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Hidden diversity for abiotic and biotic stress tolerances in the primary gene pool of rice revealed by a large backcross breeding program

A.J. Ali [a,1], J.L. Xu [a,b,1], A.M. Ismail [a], B.Y. Fu [a,b], C.H.M Vijaykumar [a], Y.M. Gao [a,b], J. Domingo [a], R. Maghirang [a], S.B. Yu [a,2], G. Gregorio [a], S. Yanaghihara [a], M. Cohen [a], B. Carmen [a], D. Mackill [a], Z.K. Li [a,b,*]

[a] *International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines*
[b] *Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, 12 South Zhong-Guan-Cun Street, Beijing 100081, China*

Abstract

Low and unstable rice productivity in many areas of Asia is associated with many abiotic and biotic stresses such as drought, salinity, anaerobic conditions during germination, submergence, phosphorus and zinc deficiency, etc. To develop rice varieties with tolerance to these stresses, we undertook a large backcross (BC) breeding effort for the last 6 years, using three recurrent elite rice lines and 203 diverse donors, which represent a significant portion of the genetic diversity in the primary gene pool of rice. Significant progress has been made in the BC breeding program, which resulted in development of large numbers of introgression lines with improved tolerance to these stresses. Promising lines have been developed with excellent tolerances (extreme phenotypes) to salinity, submergence and zinc deficiency; resistance to brown plant hopper, ability to germinate under the anaerobic condition and low temperature. Our results indicated that there exist tremendous amounts of 'hidden' diversity for abiotic and biotic stress tolerances in the primary gene pool of rice. Furthermore, we demonstrated that despite the complex genetics and diverse physiological mechanisms underlying the abiotic stress tolerances, introgression of genes from a diverse source of donors into elite genetic backgrounds through BC breeding and efficient selection (careful screening under severe stress) is a powerful way to exploit this hidden diversity for improving abiotic stress tolerances of rice. We have developed three large sets of introgression lines, which not only provide an unique platform of breeding materials for developing new rice cultivars with superior yield and stability by trait/gene pyramiding, but also represent unique genetic stocks for a large-scale discovery of genes/alleles underlying the abiotic and biotic stress tolerances of rice using genomic tools.
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1. Introduction

Rice is the staple food for more than 3 billion people in Asia, where more than 90% of the world's rice is produced and consumed. Rice production in Asia has been more than tripled in the past three decades, resulting primarily from the "Green Revolution" in the irrigated ecosystems (Khush, 2001). However, rainfed lowland rice occupies approximately 28% of the world rice area but contributes only about 16% of total rice production (Garrity et al., 1996). The yield level of rainfed lowland rice is, on average, around 2.3 t ha^{-1}, much lower than that of the irrigated systems of about 4.9 t ha^{-1}, which is due largely to many abiotic stresses such as drought, submergence, salinity, etc. For example, nearly 22 million ha of rice are affected by flash flooding and submergence in Bangladesh, Northeast India, Thailand and South Vietnam

Abbreviations: AG, anaerobic germination; BPHR, brown planthopper resistance; LTG, low temperature germination; ST, salinity tolerance; SUBT, submergence tolerance; ZDT, zinc deficiency tolerance

* Corresponding author. Tel.: +86 10 62136040.
E-mail addresses: lizhk@caas.net.cn, z.li@cgiar.org (Z.K. Li).

[1] Contributed equally to the work.
[2] Present address: National Key Laboratory for Crop Improvement, Huazhong Agricultural University, Wuhan, China.

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(Khush, 1984) and the yield loss can reach nearly 70% from submergence alone, resulting primarily from poor establishment of the crop (Widawsky and O'Toole, 1996). In both South and Southeast Asia, about 90 million ha of land (particularly along the coastal areas of Asia) suited to rice production remain uncultivated due to various soil problems such as salinity, alkalinity, strong acidity or excess organic matter, and of this area, nearly 49 million ha are saline (Poonamperuma and Bandyopadhya, 1980). Rice is very sensitive to salinity at the seedling stage. A low level of salinity with electrical conductivity (EC) of 5–6 dsm^{-1} can cause significant reduction in height, root growth and dry matter accumulation of susceptible rice lines (Pearson et al., 1966; Akbar and Yabuno, 1974). Saline soils are also commonly associated with many other problems such as mineral deficiencies (Zn, P) and toxicities (Fe, Al and organic acids), submergence, and drought (Gregorio et al., 2002).

