

Improvement of rice drought tolerance through backcross breeding: Evaluation of donors and selection in drought nurseries

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Abstract

A large-scale backcross breeding project has been undertaken to improve drought tolerance in rice. Over 160 donor cultivars from 25 countries have been used in this project, representing a significant proportion of the genetic variation in cultivated rice. These cultivars were evaluated in field experiments in the Philippines to assess their responses to drought in terms of plant height, heading date, and grain yield. Drought was imposed near heading stage, in experiments that were established either in lowland (anaerobic) fields or upland (aerobic) soil. Despite the poor adaptation of some cultivars to the tropics, it was possible to identify significant variation in plant response to drought treatments, and contrasting effects on flowering delay and growth. Subsequently, 325 BC₂F₂ bulk populations, developed by backcrossing many of these donors to one of three elite recurrent parents, were screened under drought in lowland or upland nurseries. Stress levels were managed to eliminate almost all seed set in recurrent parents, and those progeny that produced grain were selected as being putatively drought-tolerant. The selection intensity varied across years and in selection environments with more severe stress, higher selection intensity could be imposed. The number of plants selected within a population was not associated with the per se drought response of the donors in the direct evaluation, indicating the wide presence of cryptic genetic variation for drought tolerance in the apparently drought-susceptible cultivars. The genetic background of the recurrent parent affected the number of plants selected, as did the selection environment (upland versus lowland nurseries). These drought-selected introgression lines represent a useful genetic resource to develop improved cultivars for farmers in rainfed or water-scarce rice-growing regions, and also to improve our understanding of the genetic and molecular basis of drought tolerance in rice. Genetic analysis of the selected lines, reported elsewhere, indicated specific regions of high introgression. Yield evaluations of the selected lines are now underway across a range of drought scenarios.

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Keywords: Abiotic stress; Backcross; Drought; Introgression lines; *Oryza sativa*; Rice

1. Introduction

Rice is the basic food crop of Asia, providing over 30% of the calories consumed in the region (Narciso and Hossain,

2002). Rice production in Asia has increased by 2.6 times since 1961, primarily as a result of the “Green Revolution”, which dramatically increased the rice productivity in the high-input irrigated systems (Khush, 1999). The rainfed systems, which occupy about 32% of the rice-growing area in Asia, have benefited less from the Green Revolution. Drought is the largest constraint to rice production in these systems, affecting 19 million ha of upland rice and over 14 million ha of rainfed lowland rice (Evenson et al., 1996; Pandey et al., 2000). As economic development and urbanization proceed in many parts of Asia, emerging water shortages are leading to rationing of water in regions where irrigated lowland rice has

Abbreviations: BC, backcross; HI, harvest index; IL, introgression line; PET, potential evapo-transpiration; RP, recurrent parent

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traditionally been grown, and these production systems are also becoming water-limited.

Low-yielding traditional varieties are still grown in about 50% of the rainfed area of Asia, largely because of their better adaptation to different stresses and favored grain quality (Mackill et al., 1996). Farmers in drought-prone regions often favor traditional cultivars that require limited inputs in order to cope with the uncertainty of water supply. Progress in developing high-yielding, drought-tolerant rice cultivars by conventional breeding has been slow, largely because of difficulties in precisely defining the target environment, complex interactions of drought tolerance with environments, and lack of appropriate screening methodology (Cooper et al., 1999; Wade et al., 1999). Considerable work has recently been undertaken to understand the genetic basis of putative drought-adaptive traits in rice (Babu et al., 2003; Courtois et al., 2000; Price and Courtois, 1999; Price et al., 2002a,c; Robin et al., 2003), but it has been difficult to identify genetic segments with clear and repeatable effects on yield under stress. The major challenge facing plant breeders is how to combine the high-yield potential of modern rice cultivars with robust drought tolerance.

We report here the initial steps of a large-scale backcross breeding effort to improve drought tolerance in rice that was initiated at the International Rice Research Institute. The objectives of the studies described here were to characterize parental cultivars used in the backcrossing program for their response to drought in both lowland and upland managed stress in the dry season, to manage drought nurseries to reveal genetic variation in drought response within BC₂F₂ population bulks, and to assess the relationship between parental performance and the number of superior plants recovered in the selection nurseries. This project has developed large numbers of drought-selected introgression lines (ILs) in elite lowland genetic backgrounds, which will undergo subsequent evaluations for performance under drought. A similar approach has been used to address other biotic and abiotic stresses (Ali et al., 2005).

2. Materials and methods

2.1. Plant material

Three elite lines were used as the recurrent parents: two broadly adapted, high-yielding lowland *indica* cultivars (IR64 and Teqing), and the tropical *japonica* new plant type breeding line IR68552-55-3-2 (NPT; Khush, 1995). A total of 163 varieties of diverse origins were used as donors, including 72 modern varieties, 74 landraces, and 17 breeding lines from 25 countries. These donors, according to an assay based on 101 simple sequence repeat (SSR) markers (Yu et al., 2003), included 53 japonicas, 109 indicas, and 1 deep-water rice, Jalmagna, from India. A total of 322 crosses were made between the recurrent parents and donors. These were advanced to BC₂F₂ generation. This resulted in 124 IR64

BC₂F₂ populations, 75 Teqing BC₂F₂ populations, and 123 NPT BC₂F₂ populations.

