poses the interesting possibility that quantitative traits are governed by coordinated expression of groups of neighboring genes.

Ma et al. (2005) indicated that, in rice, a significant portion of the genes are organized into chromosomal domains with coexpression patterns. The average size of the regions is 100 kb.

It appears that coordinated gene expression in a chromosomal context is common.

***Sub1* locus, there are three structurally related genes *Sub1A*, *Sub1B*, and *Sub1C* present in the same QTL region, encoding ethylene-responsive factor (ERF) genes.**

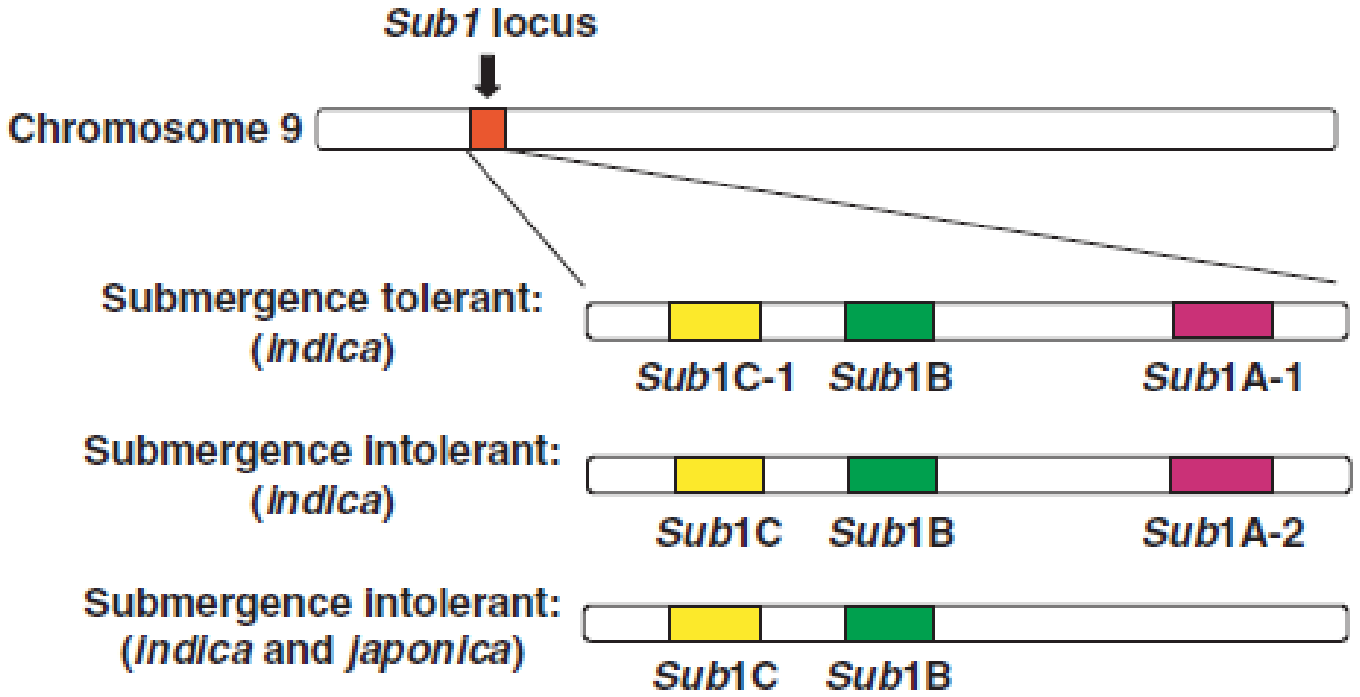


FIG. 1. *Sub1* haplotypes of *O. sativa*. The *Sub1* locus encodes two or three ethylene-responsive factors, *Sub1A*, *Sub1B* and *Sub1C*. Only submergence-tolerant accessions contain the *Sub1A-1* allele at the locus, which confers submergence tolerance to rice (Fukao *et al.*, 2006; Xu *et al.*, 2006).

Fukao, et al., Annals of Botany, 2009,103: 143–150

Sanhuangzhan 2 (SHZ-2) is durable reistance variety with broad spectrum. It has three qualitative resistant genes on chromosomes 8, 9 and 12 and 1 main-effect QTL on chromosome 8. One advanced backcross line (Texianzhan 13/SHZ-2) carrying the major-effect QTL on chr 8, exhibited resistance to rice blast disease over 14 cropping seasons.

Manosalva et al.,Plant Physiol., 2009,149: 287-296

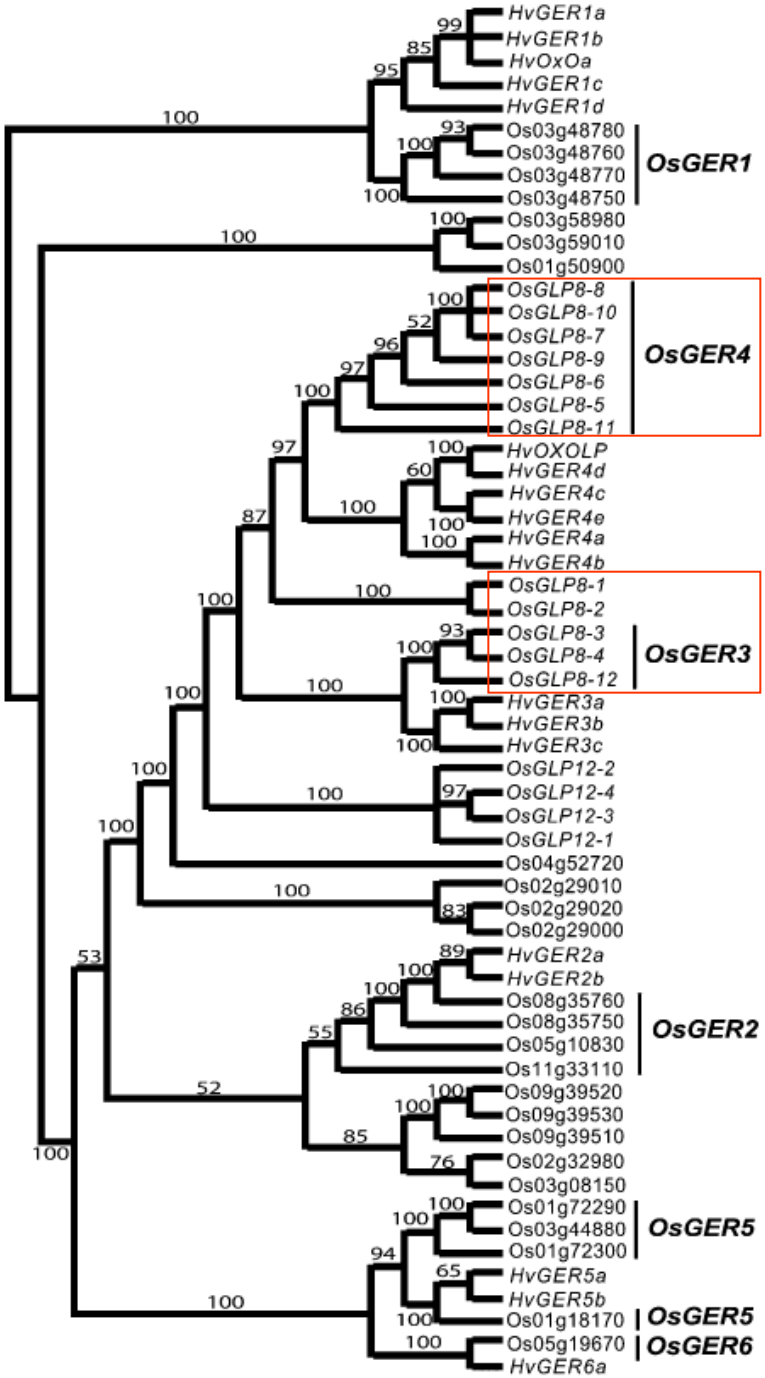


Figure 2. Phylogenetic relationships of germin box-containing proteins from rice and barley. Amino acid sequence similarities among predicted GLP proteins from rice were compared with known barley HvGER proteins (Supplemental Table S2). Rice GLP gene members were classified as known subfamilies OsGER1 to OsGER6, based on relationships with the barley HvGER proteins. Inferred amino acid sequences of 60 GLP proteins were aligned using ClustalX version 1.83. The phylogenetic tree was reconstructed using Bayesian MCMC analysis (Ronquist and Huelsenbeck, 2003). Posterior probabilities (scaled to 100) are indicated at nodes.

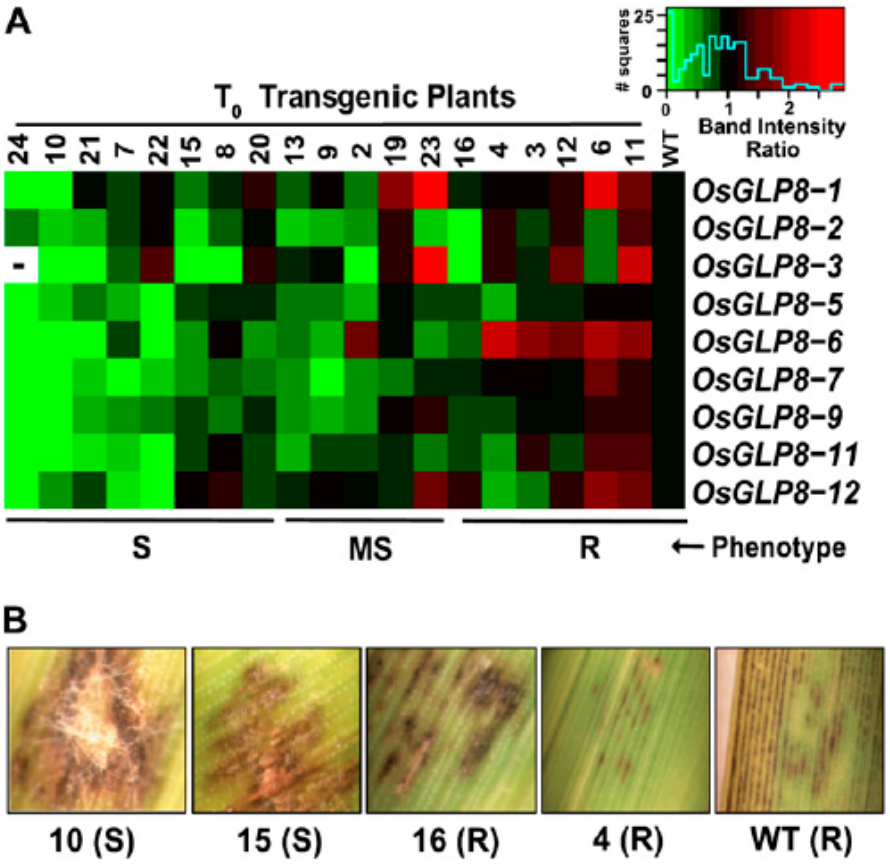


Figure 3. Rice transgenic plants silenced for chr 8 *OsGLP* gene expression show increased rice blast disease relative to wild-type (WT) Kitaake. Silencing of *OsGLP* gene expression in independent uninoculated T_0 (A) and T_1 (C) transgenic plants, as determined by semiquantitative RT-PCR, is indicated as heat maps. Each square in the heat maps indicates band intensity ratio (transgenic-wild type) for a single chr 8 *OsGLP* gene family member (row) in an independent transgenic plant (column). Color keys for each map show the range of expression (relative to the wild type; green = maximal suppression; red = maximal expression; − = missing data) and histograms with distributions of data points. Rice blast disease phenotypes for individual plants (S, susceptible; MS, moderately susceptible; R, resistant) are indicated below the heat maps. B shows the range of blast disease symptoms on individual T_0 and wild-type plants at 7 d after inoculation.