Rice genotypes are known to vary widely in their responses to these abiotic stresses, and breeding stress tolerant rice cultivars is the most efficient and economical way to overcome the problems, because management strategies to mitigate these stresses are generally beyond reach of the poor farmers in these areas. Unfortunately, breeding efforts to develop high-yielding rice cultivars adapted to the rainfed ecosystems have been less successful than for the irrigated systems. A recent survey indicated that approximately 60% of rice cultivars grown in the rainfed areas of Asia remain traditional landraces, and most modern high-yield semi-dwarf varieties are not adapted to the unfavorable rainfed environments (Pandey, personal communication). There are at least three obvious reasons for slow progress in genetic improvement of rice abiotic stress tolerance. First, past efforts in germplasm screening identified few accessions with high levels of abiotic stress tolerance for use in breeding programs. For instance, two landraces from India, FR13A and Pokkali, are among the few accessions that are highly tolerant to submergence and salinity, respectively. As a result, they have been extensively used as donors for submergence and salt tolerance in breeding programs at IRRI and other Asian countries. However, the poor agronomic performance and narrow adaptability of the two landraces have been formidable obstacles for breeders seeking to combine high-yield potential with the good abiotic stress tolerance of these landraces. Similarly, an extensive screening of over 7500 rice germplasm accessions identified only 29 accessions highly resistant to brown plant hopper (BPH) (Pophaly et al., 2001). The rapid evolution of more virulent biotypes of BPH has been a constant challenge to breeders (Ikeda and Kaneda, 1981; Panda and Khush, 1995). Second, rice tolerance to different abiotic stresses is often complex in nature and its genetic basis, as for most quantitative traits, remain poorly understood. Third, in most areas of the rainfed environments, multiple abiotic stresses coexist. For instance, drought and submergence can occur at different times during the same season in certain rainfed environments, and salinity is often accompanied with other soil problems. Thus, desirable rice varieties for rainfed environments must have tolerances to multiple stresses in addition to good yield potential and acceptable grain quality.

Domestication, artificial selection and intensive breeding of crop varieties by human have resulted in the narrowed genetic base in many crops, which renders modern crop varieties more vulnerable to biotic and abiotic stresses (Tanksley and McCouch, 1997). Base broadening (Simmonds, 1993) or gene pool enrichment has been proposed as an alternative to transfer useful genes from unadapted germplasm into elite backgrounds for developing new cultivars; this has been implemented in maize (Kannenberg and Falk, 1995; Kannenberg, 2001) and barley (Falk, 2001). However, it still remains largely unknown how much useful genetic diversity exists for complex phenotypes in the world's crop germplasm collections. Traditional landraces account for about 70% of the total rice collections in the gene banks worldwide and many of them are known to have different tolerances of abiotic stresses, but plant breeders have been reluctant to utilize these landraces, largely for three reasons (Duvick, 1984, 2002). First, slow but consistent progress can be achieved even within the narrow genetic base of many breeding populations. Secondly, outstanding commercial genotypes tend to be destroyed in crosses involving unadapted exotic parents. Thirdly, selection of germplasm as parents of the breeding programs by breeders has been largely based on the phenotype.

At IRRI, we initiated a large-scale backcross (BC) breeding effort in 1998, as part of the International Rice Molecular Breeding Program (IRMBP), to answer two important questions: (1) whether there is sufficient genetic variation for tolerances of various abiotic and biotic stresses in the primary gene pool of rice; and, if yes, (2) what is the most efficient way to exploit this useful genetic variation. We report here that there is tremendous 'hidden' diversity for abiotic stress tolerances in the primary gene pool of rice and demonstrate that BC breeding and efficient phenotyping are powerful means to exploit this hidden diversity for developing promising lines with significantly improved tolerances to many abiotic stresses.

2. Materials and methods

2.1. Parental lines

Two high-yielding varieties, IR64 (*indica*) and Teqing (*indica*), and a new plant type (NPT or IR68552-55-3-2, tropical *japonica*) breeding line, were used as recurrent parents (RPs). A total of 203 accessions from different parts of the world were used as donors in the BC breeding program (Table 1). Based on an assay with 101 simple sequence repeat (SSR) markers (Yu et al., 2003), 139 of the parents belonged to *indica*, 63 belonged to *japonica*, 2 are of intermediate types derived from *indica*/*japonica* crosses, and a deep-water rice, 'Jalmagna' from India, forms a single

Table 1
Geographical origins of the 203 parental lines in the backcross breeding program