2.2. Evaluation of parents

The parental lines were evaluated in small plots four field environments in the dry season of 2000 on the IRRI Experiment Station at Los Banos, Philippines (14°11'N, 121°15'E). Two replications were included in each environment, arranged as an alpha-(0,1) lattice. Plot size was 3 m × 3 m rows in the lowland and 3 m × 2 m rows in the upland (aerobic) field. Independent randomizations were used for each environment to minimize possible effects of inter-plot competition. Weeds in both lowland and upland environments were controlled by a combination of chemical and manual methods. Insects (particularly stemborers) were controlled chemically. The basal fertilization rate was 30 kg ha⁻¹ each of N, P, and K, with two additional 30 kg ha⁻¹ N applications, made at 44 and 66 days after sowing.

In the lowland field, seedlings were transplanted at a spacing of 25 cm × 25 cm. The field was flooded to a depth of 2–5 cm 2 weeks after transplanting. In the lowland stress treatment, the field was drained 73 days after sowing, and was not reirrigated. By 10 days after draining, soil moisture tension in the stress treatment reached 30 kPa at 15 cm depth, which is below field capacity. By 15 days after draining, some leaf rolling was observed. The free water level dropped to 70 cm below the soil surface by 10 days after the field was drained, and remained at or below that level for the remainder of the season. Total rainfall received between draining the field and harvest was 74 mm, cumulative pan evaporation (PET) during that period was 311 mm. The dynamics of stress development under this type of water management in these soils in the dry season has been reported elsewhere (Cruz et al., 1986; Tuong et al., 2002). In the upland field, seeds were sown directly in the field on raised beds at 0.9 m spacing. Two rows were sown on each bed. Water was supplied by sprinkler irrigation as required for the initial 60 days of the season. After that date, furrow irrigation was applied twice weekly to maintain the soil moisture tension below 20 kPa in the control treatment to a depth of 30 cm. In the stress treatment, the frequency of irrigation was reduced to every 7–8 days, allowing the soil moisture tension to reach 70 kPa at 15 cm depth and 50 kPa at 30 cm depth between irrigation events. Relationships between soil and plant water potentials and the dynamics of stress development in droughted rice grown in this field during the dry season have been reported elsewhere (Turner et al., 1986).

Data collected in each experiment included plant height, anthesis date, tiller number, straw production, grain yield, and panicle harvest index (PHI). PHI is the ratio of grain weight to panicle weight, and it is closely correlated with spikelet fertility in rice. In the lowland experiments, data were also collected on yield components from a sample of

eight fully bordered hills. In the upland field, scores of plant stand and type were collected in the control treatment, and scores of leaf rolling and leaf drying were recorded in the stress treatment. Leaf relative water content (RWC) was also measured in the upland stress treatment, and stomatal conductance was measured for a subset of 73 entries using a Li-Cor 1600 porometer. The cultivars were measured on four different dates in order to confine readings to the period from 10:00 to 12:00, and results were expressed relative to a repeated check that was measured every 15th reading.

2.3. Stress nurseries

In the dry season of 2001, 157 bulk BC₂F₂ populations (64 IR64 populations, 31 Teqing populations, and 62 NPT populations) were screened in a lowland stress nursery. Seeds of the bulk BC₂F₂ populations were sown in seedling trays and 20-day seedlings were transplanted into the field as single plants at 20 cm × 25 cm spacing. Each bulk occupied eight 5-m rows, and the recurrent parent was sown on either side of each plot (each BC₂F₂ population had 200 plants). The lowland field was flash flooded as needed until 2 weeks after transplanting, and then the water level in the field was maintained at a depth of 5 cm. Water stress was imposed by draining the water 50 days after sowing, with no subsequent irrigation. Rainfall contributed 132 mm in the period from draining to harvest, and PET was 361 mm. Stress was severe and the recurrent parents did not produce fertile panicles. Those BC₂F₂ plants that produced seed were selected.

In the upland 2001 nursery, the soil was prepared dry, and then flash flooded before a final superficial cultivation to provide favorable conditions for transplanting, which was conducted as in the lowland nursery. Subsequently, the field was surface irrigated every 7–10 days. This allowed the soil moisture at 15 and 30 cm depth to decline to 70 and 50 kPa, respectively, between irrigation events. A total of 51 BC₂F₂ bulks (20 IR64 populations and 31 Teqing populations) were screened. In this nursery, one of the recurrent parents (IR64) did not produce seed, but the other (Teqing) did. Selection of superior BC₂F₂ plants was based on greater leaf area development, good grain filling, and superior grain yield relative to the parents in adjacent rows (Teqing populations) or successful grain production (IR64) in the upland field. If superior plants occurred in a group, they were assumed to be due to a more favorable water environment in that particular area, and they were not selected. In some cases, flowering was delayed in the majority of plants, but some plants flowered early; these were also selected.

In the lowland drought nursery of 2002, 168 additional bulk BC₂F₂ populations (60 IR64 populations, 47 Teqing populations, and 61 NPT populations) were screened in a lowland field managed similarly as in 2001, except that the number of plants per BC₂F₂ population was 250, and the last irrigation was applied 61 days after sowing (11 days later than in 2001). Only 25 mm of rain fell during the period from draining to harvest, and PET was 429 mm. While the

soil moisture tension at 15 cm depth reached 60 kPa by the time of flowering, the soil moisture status at 30 cm remained near field capacity in this season. The stress level eliminated most seed set in all three recurrent parents, but stress was less severe than in 2001. All BC₂F₂ plants that produced seed were selected.