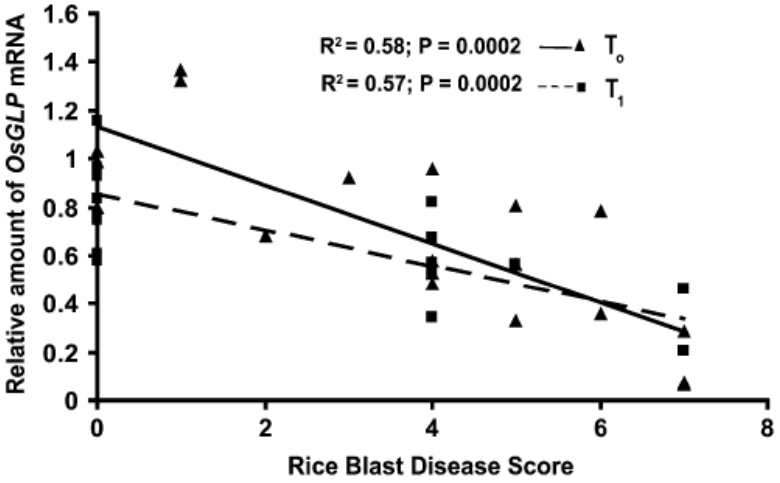


Figure 4. Reduced expression of rice chr 8 *OsGLP* gene members correlates with increased rice blast disease in both T_0 and T_1 plants. Rice blast disease score was assessed in individual T_0 and T_1 transgenic plants at 7 d after inoculation using a scale from 0 (no mycelia or colonization) to 7 (extensive mycelial growth and colonization). Total chr 8 *OsGLP* gene expression for each T_0 and T_1 independent plant was the sum of the relative amounts of mRNA for each constitutively expressed *OsGLP* (band intensity ratio of transgenic-wild type) and normalized with the band intensity of the internal control EF1-α for each plant.

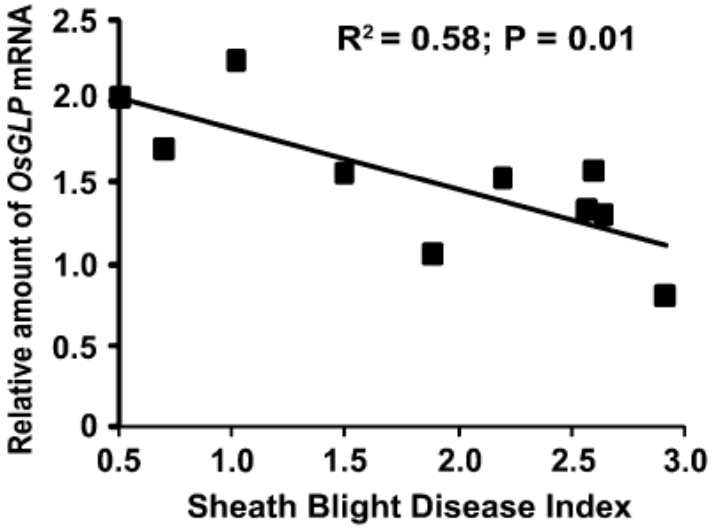


Figure 6. Reduced expression of rice chr 8 *OsGLP* gene members in individual silenced T_1 plants correlates with increased sheath blight disease. Sheath blight disease index was assessed at 14 d after inoculation as described (Jia et al., 2007), and total relative *OsGLP* mRNA values were determined as in Figure 4.

Table I. *Expression/silencing of OsGER4 subfamily members correlates with rice blast disease ($P \leq 0.05$; boldface)*

Regressions of rice blast disease score by *OsGLP* gene band intensity ratio; $n = 19$ individuals per generation. –, Not expressed.

OsGLP	T_0 Transgenic Plants				T_1 Transgenic Plants			
	Slope[a]	r^{2a}	P^a	P^b	Slope[a]	r^{2a}	P^a	P^b
8-1	−1.30	0.14	0.133	0.382	–	–	–	–
8-2	−2.51	0.19	0.074	0.289	−1.51	0.04	0.417	0.189
8-3	−0.92	0.12	0.160	0.257	–	–	–	–
8-5	−4.06	0.25	**0.035**	0.587	−5.09	0.41	**0.003**	0.253
8-6	−2.67	0.72	**<0.0001**	0.067	−5.79	0.66	**<0.0001**	**0.014**
8-7	−4.20	0.62	**0.0001**	0.771	−3.64	0.47	**0.001**	**0.032**
8-8	–	–	–	–	−3.95	0.29	**0.018**	0.570
8-9	−4.90	0.48	**0.001**	0.625	−5.61	0.39	**0.004**	0.449
8-11	−3.50	0.39	**0.006**	0.876	−2.38	0.22	**0.043**	0.397
8-12	−1.57	0.11	0.182	0.425	−1.40	0.01	0.697	0.099
Overall *P* value for the full model				**0.025**				**0.003**

[a]Single linear regression. [b]Multiple regression.

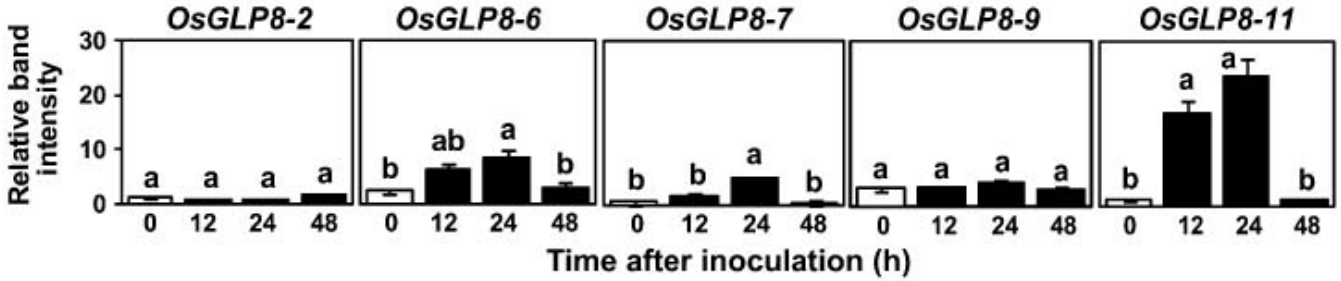


Figure 5. Induction of *OsGLP* genes after inoculation with *M. oryzae*. Three-week-old wild-type Kitaake plants were inoculated with *M. oryzae* isolate Che86061 (10^5 spores mL^{-1}), and leaves were sampled for RNA at 12, 24, and 48 h after inoculation (*x* axis). Plants at time 0 were not inoculated. Expression of selected *OsGLP* genes was screened by RT-PCR, and gel band intensities were quantified and normalized against the reference gene, *EF1-α* (*y* axis; relative band intensities are in arbitrary units). Time point means ($n = 3$ biological repetitions) for each gene were compared with SAS and Proc GLM using the LSD method with a Student-Newman-Keuls test.

Summary

It is possible that phenotypic expression of QTL is not always caused by a single gene but controlled by the coordinated expression of groups of genes. And the chromosomal domains and associated expression patterns are transmitted from one genome to another.

Each clustering gene may be synergic (blast resistance mentioned above) or antagonistic effect on phenotype, so it is important to fine-map and resolve QTLs into single genes. If gene effect of the cluster is synergic, it is helpful to introgress the cluster into elite genetic background.

- **Effects of over-expression of transcriptional factors on abiotic stress tolerance**

Recent work on over-expression of transcriptional factors has provided important insights on individual genes that may play a role in DT.

Stomatal pores regulate gas exchange for photosynthesis and the loss of water by transpiration. The engineering of stomatal closure as a means to reduce water loss is an attractive approach to improve the performance of plants under water limitation, thereby meeting the pressing need of developing crops with higher water use efficiency (WUE).

It should be taken into account for decline of photosynthesis due to diminished gas exchange when designing plants with reduced water loss through enhanced stomata closure.

1 Water use efficiency (WUE)

A ratio of biomass produced to the water used, by enhancing photosynthetic assimilation and reducing transpiration.

Drought avoidance is one of the most important mechanisms of DT, among which drought avoidance mechanisms tend to conserve water by promoting WUE. So, WUE is a trait of importance to all crops in a water-limiting environment.

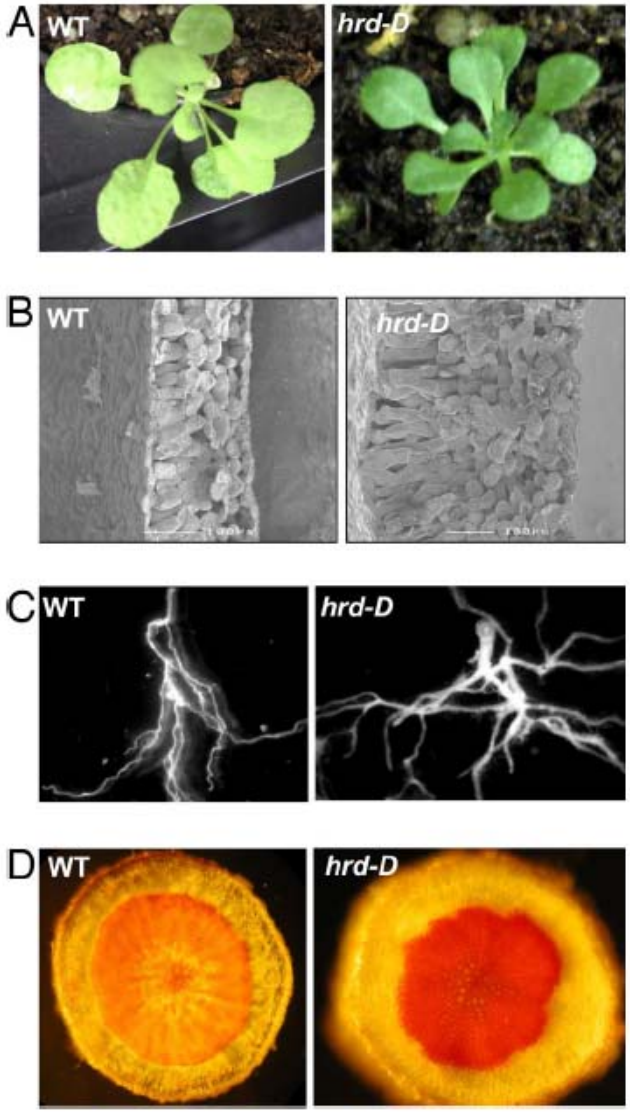


Fig. 1. The *hrd-D* mutant phenotype in *Arabidopsis*. (*A*) Rosette leaf phenotype of WT and *hrd-D* mutant with smaller, slightly curled, thicker deep-green leaves. (*B*) Cryo-fracture scanning electron microscopy section of leaves of WT and *hrd-D* mutant, showing more mesophyll cell layers. (*C*) Root structure of WT and *hrd-D* mutant, showing more profuse secondary and tertiary roots at the root base. (*D*) Cross-section of WT and *hrd-D* roots, showing increased cortical cell layers (lighter stained) and compact stele in the mutant.