Nation	EGP*	DGP*	Total	Nation	EGP	DGP	Total
Bangladesh	2	1	3	Malaysia	2	6	8
China	18	24	42	Myanmar	3	5	8
Egypt		1	1	Nepal	1	9	10
France		1	1	Pakistan		3	3
Guinea		2	2	Peru		1	1
Hungary		1	1	Philippines	2	5	7
India	10	28	38	Portugal		1	1
Indonesia	2	4	6	Sri Lanka	3	2	5
Iran	2	4	6	Suriname		1	1
IRRI (Philippines)	6	8	14	Taiwan (China)	2	1	3
Italy		1	1	Thailand		4	4
Japan	1	2	3	USA		4	4
Korea	4	1	5	Vietnam	2	12	14
Madagascar		1	1	Others		10	10

* EGP = elite gene pool (commercial cultivars), DGP = donor gene pool (landraces, breeding lines or commercial cultivars from non-target areas).

solitary group. Of these donors, 64, 77 and 62 accessions were crossed with IR64, Teqing and NPT, respectively, with 32 donors common to all the three RPs.

The procedure of the BC breeding program is shown in Fig. 1. Briefly, the three RPs were crossed with all donors to produce the F_1s. The F_1s were backcrossed with their RPs to produce the BC_1F_1s. A total of randomly selected 25 plants from each BC_1F_1 line were backcrossed with each RP to produce 25 BC_2F_1 lines each having more than 10 independent plants. In this way, the 25 BC_2F_1 lines from each cross are expected to carry more than 62.5 genome-equivalents of the donor genome or with more than 99% of the chance that all donor segments will be present in the BC_2F_1 lines by at least once. From each of the crosses, 25 BC_2F_1 lines were planted in the following season and seeds from individual plants of 25 BC_2F_1 lines from each cross were bulk-harvested to form a single bulk BC_2F_2 population. In addition, 30–50 superior high-yielding BC_2F_1 plants from each cross were further backcrossed with the RPs to produce the BC_3F_1 lines and likewise BC_4F_1 lines. Similarly, BC_3F_2 and BC_4F_2 bulks were generated by harvesting seeds of all BC_3F_1 and BC_4F_1 plants from each cross.

2.2. Screening of bulk BC populations for abiotic stress tolerances

The BC bulks were screened for their tolerance to different abiotic stresses, including salinity, submergence, anaerobic germination, zinc deficiency, etc. Not all bulks

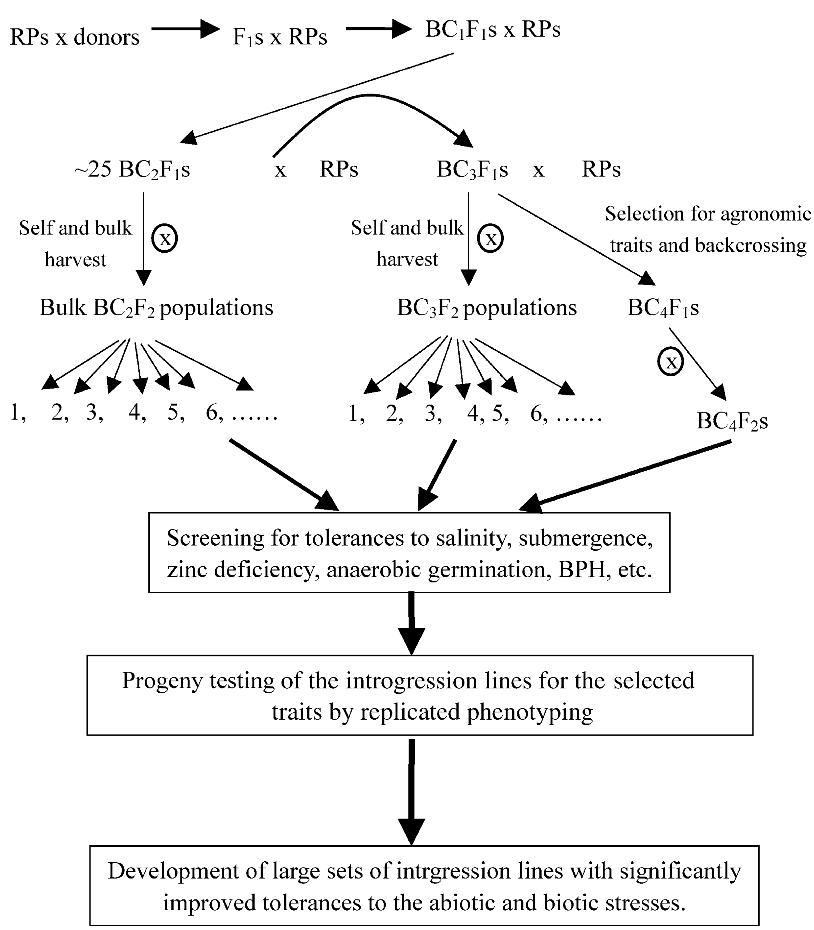

Fig. 1. Procedure of the backcross breeding for development of rice introgression lines for abiotic and biotic stress tolerances. RPs are recurrent parents (IR64, Teqing and NPT).

were evaluated for all stresses because of limitations in space and seed availability.