2.4. Characterization of selected lines for plant type and maturity in flooded conditions

Seeds from the selected BC₂F₂ plants were sown as BC₂F₃ families in the following wet season and transplanted into a lowland field. BC₂F₄ progenies were harvested for subsequent evaluation. In order to assess any changes in maturity that occurred under selection in the stress nurseries, F₄ families derived from plants selected in stress nurseries in 2001 were sown and transplanted into fully irrigated conditions in 2002, as described for previous years. Flowering date and plant height were recorded. Yield evaluations under drought are underway for all selected lines; these results will be reported elsewhere.

3. Results

3.1. Performance of parental lines in contrasting environments

3.1.1. Lowland evaluation

Significant differences were observed among the parental lines for plant height, anthesis date, tiller number, straw and biomass production, yield, and all yield components. Water level significantly affected all traits. The interaction between water level and cultivar was significant for plant height, grain yield, the proportion of productive tillers, biomass production, and thousand grain weight (TGW). Under lowland irrigated conditions, most adapted lines with yield significantly above average were *indica* types from South and Southeast Asia (Table 2). In contrast, most lines with significantly below-average yields were tropical or temperate *japonica* lines. These included Azucena, Calcag, and Milagrosa (Philippines), Molok (Indonesia), Haoannong and Yunguang8 (China), UZ-Rosz275 (Uzbekistan), Khazar (Iran), Nipponbare and UP15 (Japan), Pokhreki (Nepal), and M202 (USA). Under both water levels, there were some entries that produced no grain; these were omitted from subsequent analyses. The yield components that were significantly correlated with yield, explaining 20% or more of the observed variation ($r > 0.40$), included panicle number and grains per panicle. Biomass and harvest index (HI) were also correlated with grain yield, but there was no association between heading date and yield.

On an average, the lowland stress reduced grain yield to 75% of the control value. Cultivars with greater yield potential tended to be proportionately more affected by

stress than low potential or poorly adapted cultivars, and the correlation between yield reduction (stress yield/control yield) and control yield was -0.43 . In order to assess yield response to water regime independent of yield potential, standardized residuals were calculated from the regression of stress yield on control yield. These residuals were not correlated with control yields. Based on standardized residuals, 12 superior cultivars were identified for lowland drought conditions (Table 2). Three additional cultivars were considered superior because of yields greater than 3.3 Mg ha^{-1} in the stress treatment. This set of superior lines included six lines that also performed well in the control plots (Table 2). Low-yielding lines under stress included Calcag, Nipponbare, and Haoannong, also identified in the control treatments, along with AP423, Phalguna, and Swarna (India), TB154E-TB-2 (Indonesia), Ilmibyeo (Korea), and Tai-Nong67 (Taiwan). Important yield components under lowland stress were percentage of fertile spikelets and grains per panicle. As in the control treatment, biomass and HI were also significantly correlated with grain yield.

Yield under full irrigation was positively correlated with yield under stress ($r = 0.57$, $P < 0.001$). Nonetheless, a wide range of yield responses was observed—some lines yielded significantly more grain under stress than in the control, while others produced only 20% as much (Table 1). The yield increase observed with drought in some lines may have been associated, at least in part, with reduced disease incidence or lodging under stress. Diseases observed included leaf blight and rice tungro virus, but field notes did not indicate any consistent relationship between poor control yields and these factors.

Despite the terminal stress imposed, late-maturing lines were not significantly more affected by stress than early lines, and yield under stress was not correlated with heading date. This may have been due to inclusion of some temperate cultivars that flowered very early and had low yields due to their poor adaptation. The stress treatment delayed heading by only 1 day, on an average, but across cultivars the

response of heading date to drought ranged from 13 days acceleration to 14 days delay. Heading was accelerated by 4 or more days in 11 of the entries. On an average, the lowland stress reduced height by only 4 cm (3%), ranging from a 43 cm reduction to 22 cm increase in height. Yield responses to stress were weakly correlated ($r = 0.32$, $P < 0.01$) with height responses. In most cultivars, straw mass was only mildly affected by stress, but for cultivars producing more than about $6 \text{ Mg straw ha}^{-2}$, there was a separation into two groups (data not shown). One group was unaffected by stress, while in the other group straw production was severely reduced by stress. There was no apparent relationship between maturity and this response. The highly sensitive group included the flood-tolerant rice FR13A, along with other tall *indica* cultivars.

3.1.2. Upland evaluation

The upland stress treatment was severe and reduced grain yield to 48% of the upland control. Significant differences were observed among cultivars for grain yield across water levels, panicle harvest index within and across water levels, plant height, tiller number, and flowering date. There was a highly significant effect of water level on all these traits except tiller number, but the interaction between water level in the upland experiment and cultivar was not significant, and results are presented as average yield across the two water levels in the upland experiment. As in the lowland experiment, lines that produced no grain were excluded from the analysis. Yield across water levels in the upland experiment averaged 20% of the lowland continuously flooded experiment. Yield was closely correlated with spikelet fertility (estimated from panicle harvest index (PHI); $r = 0.70$, $P < 0.01$).