The *Arabidopsis HARDY* (*HRD*) gene, an AP2/ERF-like transcription factor, identified by a gain-of-function *Arabidopsis* mutant *hrd-D* having denser roots with enhanced strength, branching, and cortical cells.

***HRD* overexpression in *Arabidopsis* produces deeper green leaf color, thicker leaves with more chloroplast-bearing mesophyll cells.**

Karaba et al., PNAS, 2007, 104(39): 15270-15275

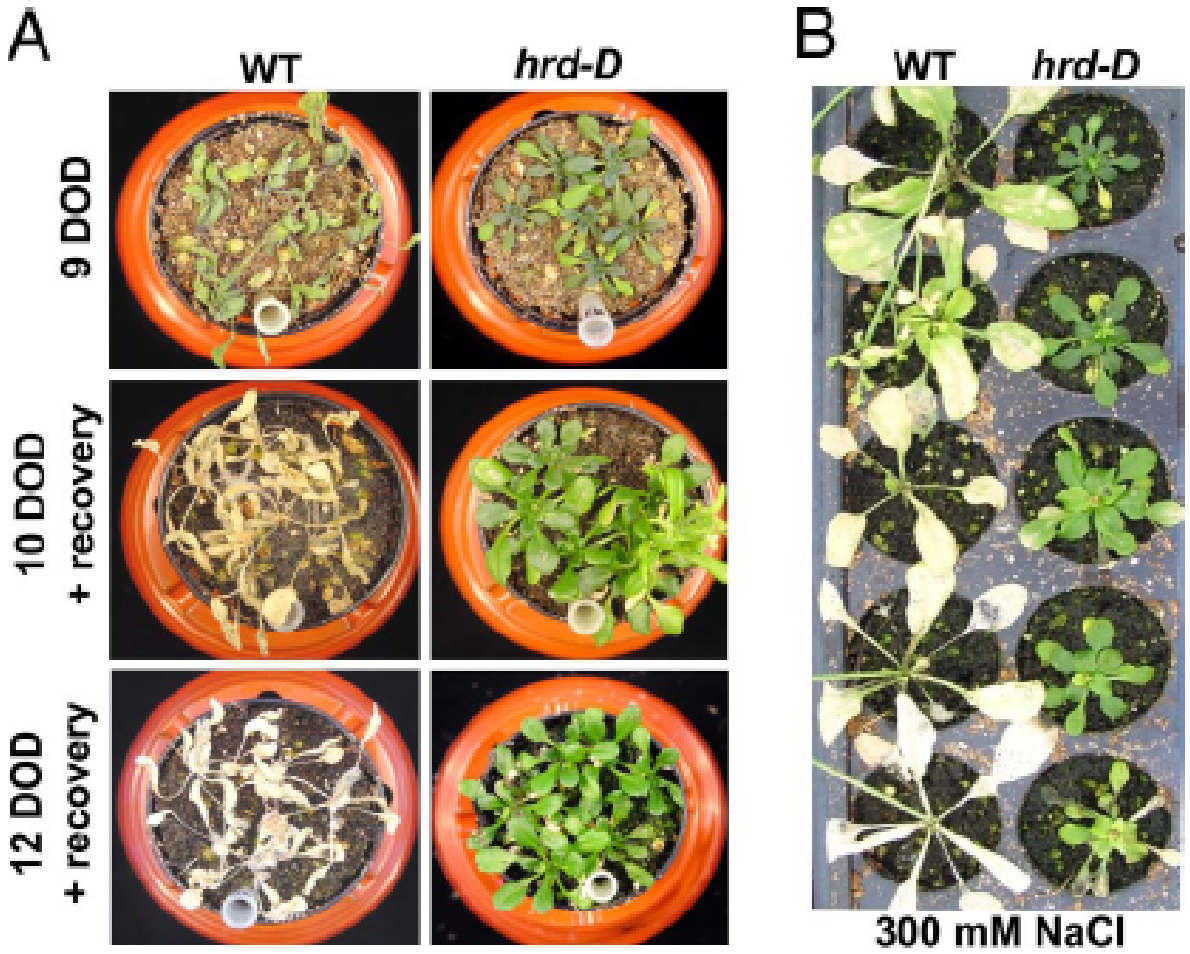


FIg. 3. Stress tolerance/resistance by overexpression of *HRD* in *Arabidopsis*. (*A*) Drought-resistance tests of *Arabidopsis* WT and the *hrd-D* mutant line, treated for 9–12 days without water. The first row is at 9 days of dehydration (DOD), followed by plants treated for 11 and 12 DOD that were subsequently watered to reveal surviving plants. (*B*) Mutant *hrd-D* and WT *Arabidopsis* treated at 300 mM NaCl concentrations, showing bleached/dead plants and surviving *hrd-D* plants. → **Salt tolerance**

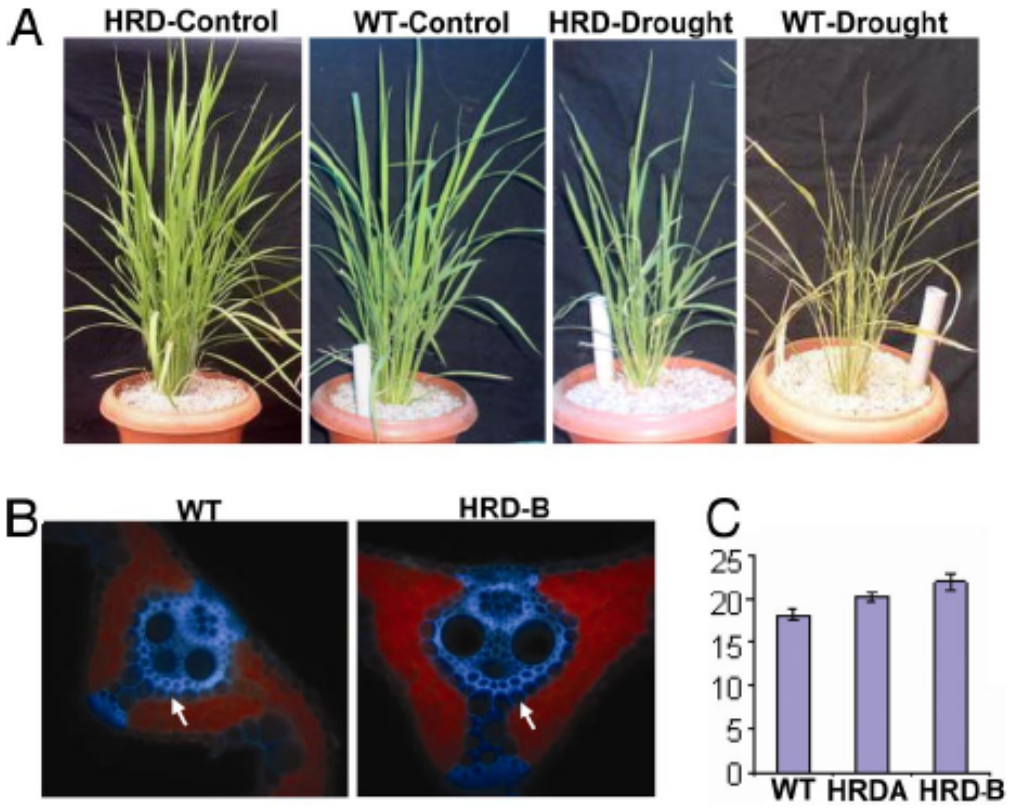


Fig. 4. Phenotype of *HRD* overexpression in rice. (*A*) Rice *HRD* overexpression line compared with WT Nipponbare under well watered (control) and water-stress (70% field capacity) conditions. (*B*) Leaf cross-section of WT and *HRD* overexpression lines, observed under fluorescence microscope, revealing red chlorophyll fluorescence and blue vascular bundles surrounded by the bundle sheath cells marked with an arrow. (*C*) Number of bundle sheath cells in WT compared with *HRD* overexpressors, which show significant increase ($n > 5$, $P = 7.5 \times 10^{-10}$).