2.2.1. Seedling salinity tolerance (ST)

Surface-sterilized seeds of individual BC_2F_2 bulks were germinated in Petri dishes at 30 °C for 48 h. Two pre-germinated seeds were placed in holes made on styrofoam sheets with nylon bottoms, floating on distilled water in plastic containers (Gregorio et al., 1997). For each of the BC_2F_2 bulks, 160–250 seedlings were screened along with respective RPs, Pokkali (tolerant check) and IR29 (susceptible check). Three days after the seedlings were established, distilled water was replaced by salinized nutrient solution with EC 6 of dsm^{-1} (Yoshida et al., 1976). Three days later, the salinity level was increased to 12 dsm^{-1} by adding NaCl to the nutrient solution. This level was maintained for up to 2 weeks. Then, the EC level of the nutrient solution was raised to 18 dsm^{-1} for 1 week, and then to 24 dsm^{-1} for another week for all NPT BC_2F_2 populations. Salinity was increased to 30 dsm^{-1} for the IR64 and Teqing BC_2F_2 populations. The solution was replenished every 5–8 days and the pH was adjusted to 5.0 daily. The screening was carried out up to 31 d from the initial salinization. The surviving plants were then transferred to a screen house to produce BC_2F_3 seeds. All selected ST plants were confirmed using progeny testing of their BC_2F_3 lines under the same conditions in the growth chamber. The final confirmation of the selected ST BC_2F_4 materials was conducted in a hot spot at Iloilo province under field conditions with seawater intrusion during high tides (approximately between EC 16–18 dsm^{-1}. Each entry was grown in a single row of 15 hills at a spacing of 20 cm × 20 cm with the RPs, Bicol (tolerant check) and IR29 (susceptible check) inserted in every 20 rows in an augmented field design. The initial and final scores of survival rate (%) were recorded at 30 and 90 d after transplanting using the standard evaluation system of IRRI (SES, IRRI, 1996).

2.2.2. Zinc deficiency tolerance (ZDT)

Screening of BC_2F_2 bulks for ZDT was conducted in a highly zinc deficient soil at Tiaong, Philippines, which has been used for screening by IRRI for more than 25 years. Zinc deficiency in wetland soils such as those used for this study is associated with highly reduced soil conditions caused by continuous flooding. The soil at the testing site is a Typic Hydraquent clay loam with pH 7.8, 3.7% of organic content, and about 0.08 mg $kg^{-1}$of extractable Zn. Three-week-old seedlings raised in the normal nursery were transplanted into 3-m rows at 20 cm × 20 cm spacing. Since the severity of the stress depends on the frequency of soil wetting, a moderate stress level was maintained by briefly draining the soil before the start of the trail. The BC_2F_2 bulks were screened under this moderate level of zinc deficiency stress in the wet season of 2001 by planting 200 plants per BC_2F_2 population. Seeds was harvested from surviving individual BC_2F_2 plants. The resulting BC_2F_3 lines were progeny tested in the dry season of 2001–2002 under more severe zinc deficiency at Tiaong in an augmented field design with 16 plants in a single row per BC_2F_3 line, with a single row of their RPs planted every 10 rows of BC_2F_3 lines as checks. The percentage of surviving plants per row at the tillering stage and spikelet fertility of each line at maturity was visually scored for the BC_2F_3 lines.

2.2.3. Anaerobic germination (AG)

A total of 130 BC_2F_2 populations was screened for AG tolerance under a low oxygen condition by directly seeding 40 dry seeds in plastic containers immediately followed by submerging in 10 cm water for 21 d. The percent survival (seeds that germinated and seedlings emerged out of the submerged condition) was recorded for each BC population and the surviving plants were transferred to the field for seed production.