The top 11 lines in the upland experiment included 9 *indica* and 2 *japonica* cultivars (Table 2). The set of superior cultivars varied widely in plant type, ranging from low-tillering, semi-dwarf types to tall, and leafy types. There was no apparent association between plant type and PHI or yield. PHI was, however, negatively correlated with straw

Table 1

Summary statistics of the performance of 179 parental cultivars under continuously flooded lowland conditions (L irr), lowland conditions with stress imposed beginning near heading (L stress), under upland (aerobic soil) conditions with frequent irrigation (U irr), or under upland conditions with restricted irrigation (U stress)

Water level	Grain yield (g/m^2)		Plant height (cm)		Flowering date (days)	
	Mean \pm S.D.	Range	Mean \pm S.D.	Range	Mean \pm S.D.	Range
Lowland irr	250 \pm 107	37–538	103 \pm 22	40–157	82 \pm 12	48–106
Lowland stress	192 \pm 90	23–449	99 \pm 20	45–145	83 \pm 13	50–106
Upland irr	50 \pm 30 ^a	0–141 ^a	75 \pm 15	38–135	86 \pm 12	52–116
Upland stress	50 \pm 30 ^a	0–141 ^a	66 \pm 14	45–123	88 \pm 12	54–112
Percent change L	–16 \pm 42	–94–152	–3 \pm 10	–28–37	1 \pm 15	–18–18
Percent change U ^b	–83 \pm 21	–100–58	–11 \pm 10	–30–30	4 \pm 7	–15–27

No significant cultivar-by-water interaction was observed for yield in the upland experiment, grain yield is presented as the average measured across the two irrigation regimes.

^a Grain yield reported for the upland experiments is the average of both irrigation levels.

^b For yield, this is percent change relative to the lowland irrigated lowland. For other traits, this is the percent change from the upland irrigated treatment to the upland stress treatment.

Table 2

Cultivars selected for superior yield in contrasting water environments at Los Baños, Philippines in the 2000 dry season

Lowland control		Lowland stress		Upland	
Name	Origin	Name	Origin	Name	Origin
Ajaya	India	ASD16 ^a	India	ASD18	India
Babaomi	China	Bg 300 ^a	Sri Lanka	CS94	Vietnam
Bg 94-1	Sri Lanka	Bg 304 ^a	Sri Lanka	Daintun 502 ^a	China
Chorofa	Philippines	CS94	Vietnam	Doddi	India
CS94	Vietnam	Doddi	India	Gayabyeo	Korea
IR6	Pakistan	HR 98 ^a	India	IRAT216	Ivory Coast
IR64	Philippines	IR66897B ^a	Philippines	Jhona349	India
IR68835	Philippines	OM1723 ^a	Vietnam	Khumal 4	Nepal
IR69513	Philippines	Tarom molaii ^a	Iran	Lemont	USA
Khao Daeng	Thailand	TGMS29 ^a	India	OM997 ^a	Vietnam
MR 159	Malaysia	TKM9 ^a	India	Q5	Vietnam
OM1706	Vietnam	Waixuan 35	China	Shwe Thwe Yin Hyv	Myanmar
OM1723	Vietnam	Y134 ^a	China	TKM9	India
PSBRc28	Philippines	Yunhui 290 ^a	China		
TKM9	India	Zale ^a	Myanmar		
Waixuan 35	China				

^a Indicates that selection was on the basis of standardized residuals >1.6 from the regression of stress yield on yield in the lowland control. Other selections were on the basis of yield per se being significantly above average ($P < 0.05$) for the experiment.

production in the control ($r = -0.49$; $P < 0.01$), and also with flowering delay in stress plots relative to the control ($r = 0.29$; $P < 0.01$). Of the cultivars tested, 34 produced no seed under stress. Yields in the upland experiment were only weakly correlated with yields under fully irrigated lowland conditions ($r = 0.26$; $P < 0.01$). Therefore, upland yield per se was taken as the indicator of adaptation to upland stress. Only two additional lines were identified as superior when the selection was based on standardized residuals from the regression of upland yield on lowland control yield (Table 2).

Drought stress in the upland delayed heading by an average of 3 days. Individual lines responded differently, ranging from 15 days earlier heading to a 20 days delay in heading. An acceleration of heading date of more than 4 days was observed in 13 cultivars, but there was no consistent relationship observed between accelerated head-

ing and yield. In contrast to the lowland stress treatment, late-maturing cultivars suffered greater yield reduction than earlier ones, even though the stress was fairly continuous and encompassed the sensitive flowering and early grain filling periods for all cultivars. On an average, the stress reduced height by 8.9 cm (12%) and only six *japonica* lines showed increased height under the stress. These were Azucena (Philippines), Caozhao 2 (China), Pahenle (Nepal), Type 3 and Ap423 (India), and Razza 77 (Italy). Stomatal conductance differed significantly among the parental lines on three of the four dates that measurements were made (data not shown). There was, however, no significant correlation between stomatal conductance and other traits, such as RWC or leaf rolling, or with yield. RWC did not differ significantly among cultivars. PFW differed among cultivars, but this trait was not correlated with other measurements of traits or yield performance.