***HRD* overexpression in rice increase leaf biomass and bundle sheath cells that probably contributes to the enhanced photosynthesis assimilation and efficiency.**

These drought-tolerant rice plants exhibit increased shoot biomass under well irrigated conditions and an adaptive increase in root biomass under drought stress.

water use efficiency (WUE)

mean transpiration rate (MTR)

net carbon assimilation rate (NAR)

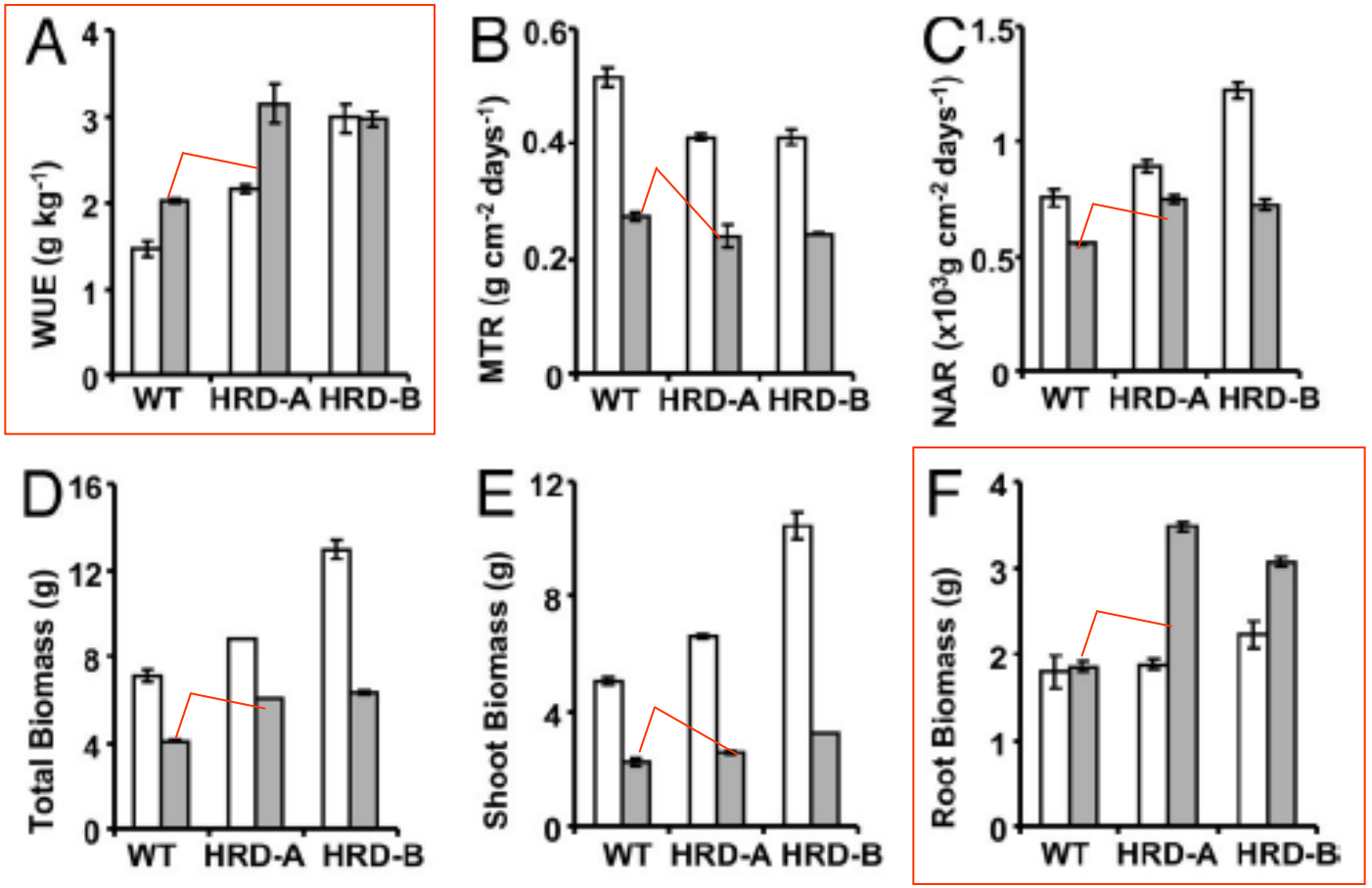


Fig. 5. Physiological analyses of rice *HRD* overexpression lines showing improved WUE. (*A* and *B*) The *HRD* lines and WT Nipponbare tested under well watered (white) and drought stress (shaded) conditions. Bars indicate SE ($n > 3$). All parameters are significant at 1% with calculated *P* values shown for *HRD* vs. WT. (*A*) WUE by gravimetric determination ($P = 1.6 \times 10^{-04}$). (*B*) MTR ($P = 2 \times 10^{-2}$). (*C*) NAR ($P = 2.27 \times 10^{-5}$). (*D*) Total biomass ($P = 9.9 \times 10^{-10}$). (*E*) Shoot biomass ($P = 7.4 \times 10^{-6}$). (*F*) Root biomass ($P = 1 \times 10^{-7}$). (*G*) Instan-

Summary

Overexpression of *HRD* gene in rice generates:

- **Reduction in specific leaf area (leaf area per unit dry weight), suggesting increase in leaf thickness or tissue density, namely more and better mesophyll cells, resulting in high photosynthetic efficiency**
- **Lower stomatal conductance, resulting in reduced transpiration rate**

More photosynthetic capacity and less transpiration (high WUE)

- **Increase root biomass under drought stress, indicating an ability to harvest the scarce water.**

DT

Improved WUE and DT contribute to maintaining yield under drought stress

Restricted transpiration may not result in improved DT in a competitive environment for water

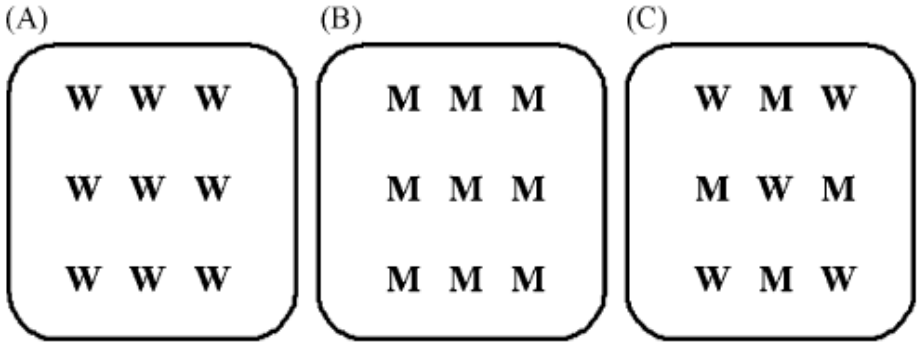


Fig. 1. Sowing grids of wild type (W) and *cbp20* mutant (M) plants. 9 plants were grown in each pots at the patterns indicated with an average distance of 6.5 cm. The weights of pots ranged between 900 and 1100 g at field capacity.

A: Wild type behaved wilting 1 week after water deprivation.

B: *cbp20* and *era1* mutant remained greener and more turgid

C: Mutants did show wilting and characteristics of water shortage very similar or indiscernible from the wild type plants in the same pot (but better than A).

ABA oversensitive *cbp20* and *era1* Arabidopsis mutants

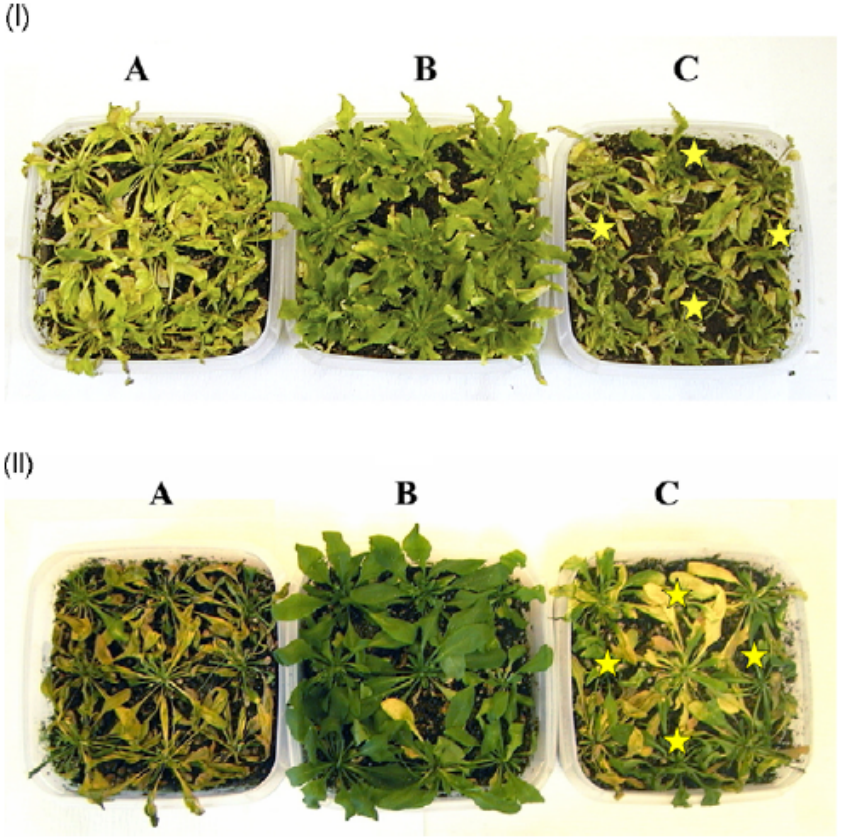


Fig. 2. Rosette leaves of wild type and *cbp20* mutant as well as wild type and *era1* mutant plants (panel I and II, respectively) in the sowing grids after 7 days of water deprivation, flowering stems removed. A—wild type plants (“pattern A”), B—mutants (“pattern B”), and C—mixed plants (“pattern C”, yellow asterisks mark mutant plants). The experiment was repeated five times for *cbp20* and three times for *era1* with similar results, one representative is shown.

Bacso et al., Plant Science, 2008, 174:200–204

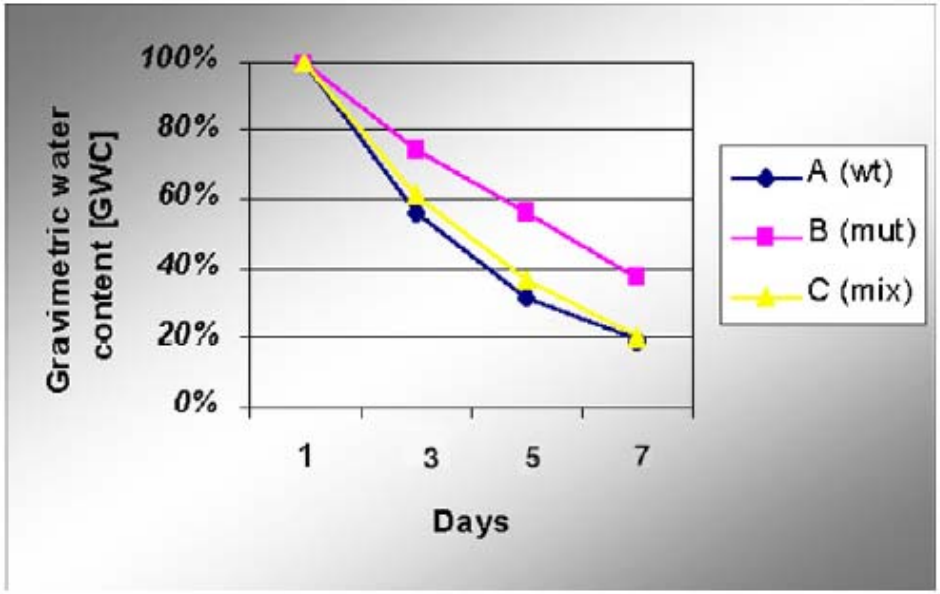


Fig. 6. Water content of soil in the pots containing wild type and *cbp20* mutants determined by gravimetric method. For figure legends see Fig. 3.