Table 3

Summary of selection for drought tolerance undertaken in bulks of BC₂F₂ progeny in 2001 and 2002 dry seasons at Los Baños, Philippines

Year	Selection environment	Recurrent parent	Number of populations		Number of plants selected per population		SI ^a (%)
			N ^a	n ^a	Range	Total	
2001	Lowland	NPT	62	32	0–9	62	0.50
2001	Lowland	IR64	64	64	2–23	583	4.55
2001	Lowland	Teqing	31	31	3–15	279	4.50
2001	Upland	IR64	25	25	5–29	326	7.41
2001	Upland	Teqing	22	22	2–22	182	4.70
2002	Lowland	NPT	51	42	0–66	835	8.18
2002	Lowland	IR64	60	59	0–110	2192	18.26
2002	Lowland	Teqing	47	28	0–30	210	2.24
Total			362	303		4669	6.8 (average)

SI is the selection intensity.

^a N is the total number of BC₂F₂ populations screened and n is the number of BC₂F₂ populations with surviving plants.

3.1.3. Performance of recurrent parents

Two of the recurrent parents, NPT and IR64, were sensitive to water stress and suffered yield losses of 56 and 29% under the lowland stress, and of 100 and 86% in the upland experiment. These two cultivars were about 10% shorter in the lowland stress treatment than in the control, and heading was delayed by 2–5 days. Teqing appeared to have a moderate level of tolerance, with a yield increase of 29% and a height increase of 3 cm under the lowland stress. It suffered 55% yield loss, 34 cm height reduction and 14 days heading delay under the upland stress compared to the lowland control.

3.2. Screening segregating BC populations for drought tolerance

Progeny of 157 different crosses underwent selection in 2001. In the lowland screen, flowering was delayed by stress, and the average anthesis dates observed were 103 days for IR64 and 112 days for Teqing, compared to 88 and 91 days for the recurrent parents grown in control plots. The number of plants selected in each bulk ranged from 0 to 23, resulting in a total of 924 selected BC₂F₂ plants from 137 populations (Table 3). There was a weak but significant negative correlation between anthesis date in the bulk and the number of plants selected ($r = -0.45$ in IR64, $r = -0.34$ in Teqing; $P < 0.05$). The number of plants selected in each BC population depended on the genetic background; all donors produced at least two surviving plants in the IR64 and Teqing backgrounds, whereas only 32 donors (52%) produced survivors in the NPT background (Table 4). On average, the selection intensity was 4.6% (range 1.0–11.5%) for the IR64 populations, 4.5% (range 1.5–7.5%) for the Teqing populations, and 0.5% (range 0–4.5%) for the NPT populations (Table 3). There was no significant correlation observed between the performance of the donor in the lowland stress evaluation and the number of plants selected for any of the recurrent parents (Table 4). For Teqing bulks, the number of superior plants was positively correlated with the tiller number of the donor in the control; for IR64 bulks, the number selected was negatively correlated with tiller number under stress.

In the lowland screening of 2002, 158 BC₂F₂ populations were screened, resulting in 3218 selected plants, for an average selection intensity of about 8% (Table 3). Many more plants were selected in NPT and IR64 backgrounds than had been selected in 2001, because stress was imposed later and was less severe, allowing more plants to survive. The IR64 bulks flowered, on an average, by 84 days, compared to 88 days for NPT and 93 days for Teqing. Within each recurrent parent, the number of plants selected was again negatively correlated with the flowering date of the bulk ($r = -0.46$ for IR64 and NPT, $r = -0.75$ for Teqing; $P < 0.05$). Promising donors producing more surviving plants included Pokhrel and Kholi marshi (Nepal), Doddi (India), and IRBB60 (Philippines) in the IR64 background, and Chipda (India) in the NPT background (Table 5). There was no association

Table 4

Number of BC₂F₂ plants selected under lowland water stress during the 2001 dry season in backcross populations derived from different recurrent parents, and the effect of drought stress on yield of the donor cultivars in the lowland evaluation conducted in 2000 (see text for details)

Donor name	Drought effect ^a	Recurrent parent		
		IR64	Teqing	NPT
Amol 3 (Sona)	−0.22		11	1
Babaomi	−1.58	7		0
Basmati	0.22	15	11	1
Basmati 370	0.77	9		1
Bg 300 ^L	1.88	14	9	0
Bg 304 ^L	1.84	4	4	
Bhavani	1.24		15	1
Binam	0.56	14	12	1
BR11	−0.42	7	6	
BR24	−0.09	11	9	4
C418	−0.85	9		1
C70	1.04	5		2
Cheng-Hui 448	−0.37	7	9	0
Cisanggarung	0.11	9	11	
Cisedane	−0.70	8		1
CS94 ^{L,U}	1.51	6		3
Feng-Ai-Zan	13	10		0
FR13A	−	12	12	0
Gang 16	0.39		7	0
Gayabyeo ^U	0.15	15		1
Haoannong	−0.51	10		7
Hei-He-Ai-Hui	−	7		0
IR50	0.91	4		1
IR6	−0.28		5	1
IR72	0.27	20		3
Jalmagna	−	6	10	
Jhona 349 ^U	0.29	18		2
Jiangxi-Si-Miao	−	7		1
JP-5	−0.40	4		0
Karnal Local	0.83	9		0
Khazar	−0.46	19		2
Lao-Hu-Dao	−0.80	14		0
Lemont	−	10	7	0
Madhukar	−1.24	9		1
MR 106	−0.81	3		0
MR 159	−0.58	2	15	0
MR 167	0.05	4	5	1
NAN29-2	−	15		0
OM1706	−	3	8	1
OM1723 ^L	1.68	5	7	1
OM997 ^U	0.26	6		2
Shen-Nong 89366	−	12		2
Shwe Thwe Yin Hyv ^U	0.25	17	10	0
Tarom molaii ^L	2.46	6	6	0
TB154E-TB-2	−1.42		3	1
TKM 9 ^L	1.62	12		1
Type 3	1.37	19	10	0
X22	−2.12	3		2
Y134 ^L	1.66	6		1
Yunhui 290 ^L	1.85		12	0
Zihui 100	−	6		9
Mean	0.56	9.3	8.9	1.2

Empty cells indicate that no bulk was evaluated for that cross. (−) indicates missing data. Superscripts (^{L,U}) indicate donor that was identified as superior under lowland stress (L) or upland (U) conditions in the 2000 evaluation of donors.

^a Drought effect is the standardized residual from the regression of stress yield on control yield.

Table 5

Number of BC₂F₂ plants selected under lowland water stress during the 2002 dry season in backcross populations derived from different recurrent parents, and the effect of drought stress on yield of the donor cultivars in the lowland evaluation conducted in 2000 (see text for details)

Donor name	Drought effect ^a	Recurrent parent		
		IR64	Teqing	NPT
ASD 16 ^L	1.87		10	0
ASD18 ^U	0.72	59	0	54
B4122	−0.30	37	2	1
Budda	0.82	75	11	
Chipda	−1.04	47	0	85
Chorofa	−2.20		1	20
Dacca 6	−0.22	20	19	
Dhan4	−1.74		1	0
Doddi ^L	−	81	2	
Gajale	−0.14	61	22	
Giza 14	−0.03	29	4	
Guang122	0.17	52	0	
Hei Mi Chan	0.45	52	2	
IRBB60	−0.10	110	0	66
Jalmagna	−		0	28
Jumli Marshi	−	72		40
Khole	−0.87			
Marshi		84	30	14
Khumal 4 ^U	1.04	0	0	0
LA 110	1.07	25	5	47
M202	−	46		45
Minghui63	−0.93	23	0	
Moroberekan	−	13		11
MR 77	−0.66	31	0	
Palung 2	−	33		36
Pokhrel	0.51	119	7	30
Pusa (Basmati 1)	−1.39	15	4	
Rasi	−	63		9
Rusty Late	−0.80	38	2	4
Sadajira 19	−	55	0	
Shwewartun	−	5	0	6
SLG-1	−	26		0
SML242	−	6	0	5
Tek Si Chut	−0.27	13	0	
TGMS29	2.07	22		3
TKM 6	0.50		11	5
UP 15	−		22	24
UPR191-66	−1.77	68	0	24
UZ-Ros 275	−0.32		6	37
Vary Lava 16	−	24	0	5
Zale ^L	1.67	2	0	
Ziri	−0.55	10	5	0
Mean	−0.09	42	5	21

Empty cells indicate that no bulk was evaluated for that cross. (−) indicates missing data. Population size was 250 plants. Superscripts (^{L,U}) indicate donor that was identified as superior under lowland stress (L) or upland (U) conditions in the 2000 evaluation of donors.

^a Drought effect is the standardized residual from the regression of stress yield on control yield.

between the number of plants selected and the performance of the donors under lowland stress in the 2000 evaluation.

In the upland screening of 2001, the average anthesis date (across water levels) of IR64 bulks was 75 days, compared to 87 days for Teqing bulks. There was no apparent association between the number of superior plants identified and the

Table 6

Numbers of drought-tolerant plants selected in the IR64 and Teqing BC₂F₂ populations in the upland nursery in the 2001 dry-season

Donor name	Yield in upland (Mg ha ^{−1})	Recurrent parent	
		IR64	Teqing
Ai-Zi-Dao	0.12		4
Babaomi	0.67	10	10
Basmati	0.35	7	10
Bg 300 ^L	0.32	12	5
Bg 304 ^L	0.26	12	
Bhavani	0.11	16	10
Binam	0.42	19	13
BR24	0.39	27	7
Cheng-Hui 448	0.51	12	3
Cisanggarung	0.52	7	
CR203	0.80		10
CS94 ^L	0.92	8	
Diantun 502	0.84	5	
Feng-Ai-Zan	0.27	19	
FR13A	0.03	16	15
Gang 16	0.34		22
Haoannong	0.10	13	
IR6	0.31	6	4
Lemont	1.05	9	
MR 159	0.24		8
MR 167	0	9	
OM1706	0.16	6	4
OM1723 ^L	0.72	17	6
Shwe Thwe Yin Hyv	1.17	29	
Tarom molai ^L	0.66	19	3
TB154E-TB-2	0.78		5
Type3	0.16	15	12
X21	0.25	7	
Yunhui 290 ^L	0.45		10
Yu-Qiu-Gu	0.30		14
Zhong-You-Zao 81	0.62	10	2
Mean	0.45	13.0	8.4

Superscript (^L) indicates that donor that was identified as superior in lowland stress conditions in the 2000 evaluation of donors.

average anthesis date for the bulk in IR64, but in Teqing there was a negative correlation between these values ($r = -0.51$; $P < 0.05$). The number of lines selected per bulk ranged from 0 to 27 (Table 6). There was no correlation between parental performance in the upland evaluation of 2000 and the number of plants selected. In contrast to the lowland evaluation, there were a number of cases where superior plants were identified in the IR64 cross, but not in the Teqing cross, perhaps because of difficulty in identifying lines superior to Teqing itself, which performed fairly well in this screening environment.