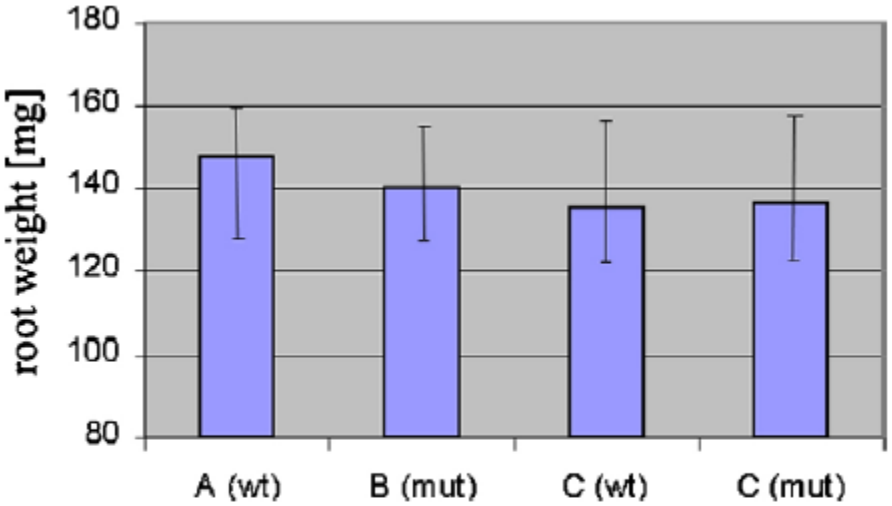


Fig. 3. Fresh weights of the roots of mutant (*cbp20*) and wild type plants at different positions. For the method of measurements see Section 2.

Roots of neighboring plants (wild type) desiccated the soil in the vicinity of the mutants whose water potential declined rapidly following their neighbors, resulting in that pots with both wild type and mutant plants lost water at a rate close to ‘‘pattern A’’ pots, with GWC 20% at day 7 of the experiment.

The fresh weight of roots of the mutants did not differ significantly from that of wild type in any sowing pattern.

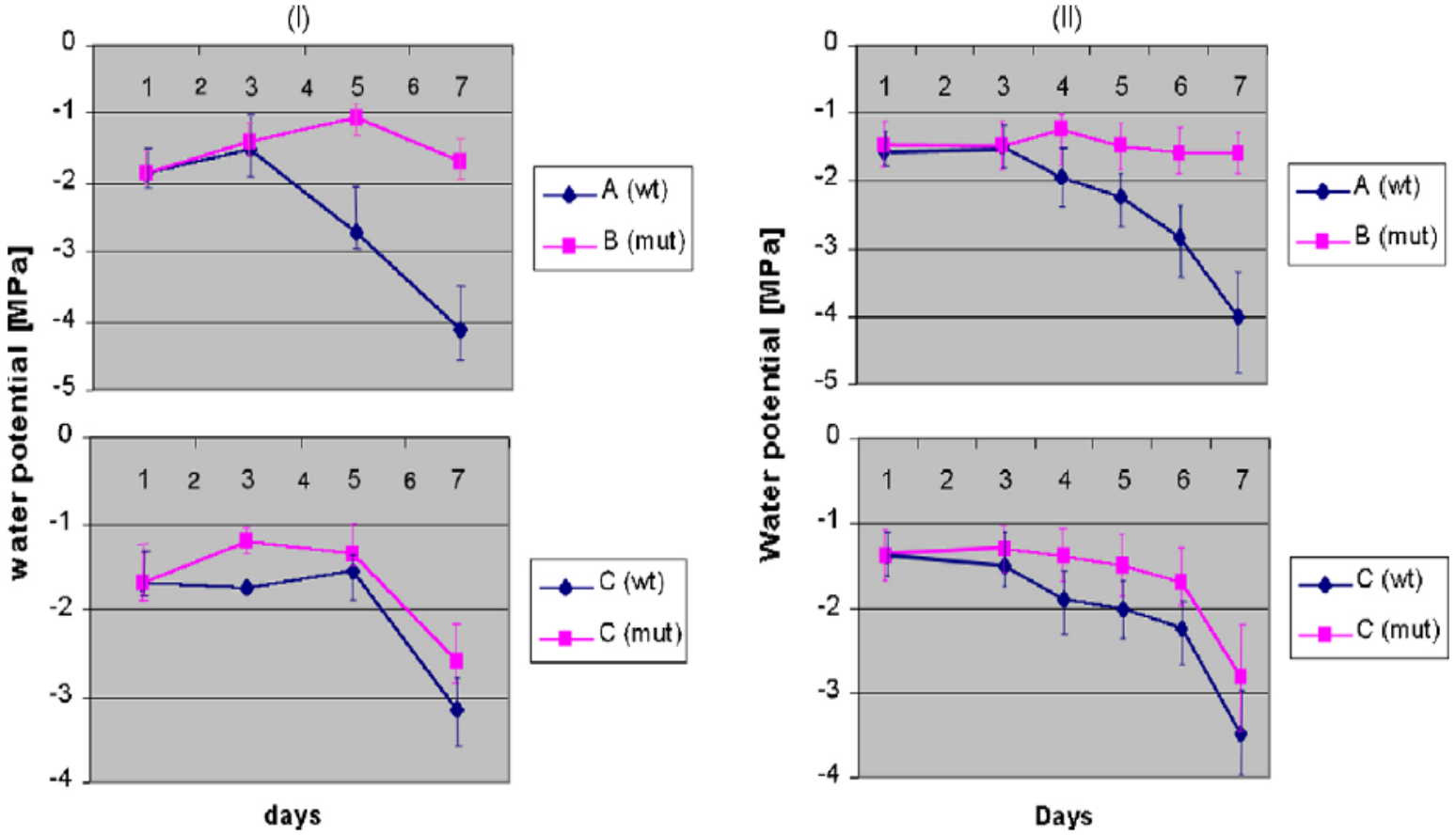


Fig. 4. Changes in leaf water potential at different positions in the sowing grids of wild type and *cbp20* as well as wild type and *era1* mutant plants (panel I and II, respectively). A—wild type plants ("pattern A"), B—mutant plants ("pattern B"), C (wt)—"pattern C" wild type plants, and C (mut)—"pattern C" mutant plants. The experiment was repeated five times for *cbp20* with similar results, one representative is shown.

Wild type plants (pattern A): wilted in about 7–8 days, reaching leaf water potential 4 to 5 MPa
cbp20 mutant plants (pattern B): kept more water, plant water potential and LWC did not sink considerably.
Pots with both wild type and mutant plants: lost water at a rate close to pattern A pots.

Summary

The sensitivity of the water tolerant phenotype may restrict the potential to use this mutant class in agronomy. However, by excluding competition for water as much as possible, e.g. in monoculture, may indeed keep the moisture content of the soil higher allowing the development of DT.

Closed stomata mutants will not be found by traditional screen because as wild type plants dry the soil, emerging mutants will wilt rapidly following the rest of the population. Screening such mutants may be more efficient, e.g. by thermal imaging, where stomatal mutants are selected by the changed temperature of the leaf due to altered transpiration rates or individually planting.

2 *SNAC1* gene

***SNAC1* (*STRESS-RESPONSIVE NAC 1*) gene can be induced by drought specifically in guard cells from the DT variety IRAT109. *SNAC1*-overexpressing transgenic plants showed significantly improved drought resistance at reproductive stage under field conditions and strong tolerance to salt stress at seedling stage.**

The transgenic plants showed much delayed leaf-rolling compared with the negative control S18 and the WT.

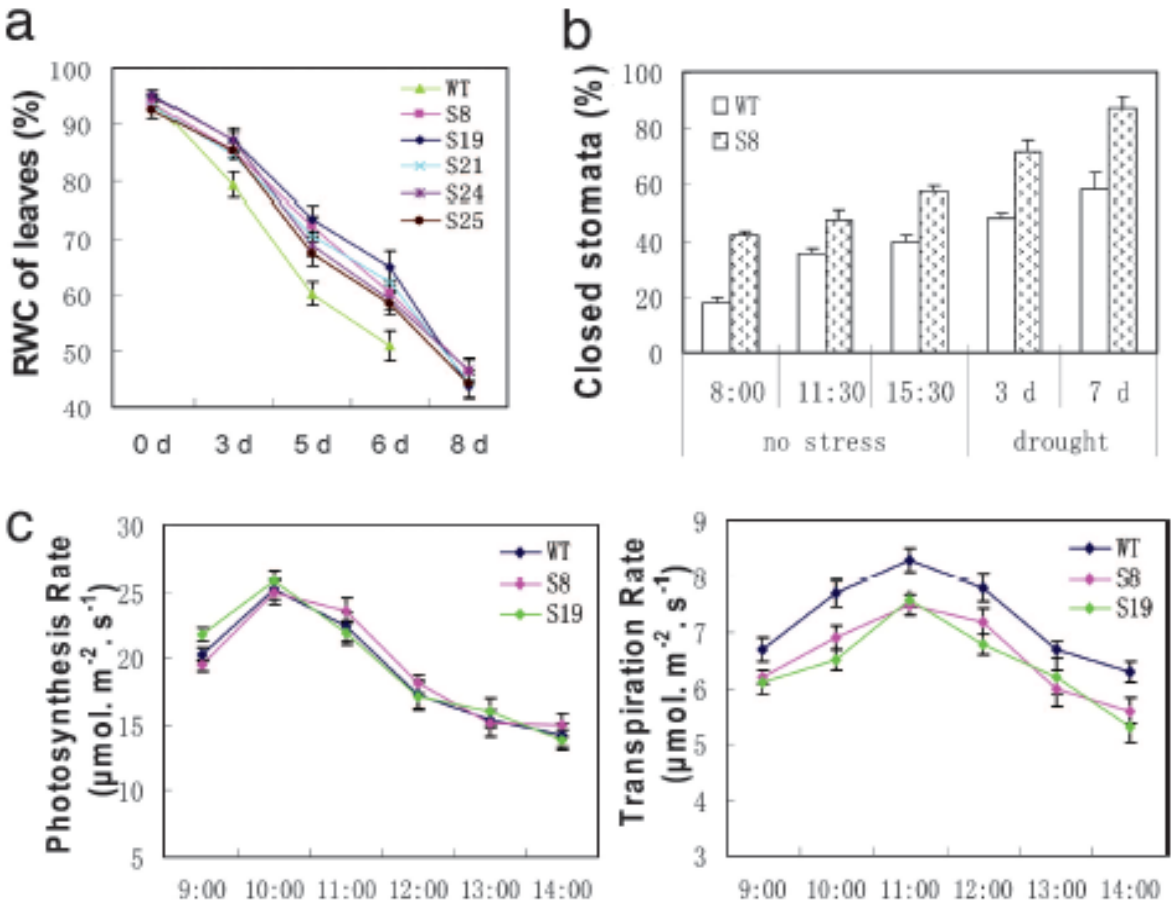
Hu, PNAS, 2006,103 (35): 12987–12992

Table 1. Spikelet fertility (%) of *SNAC1*-overexpressing transgenic rice plants under different drought stress conditions and mRWC for establishing leaf turgor pressure