The number of lines selected from any given donor was not correlated across recipient parents within a screening environment, except in the case of IR64 and NPT in the 2002 screen (Table 5). Twenty donors were represented in both upland and lowland screens in 2001. Among those 20, significant correlation was observed between the number of plants selected under lowland stress in IR64 background and the number of lines selected in the upland screen in Teqing background. Ten donors were represented as both IR64 and

Table 7

Comparison of the number of selected drought-tolerant BC progeny from 20 BC₂F₂ populations sharing the same 10 donors between the lowland (LL) and upland (UL) stresses

Donor	Recurrent parent					
	IR64			Teqing		
	LL	UL	Total	LL	UL	Total
Basmati	16	7	23 ^a	12	10	22
Bg 300	16	12	28	10	5	15 ^a
Binam	20	19	39	14	13	27
BR24	14	27	41 ^a	12	7	19 ^a
Cheng-Hui 448	8	12	20	9	3	12 ^a
FR13A	15	16	31	17	15	32
OM1706	4	6	10	9	4	13 ^a
OM1723	7	17	24 ^a	7	6	13
Tarom molaii	8	19	27 ^a	8	3	11 ^a
Type3	23	15	38 ^a	7	12	19 ^a
Total	131	150	281	105	78	183

^a Indicates significant difference between the number of plants selected in lowland and upland stress for that recurrent parent at $P < 0.05$.

Teqing crosses in both water levels (Table 7). In this set, 7 BC populations produced more drought-tolerant plants under the lowland condition than the upland situation, and the opposite was true for four other populations. The number of plants selected from Teqing bulks in the upland was significantly correlated with both the number selected in Teqing bulks in the lowland and also plants selected in the lowland in IR64 background.

3.3. Assessment of maturity and plant height in selected lines

Average flowering date of the selected lines was 1.5 days earlier than the recurrent parent for IR64 selections and 2.2 days earlier for Teqing (Table 8). Average plant height

Table 8

Plant height and days to heading of IR64 and Teqing introgression lines (ILs) selected under drought in 2001, when evaluated under fully irrigated conditions during the dry season of 2002

	Heading date (days)		Plant height (cm)	
	Recurrent parent	Selected lines	Recurrent parent	Selected lines
IR64				
<i>N</i>		386		386.0
Mean	85.6	84.1	66.2	74.3
S.D.	2.9	4.0	4.7	9.2
Minimum	80.0	70.0	56.0	54.2
Maximum	92.4	100.0	76.8	119.0
Teqing				
<i>N</i>		172		172
Mean	92.7	90.5	67.2	72.5
S.D.	2.9	4.2	6.6	8.9
Minimum	87.2	72.6	52.4	53.1
Maximum	97.8	101.2	84.2	105.3

N is the number of lines tested and S.D. is the standard deviation. Lines were evaluated in three replications, and the parents were repeated in 10 (Teqing) or 20 (IR64) copies in the experiment.

increased with selection in the two genetic backgrounds by 8 and 5 cm. The range of values observed for both traits, however, indicates that selected lines could be either earlier or later, and either taller or shorter, than parental cultivars.

4. Discussion

It has long been recognized that some rice cultivars have more stable grain yields under drought than others (Chang et al., 1982; Mackill et al., 1996). Consistent with this observation, the parental lines evaluated in this study showed tremendous yield variation in response to water level. The absolute yields in our stress scenarios resulted from both the general adaptability of the tested lines to the local dry season environment as well as a wide range of stress response strategies. For example, two clearly contrasting responses of heading date to drought were observed. In some cultivars, heading was significantly accelerated by drought. This might allow those cultivars to complete the sensitive processes of pollination and embryo development (Ekanayake et al., 1990) before stress becomes too severe. The opposite strategy was observed in other cultivars, which had a significant delay in maturity with drought. Heading delay is a common drought response observed in rice (Lilley and Fukai, 1994), which is expected to confer a benefit in those environments where stress is temporary, if development and flowering resume after the stress is relieved. Either of these responses could confer an advantage, depending on the stress environment. This observation is consistent with reports of significant interactions between cultivar yield and characteristics of the drought environment (Wade et al., 1999). In other studies, the biological basis of success in specific drought scenarios was the matching of crop water use to the timing of water supply, and the optimal plant type differed across stress environments (Pantuwan et al., 2002). Drought is clearly not a single, homogeneous stress, and the type of drought, in terms of timing and severity, must be explicitly defined in a breeding program. The drought levels and timing used in a stress nursery would be expected to identify lines with specific advantageous traits that might not be valuable in other drought scenarios.

The backcrossing breeding activities described here allowed the identification of 4669 introgression lines (ILs) from 362 BC populations (Table 2). We found a high frequency of BC progeny showing apparent transgressive performance for yield in the severe stress of the selection nurseries over the parental lines in most populations, regardless of the performance of the donors (Tables 3–6). This indicates substantial genetic drought tolerance in the donors. Moreover, this diversity appeared to be ‘hidden’ in the donors, because most donors that produced successful progeny could not be considered drought-tolerant based either on their yielding ability in small plots under managed

drought stress in the dry season or on the characteristics of the environments where they are cultivated.

Our results indicate that backcross breeding combined with direct selection for yield in stressed nurseries may be a highly effective way to improve drought tolerance in rice. The timing of stress relative to the sensitive flowering stage has a tremendous effect on the yield reduction caused by a drought event (Cruz and O'Toole, 1984; Garrity and O'Toole, 1994; O'Toole, 1982). Heading time in rice is controlled by a complex genetic system that interacts with environmental factors, such as photoperiod, temperature, and soil fertility (Li et al., 2003). This makes it difficult to create a uniform stress condition for breeding populations that are segregating for heading time, and explains in part why grain yield is frequently not associated with traits that are expected to influence drought response, as was the case for leaf relative water content and stomatal conductance in our evaluation of parental cultivars. Compared to normal segregating breeding populations of F_2 plants, BC populations have much more uniform genetic backgrounds and are less affected by the genetic 'noise' from co-segregating non-target traits, such as heading time and plant size. Thus, it was possible to apply a uniform severe stress at the critical developmental stage(s) and to select superior plants based directly on seed set compared to the recurrent parents sown in adjacent rows. In the preliminary assessment of BC_2F_4 lines selected under lowland stress, however, we did note that some early lines were selected. This was expected because a terminal stress treatment was imposed and some variation in maturity remained in the BC_2 populations. In the upland selections, the drought treatment was not expected to favor earliness, but an association between earliness and greater drought tolerance was observed in the upland evaluation of the donors, and this has been reported in other studies as well (Lafitte and Courtois, 2002). In upland fields, earliness cannot be distinguished from reduced flowering delay, which can itself serve as an indicator of drought tolerance. It is, therefore, inevitable that both a lowland screen with terminal stress and an upland screen with continuous stress will result in the identification of some lines that are early but not particularly drought tolerant. This also provides an explanation why comparatively fewer drought-tolerant Teqing BC progeny were selected in the lowland stress of the 2002 dry season (Table 3)—later heading of Teqing meant that these lines were under greater stress at flowering than IR64 or NPT crosses. Nevertheless, our results indicate that a severe stress that reduces the yield of most plants by 75–100% clearly distinguishes individuals that are superior to the adjacent recurrent parent despite background environmental variation. One potential drawback to this approach is that the level of stress needed to expose genetic variation in unreplicated, single plant screens may be unrealistically severe. In addition, the link between performance under managed drought in a dry season screen and yield under natural drought in the regular growing season has not been clearly established. Further evaluations

of the selected lines are being conducted to establish the gains resulting from selection.

Another shift that occurred in some selected plants was toward greater height, as the donors included both tall and semi-dwarf types. The relationship between plant height and drought tolerance has been debated for some time, and is particularly relevant in the present study because of the presence of the semi-dwarf gene, *sd1*, in all three recurrent parents. The *sd1* gene, which affects gibberelic acid synthesis (Ashikari et al., 2002), was reported to have pleiotropic effects on yield-related traits, such as sink size (Murai et al., 2002). In addition, the semi-dwarf habit is often associated with characteristic high tillering and shallow rooting, due either to pleiotropic effects or genes closely linked to *sd1*. Nonetheless, earlier studies indicated that, while most deep-rooted varieties were tall, some short varieties with deep roots could be identified (Yoshida et al., 1982). In some studies of root depth in crosses where one parent carried *sd1*, QTLs for plant height and root depth co-localized (Yadav et al., 1997), but in others these two traits did not appear to be linked (Price et al., 2002b). As in the case of maturity, the screening environments cannot distinguish between plants that are tall because they are accessing water at depth, and thus their expansive growth is less affected by water deficit, or plants that are inherently tall. Possible pleiotropic effects of *sd1* on root depth and other yield-related traits and the genetic basis underlying the association between height and root depth are important questions to be resolved.

The lowland stress and upland stress screening protocols applied in this study are expected to have very different effects on the segregating BC populations and the traits that provide an advantage. In the lowland terminal stress, continued development toward flowering, successful pollen formation and fertilization, continued photosynthesis, and effective mobilization of stored reserves are essential. In aerobic systems with chronic stress, emphasis is on continued expansive growth for leaf area development and root growth into moist soil, limited degeneration of potential spikelets, and continued photosynthesis. The three large sets of drought-selected introgression lines generated in these studies have two unique characteristics. First, all sister lines within a single set of ILs are in the same elite genetic background but each has a few introgressed genomic segments from a known donor. These will be valuable for genetic studies to identify the effect of specific introgressions on performance under drought and other stresses. Second, the three sets of ILs together contain a wide range of drought-tolerant types and genes from 122 donors of diverse origin. Further improvement of drought tolerance by trait/gene pyramiding through crossing promising sister ILs is in progress. These ILs provide a unique set of genome-wide genetic stocks for large-scale gene/allele discovery and functional genomic research of drought tolerance in rice.

Acknowledgements

We thank the Rockefeller Foundation for postdoctoral fellowships awarded to C.H.M. Vijayakumar, Y.M. Gao, Y. Shi, J.L. Xu, and B.Y. Fu. We acknowledge the generous financial support provided by the Rockefeller Foundation, the German Bundesministerium für wirtschaftliche Zusammenarbeit und Entwicklung (BMZ), and the 948 Program of the Ministry of Agriculture of the People's Republic of China.

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