Line	Well irrigated condition	Severe stress in sheltered field	Moderate stress in open field	Drought stress in PVC pipes	mRWC
WT	87.2 ± 3.4	0.8 ± 0.7	54.3 ± 5.6	48.2 ± 5.3	50.9 ± 1.4
S18	89.1 ± 3.5	0.9 ± 0.6	57.7 ± 5.3	46.3 ± 6.7	ND
S8	85.4 ± 4.2	24.1 ± 3.4**	74.2 ± 6.4**	65.8 ± 7.5**	46.3 ± 1.6*
S19	88.6 ± 2.8	34.6 ± 6.1**	78.3 ± 5.1**	68.3 ± 6.8**	42.2 ± 2.1**
S21	84.7 ± 3.7	23.3 ± 2.8**	75.1 ± 4.3**	71.3 ± 8.4**	44.4 ± 2.0*
S24	89.2 ± 3.4	24.0 ± 3.5**	73.4 ± 5.8**	64.5 ± 6.1**	45.5 ± 1.5*
S25	86.5 ± 3.5	23.0 ± 3.1**	74.1 ± 4.6**	65.1 ± 4.4**	43.6 ± 2.6*

S18 was used as a negative transgenic control (no expression of transgene)

All of the *SNAC1*-overexpressing plants produced significantly higher spikelet fertility than the negative control under all three treatments (severe stress, moderate stress and stress in PVC pipes).



SNAC1 **expressed in guard cells by drought stress. Significantly more stomatal pores were closed in transgenic rice than in the WT under both normal and drought-stressed conditions. Interestingly, photosynthesis rate was not significantly affected in the transgenic plants, but transpiration rate was lower in the transgenic plants than the WT. Transgenic seedlings were significantly more sensitive to ABA treatment, suggesting that the enhanced DT of the transgenic plants was at least partly due to the increased stomatal closure and/or ABA sensitivity to prevent water loss.**

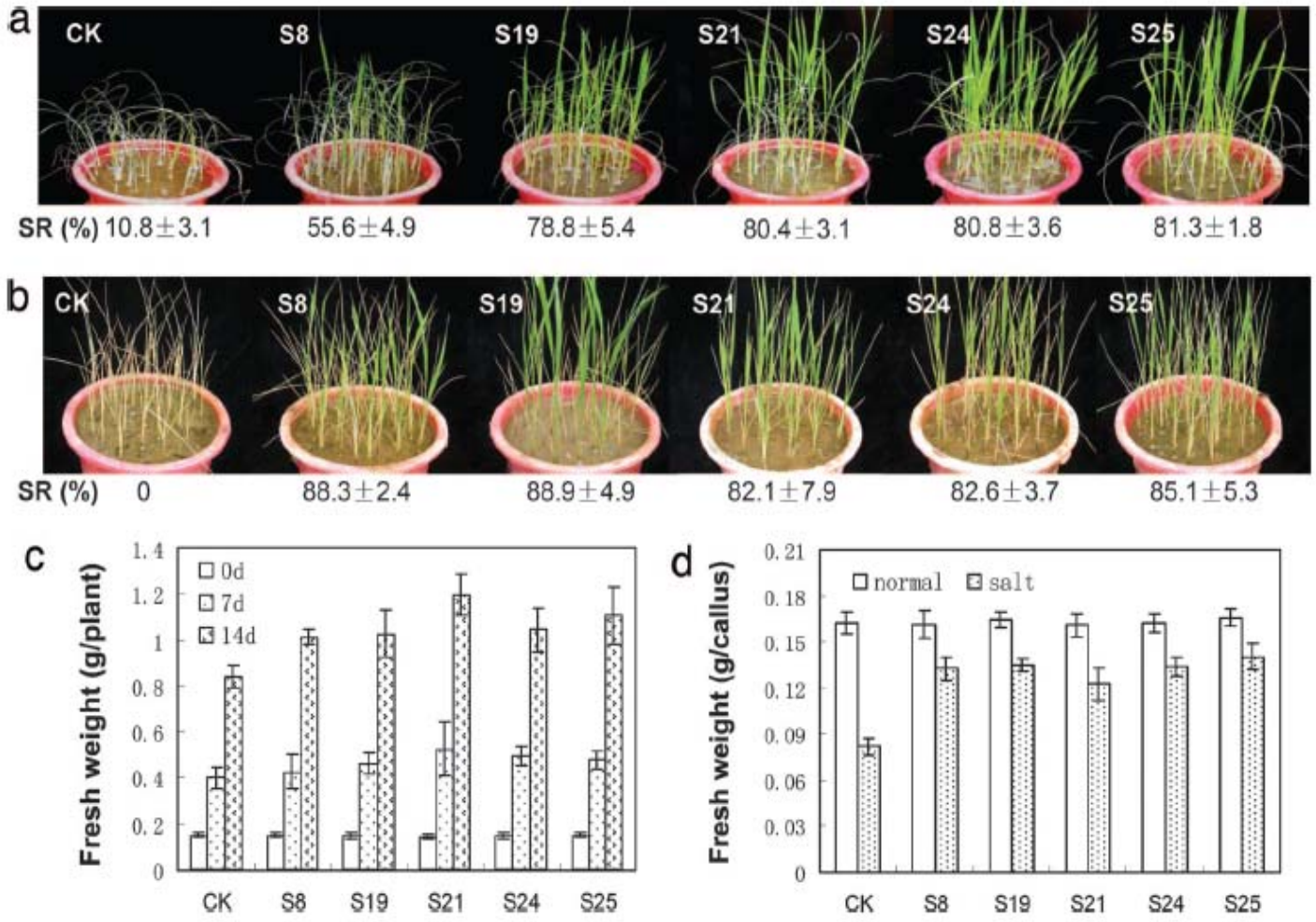


Fig. 4. Improved drought resistance and salt tolerance of *SNAC1*-overexpressing transgenic rice at vegetative stage. (*a* and *b*) Recovery of the *SNAC1*-overexpressing seedlings after drought stress (*a*; 12 days of water-withholding at four-leaf stage followed by 1 week of watering) or salt stress (*b*; 200 mM NaCl for 12 days). Survival rate is indicated below, and the values are based on three repeats (Table 3). CK, WT; SR, survival rate. (*c*) Fresh weight of hydroponic cultured transgenic seedlings measured during the recovery period of 0, 7, and 14 days after 5 days stress with 100 mM NaCl in the nutrient solution. Values are the means ± SD ($n = 10$). (*d*) Fresh weight of calli (starting with 0.1 g of callus with same size) grown in MS medium with 100 mM NaCl for 15 days. Values are the means ± SD ($n = 10$).

Transgenic plants (T_0) or families (T_1 and T_2) of *SNAC1* showed no obvious difference from the WT plants in all of the traits investigated, showing its promising use in rice breeding for DT and ST.

Summary

SNAC1 **significantly enhances DT in transgenic rice (22–34% higher seed setting than control) in the field under severe drought stress conditions at the reproductive stage while showing no phenotypic changes or yield penalty. The transgenic rice also shows significantly improved DT and ST at the vegetative stage.**

Compared with WT, the transgenic rice are more sensitive to ABA and lose water more slowly by closing more stomatal pores, yet display no significant difference in the rate of photosynthesis.

- **MAS for abiotic stress tolerance**

QTLs for the root traits detected in IR64/ Azucena DH lines for backcrossing

Donor		Primary target region			Information on QTLs at primary target[b]			
DH line	Azucena proportion[a] (%)	Chr.	Markers	Length of the interval (cM)	Trait	Interval	R^{2} [c]	Effect
P0055	33.8	1	RZ19 - RG690 - RZ730-RZ801	61.4	MRL	RZ19-RG690	8.9	3.661
					DRW	RG690-RZ730	7.5	0.028
					TRW	RZ19-RG690	9.6	0.119
P0035	38.9	2	RG437 - RG171 - RG157 - RZ318	99.2	MRL	RG171-RG157	9.9	3.812
P0295	44.9	7	RM234 - CDO418 - RZ978 - CDO38 - RG351 - RM248	42.4	MRL	CDO418-RZ978	17.7	4.896
					DRW	CDO418-RZ978	14.7	0.034
					TRW	CDO418-RZ978	4.8	0.080
P0475	37.0	9	RZ228 - RM242 - RZ12 - RM201 - RG667	30.8	MRL	RZ12-RM201	8.8	3.582
					DRW	RZ206-RZ422	5.6	0.022

MRL = maximum root length; DRW =deep root weight (root weight below 30 cm); TRW = total root weight

Large chromosomal regions bearing putative QTL associated with root length in a population derived from a cross between Azucena (deep-rooted upland cultivar) and IR64 (shallow-rooted lowland cultivar) were introgressed into the IR64 background, but the majority of lines carrying the desired introgressions failed to have deeper roots than IR64.

Shen et al., TAG, 2001, 103:75–83

Four QTLs (QTL2, QTL7, QTL9, QTL11) were chosen for improved rooting ability based on three mapping populations: IR64/Azucena (Yadav et al. 1997, Zhang et al. 1999); Bala/Azucena (Price and Tomos 1997; Price et al. 2000); and CO39/Moroberekan (Champoux et al. 1995)

Ch	Near or flanking mapped marker	QTL	R^2%	References
(a) *Targets for introgressions from Azucena*				
2	C601	Root penetration	18.0	Price et al., (2000)
		(ratio of penetrated roots to total roots).	10.8	Price et al., (2002a)
		Deep root weight (well-watered treatment).	15.8	
		Root thickness (well-watered treatment)		
7	RG650-C507	Total root weight	4.8	Yadav et al., (1997)
		Deep root weight	14.7	
		Deep root per shoot ratio	22.3	
		Deep root weight per tiller	18.7	
		Maximum root length	17.7	
8	RG28-RG1	Aroma	69.0	Lorieux et al., (1996)
9	G385	Deep root thickness (well-watered treatment)	18.1	Price et al., (2002a)
	G1085	Deep root thickness (drought-stressed)	13.0	
11	RG2	Root length (hydroponics)	29.8	Price & Tomos (1997)
	C189	Root penetration (ratio of penetrated roots to total roots)	7.2	Price et al., 2000

Above Azucena root-related QTLs have been introgressed into the *indica* cultivar Kalinga III (independent India variety), the selection made in three backcross generations and two further crosses between BC3 lines to pyramid all five target segments. Twenty-two NILs were evaluated for root traits in five field experiments in India, but only one (on ch 9) of the four target QTLs had an effect on root length.

Steele et al.,TAG, 2006, 112: 208–221

The reason for lack of effects of the introgression segments on root length and yield may be:

(1) Target QTLs were responsible for a relatively small phenotypic variation (5.6–17.7%)

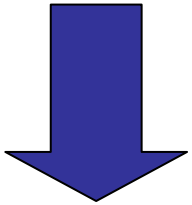
(2) Introgressed region was large, and therefore desirable genes within it could be lost because of recombination during backcrossing

(3) Many root QTLs show strong interactions with the environment, in particular the physical properties of the soil

Owing to considerable G x E, their effects on productivity under stress in the field are very difficult to determine and disappointingly few root QTLs have been found to be related to yield.

- **Our breeding strategies**

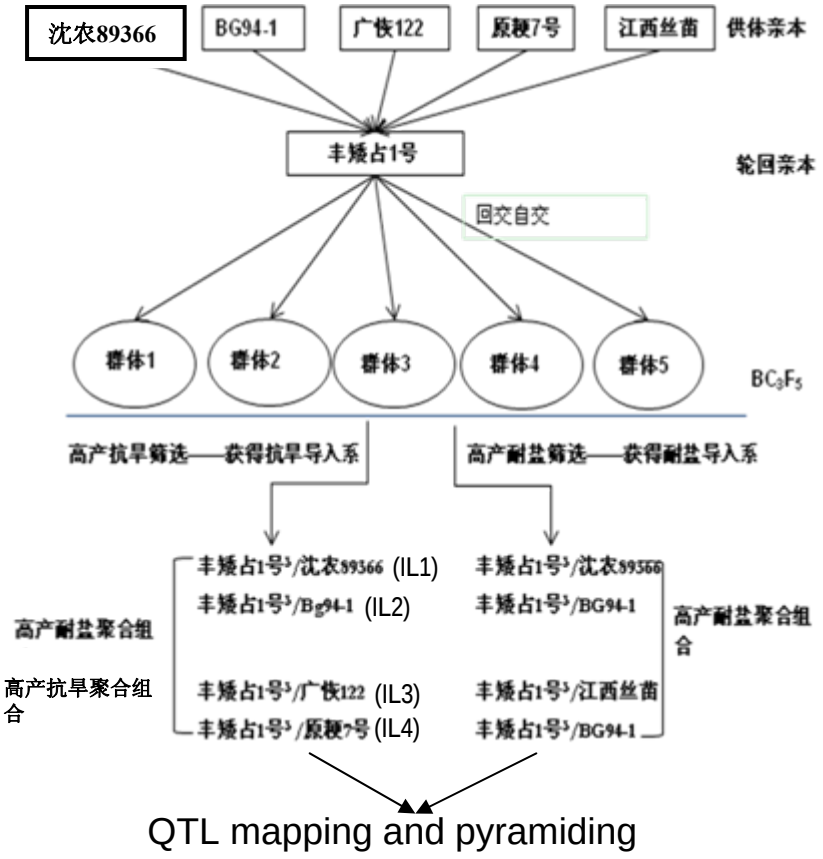
To date, no DT or ST rice varieties have been developed and released to farmers by MAS. In addition to a relatively high costs, the information about magnitude, consistency, genetic background and environment interaction effects of the target QTLs is unclear. Most rice breeders are still reluctant to apply MAS to improving complex traits such as DT and ST



So, a new high efficient strategy combining gene discovery with pyramiding breeding needs to design and apply

高产、抗旱、耐盐导入系的培育及基因发掘与聚合育种的技术路线

回交群体构建以及聚合亲本的获得



聚合群体筛选与交叉筛选以及目标性状QTL的发掘与聚合

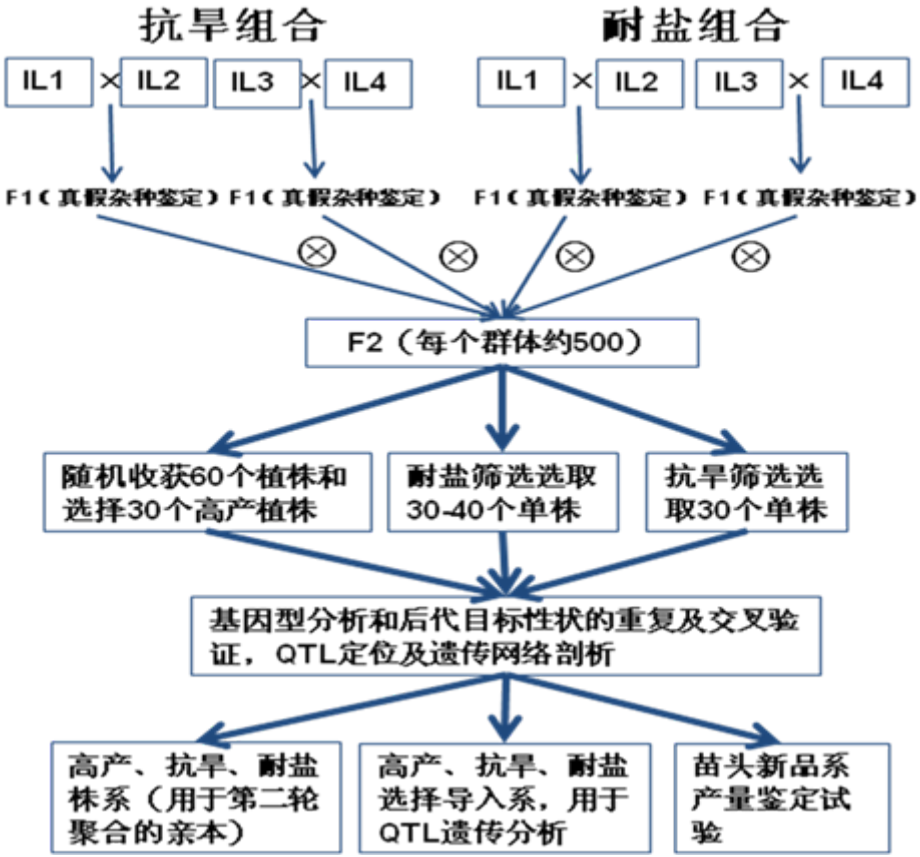


Table 1 Progeny test of high yield, DT and ST selected populations and performance of their yield-related traits in normal irrigated condition

Pyramiding population	Selected population	Test of F_3 lines				Yield-related traits under normal irrigated condition						
		No. of lines	DT (g)	ST	GY (g)	HD (d)	PL (cm)	PNP	SNP	SSR (%)	FNP	GWT (g)
(FAZ1/ Guanhui122) / (FAZ1/Yuanjing7) $(P_1/P_2)F_2$	P_1	12	19.8b	8.8c	30.1a	108.5a	25.0ab	11.4a	282.7ab	57.0abc	161.2bc	18.9a
	P_2	12	21.3b	7.0d	24.9c	108.0ab	25.2a	8.5c	306.1a	69.7a	213.5a	18.5c
	DT selection	30	23.4a	9.7ab	27.3b	106.3ab	24.4ab	9.6b	244.5c	60.3abc	147.3c	19.7a
	ST selection	33	-	10.2a	27.2b	106.0bc	24c	9.8b	246.2c	58.4b	143.6c	18.7c
	GY selection	30	-	9.3bc	29.7a	107.3a	23.9c	10.2ab	237c	57.9bc	136.7c	19.3ab
(FAZ1/ Shennong89366) / (FAZ1/Bg94-1) $(P_1/P_2)F_2$	P_1	12	17.8b	8.4b	25.6ab	107.8a	23.8ab	11.3a	217.0c	63.5a	137.6	19.9
	P_2	12	17.5b	8.3b	24.8ab	109.0a	24.4a	7.7c	261.5abc	53.9ab	141.7	19.4
	DT selection	30	20.9a	8.5b	23.6b	106.6b	24.1ab	8.9c	266.6ab	55.8ab	150.1	18.4
	ST selection	42	-	11.5a	27.2a	107.1ab	23.4ab	9.5bc	261.4ab	58.5a	153.7	18.5
	GY selection	30	-	7.9b	27.0a	106.8b	23.4b	9.9ab	268.8a	55.7b	150.5	18.8
FAZ1	CK	12	15.1	7.1	24.5	108.1	23.1	10.9	248.5	56.3	135.9	18.8

DT: drought tolerance, expressed by grain yield per plant under drought stress; ST: salt tolerance, expressed by survival days of seedlings under salt stress; GY: grain yield per plant under normal irrigated condition; HD: heading date; PL: panicle length; PNP: productive panicle number per plant; SNP: spikelet number per panicle; SSR: seed setting rate; FNP: filled grain number per panicle; GWT: 1000-grain weight.

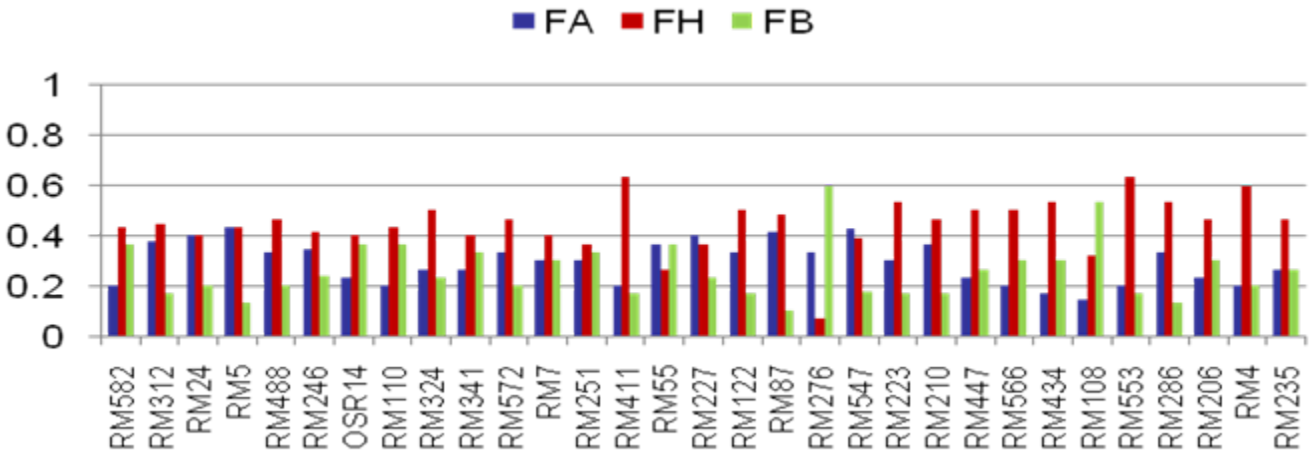
Same letter stands for no difference among means while consecutive and interval letters for differences significant at the levels of 0.05 and 0.01, respectively. Underlined data represent grain weight of parents and single plants selected from the populations.

Table 2 Number and trait performance of lines significantly different from the check, FAZ1 in the high yield, DT- and ST-selected populations

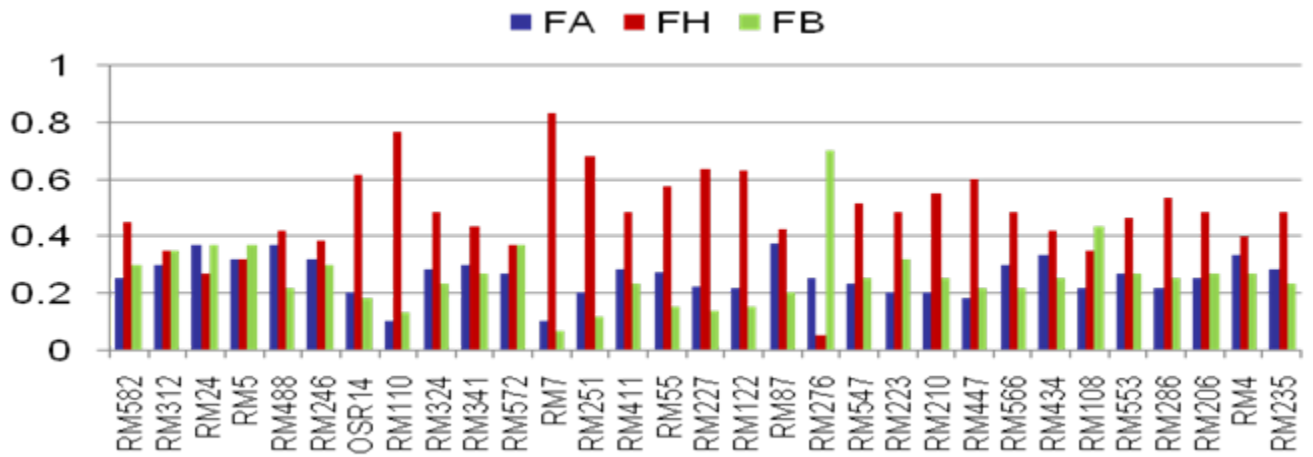
Selected pop.	Comp. with check	HD		PL		PNP		SNP		SSR		FNP		GWT		GY	
		M	%	M	%	M	%	M	%	M	%	M	%	M	%	M	%
Pop1-DT	De	105 (2)	-3.3	(0)		9 (3)	-15.7	219 (2)	-11.7	(0)		106 (2)	-21.7	(0)		22 (1)	-9.5
	In	(0)		245 (2)	4.9	(0)		280 (1)	12.7	72 (2)	33	157 (2)	15.6	19 (1)	4.5	32 (2)	31.9
Pop1- ST	De	105 (4)	-3.1	(0)		9 (1)	-14.7	230 (3)	-7.3	(0)		123 (1)	-9.7	(0)		22 (2)	-11.5
	In	(0)		25 (3)	5.1	(0)		(0)		69 (2)	29	155 (1)	13.8	19 (1)	1.2	33 (2)	34.8
Pop1-HY	De	105 (2)	-2.9	(0)		7 (2)	-34.5	(0)		(0)		120 (2)	-11.6	(0)		21 (1)	-15.6
	In	112 (1)	3.6	24 (3)	4.5	(0)		(0)		75 (1)	40	167 (3)	22.8	19 (1)	4	34 (2)	40.3
Pop2-DT	De	106 (3)	-1.9	22 (3)	-4.7	9 (5)	-21.4			(0)		(0)		(0)		19 (2)	-21
	In	(0)		26 (1)	9.3	(0)		301 (1)	21	(0)		195 (1)	43.7	19 (2)	2.8	(0)	
Pop2- ST	De	106 (3)	-1.6	23 (1)	-2.4	7 (1)	-34.5	(0)		47 (1)	-12.5	(0)		16 (2)	-12.7	17 (2)	-30.1
	In	(0)		24 (1)	3.9	(0)		(0)		62 (4)	16.1	186 (4)	36.5	20 (4)	6.5	(0)	
Pop2-HY	De	(0)		(0)		9 (3)	-18.2	(0)		(0)		(0)		(0)		19 (1)	-21.2
	In	(0)		24 (2)	3	(0)		269 (1)	8.2	(0)		158 (1)	16.3	20 (2)	7.2	(0)	
CK		108		23		11		248		54		136		18.8		25	

Table 3 Promosing pyramided lines selected from intercross or repeated screening for high yield and salt tolerance

Selected pop.	Intercross or repeated screening trait	No. of selected lines	Line #	Yield of introgression line (g)			Salt tolerance of introgression line at the seedling stage					
							No. of survival days			Score of salt toxicity of leaves		
				Trait value	Check of higher value parent	±% comp. with check	Trait value	Check of higher parent	±% comp check	Trait value	Check of higher parent	±% comp check
DT selected (30)	HY	1	QP49	**43.5**	**30.1**	**44.8**	10	8.8	13.6	4.5	5.5	18.2
	ST	11	QP47	31.8	30.1	5.5	**11**	**8.8**	**20.6**	**4.5**	**5.5**	**18.2**
			QP48	29.8	30.1	-0.9	**11**	**8.8**	**22.9**	**4.5**	**5.5**	**18.2**
			QP63	24.3	30.1	-19.3	**12**	**8.8**	**36.4**	**4.5**	**5.5**	**18.2**
			QP60	26.3	30.1	-12.6	**12**	**8.8**	**31.8**	**4**	**5.5**	**27.3**
			QP61	28.8	30.1	-4.3	**11**	**8.8**	**30.3**	**4**	**5.5**	**27.3**
			QP36	28	30.1	-7	**11**	**8.8**	**29.5**	**4**	**5.5**	**27.3**
			QP37	28.2	30.1	-6.3	**11**	**8.8**	**29.7**	**5**	**5.5**	**9.1**
			QP62	26.1	30.1	-13.1	**11**	**8.8**	**23.4**	**4.5**	**5.5**	**18.2**
			QP64	24.8	30.1	-17.6	**11**	**8.8**	**23.4**	**5**	**5.5**	**9.1**
			QP59	25.8	30.1	-14.3	**11**	**8.8**	**20.6**	**4.5**	**5.5**	**18.2**
			QP35	21.2	30.1	-29.5	**10**	**8.8**	**18.9**	**5**	**5.5**	**9.1**
HY selected (30)	HY	2	QP163	**38.6**	**30.1**	**28.4**	9.6	8.8	9.1	5	5.5	9.1
			QP167	**36.6**	**30.1**	**21.8**	11.4	8.8	29.5	4	5.5	27.3
	ST	7	QP171	35.8	30.1	18.9	**10**	**8.8**	**17.1**	**4.5**	**5.5**	**18.2**
			QP169	32.1	30.1	6.7	**12**	**8.8**	**33**	**4.5**	**5.5**	**18.2**
			QP168	25.4	30.1	-15.6	**13**	**8.8**	**51.1**	**4**	**5.5**	**27.3**
			QP166	28.3	30.1	-6	**11**	**8.8**	**29.1**	**4**	**5.5**	**27.3**
			QP164	23	30.1	-23.4	**11**	**8.8**	**25.7**	**4**	**5.5**	**27.3**
			QP170	17.4	30.1	-42.2	**11**	**8.8**	**25.1**	**4.5**	**5.5**	**18.2**
			QP165	24.5	30.1	-18.7	**11**	**8.8**	**20.6**	**4**	**5.5**	**27.3**
ST selected (33)	HY	2	QP327	**36.6**	**30.1**	**21.6**	NA	NA	NA	NA	NA	NA
			QP337	**34.9**	**30.1**	**15.9**	NA	NA	NA	NA	NA	NA



Drought selected F_2



Random F_2

Frequency distribution of genotypes at polymorphic loci in the selective and random populations selected from pyramiding populations for high yield, DT and ST

Table 4 QTLs for HY, DT and ST detected in the selective populations by marker distorted segregation

	Marker	Ch.	Position (cM)	DT selection		Yield selection		ST selection	
				X^2	*P*	X^2	*P*	X^2	*P*
(FAZ1/ Guang122)/ (FAZ1/ Yuanjing7)	**RM486**	**1**	**153.5**	**27.3**	**0**	**18.8**	**0.0001**	**25.8**	**0**
	OSR14	2	6.9	7.8	0.0207				
	RM471	4	53.8					13.5	0.0012
	RM584	6	26.2			7.7	0.0209		
	RM3	**6**	**74.3**	**13.7**	**0.0011**	**7.5**	**0.0231**		
	RM547	8	58.1	27.9	0	20.0	0	31.0	0
	RM21	11	85.7	10.8	0.0046				
	RM4	12	5.2			11.9	0.0026		
	RM2	NONE	NONE	8.1	0.0176				
(FAZ1/ Shennong89366)/ (FAZ1/ Bg94-1)	**RM297**	**1**	**155.9**	**6.5**	**0.039**	**10.4**	**0.0054**	**9.9**	**0.007**
	RM324	2	66					6.3	0.0426
	RM55	3	168.2			6.5	0.0386		
	RM3	**6**	**74.3**	**9.5**	**0.0087**	**13.4**	**0.0012**	**7.7**	**0.0213**
	RM444	9	3.3					56.4	0
	RM4A	12	5.2			6.3	0.043		
	RM235	12	91.3	12.6	0.0019				

结　论

（1）从不具抗旱和耐盐的水稻品种的回交导入后代，经过抗旱和耐盐的严格鉴定和聚合，能够实现了高产、抗旱和耐盐的结合。表明通过回交和针对抗逆性的严格鉴定是挖掘这种有利隐蔽基因的有效途径。

（2）在高产抗旱或耐盐聚合群体的高产、抗旱和耐盐选择后代，水田条件下各种农艺性状均有较大分离，为选育到适应不同生态条件下的节水抗旱、耐盐的高产水稻品种奠定基础。

（3）发现经抗旱和耐盐筛的个体在全基因组范围的杂合基因型频率显著降低，相对于随机群体，后代表型稳定有明显加快的趋势。为此，在开展高产抗旱和高产耐盐育种中，先筛选抗旱和耐盐性，再从获得的抗旱和耐盐后代鉴定产量性状，不但能将高产与抗逆性结合起来，而且能加速育种材料的稳定速度。

（4）无论是高产抗旱还是高产耐盐聚合的旱选后代，均能交叉筛选出较多的耐盐株，而且耐盐水平与盐选后代的耐盐性相当。这启示我们，在高产、抗旱、耐盐聚合育种中，先进行抗旱筛选，再对旱选材料进一步鉴定耐盐性和高产性状，可能比较容易获得高产、抗旱和耐盐的个体，从而使抗逆育种事半功倍。

Thanks for your attention!