2.2.4. Submergence tolerance (SUBT)

Screening for SUBT was conducted in the submergence facility of deepwater ponds using the standard method developed at IRRI, Philippines (Mohanty and Khush, 1985; Haque et al., 1989). A total of 179 BC_2F_2 and 51 BC_4F_2 populations was screened for SUBT. One hundred seeds from each BC_2F_2 population were directly sown on the seedbed of the submergence facility. Thirty days after germination, the seedlings were submerged in 1.3-m deep water for a period of 14 d or until the susceptible check showed severe symptoms of damage. Water was then drained and the surviving plants were allowed to grow to maturity. For screening of the BC_4F_2 populations, 300 seeds per population were used. Seeds from the surviving plants were harvested and the progeny was tested under the same conditions in the following season to confirm the tolerance of the selected SUBT lines.

2.2.5. Low temperature germination (LTG)

A total of 39 BC_4F_2 bulks involving *indica*/*japonica* crosses was subjected to LTG screening at 10 °C for 19 d in a controlled growth chamber under dark conditions using the method of Bertin et al. (1996) with minor modifications. The experiment was carried out in two replications by placing 50 sterilized seeds in a Petri plate, which was immediately transferred into the growth chamber after wetting the seeds with distilled water equilibrated at 10 °C. The number of germinated seeds per bulk was recorded on 19 d after seeding.

2.2.6. Brown planthopper (BPH)

A total of 193 BC_2F_2 populations was screened for BPH resistance using the BPH screening facility at IRRI. One hundred pre-germinated seeds per BC_2F_2 bulk were sown in plastic trays (40 cm × 60 cm) half-filled with garden soil along with TN1 (susceptible check) and RPs. Ten days after sowing, each seedling was infested with two 2nd to 3rd instar nymphs using the local BPH population. A plastic insect cage was installed on each tray to contain the insect population. When TN1 and RPs were killed, surviving plants from each BC_2F_2 population were counted and transferred to the field.

2.2.7. Data analyses

Standard Z tests (Larsen and Marx, 1981) were performed to compare the differences in selection frequency and survival rate between different BC populations screened for the same traits, in which H_0: $P_X = P_Y$, H_1: $P_X \neq P_Y$, where P_X and P_Y are the selection frequencies or survival rates of BC populations X and Y. H_0 is rejected whenever $(P_X - P_Y)$/S.D. is either $\leq -Z_{\alpha/2}$ or $\geq Z_{\alpha/2}$, where S.D. = $\{(P_X + P_Y)(1 - P_X + P_Y)(n + m)/nm\}$, and n and m are the population sizes of BC populations X and Y, respectively.

3. Results

3.1. Salinity tolerance (ST)

Screening of 175 BC_2F_2 populations resulted in a total of 1292 surviving BC_2F_2 plants under the salinity stress that killed the RPs (Table 2). These included 490 IR64-derived lines, 428 Teqing-derived lines and 374 NPT-derived lines. The average selection intensity was 3.95, 3.69 and 3.40% for IR64, Teqing and NPT BC populations, respectively. Although ST BC progeny were identified in all BC populations, some donors produced more ST plants in all three RPs. These promising donors included OM1706, OM1723, FR13A, Nan29-2, Babaomi and Khazar. Some donors (BG300, Cisedane, Pahk Maw Peun Meuang and TKM 9) produced more ST plants in the *indica* (IR64 and Teqing) genetic background than the *japonica* background (NPT). Progeny testing of the selected ST BC_2F_3 lines from 68 BC populations indicated that over 90% of the single plant selections from the BC_2F_2 populations had indeed improved ST except for some lines in the NPT background.

Salinity tolerance of the selected BC_2F_4 lines was confirmed in saline soils under natural field conditions in Iloilo, Philippines in 2002. Most selected ST BC progeny survived and set seeds under the field stress level of EC 16–18. Of these, 22 BC_2F_5 lines with a ST score of 2–3 at the seedling stage showed a high level of ST when even the tolerant check, Bicol, all donors and RPs were severely damaged or dead (with SES scores ranging from 7 to 9). The donors of the 22 promising lines included Y134, Yue-Xiang-Zan, Zhong413, and 93072 from China, TKM 9 from India, Shwe-Thwe-Yin-Hyv from Myanmar, BG300 from Sri Lanka, OM997 from Vietnam, M401 from USA, and Pahk Maw Peun Meuang from Korea (Table 10).

3.2. Zinc deficiency tolerance (ZDT)

Under the stress at Tiaong where none of the RPs or donors could survive, 1161 surviving BC_2F_3 plants were identified from 129 BC_2F_2 populations screened (Table 3), though many of the surviving progeny showed relatively high spikelet sterility. BC_2F_4 populations bulk-harvested from the surviving plants from 42 populations were progeny tested under a more severe stress at Tiaong (using soils that had been maintained under flooded conditions throughout the year) in the following season. The Teqing and IR64 BC populations showed survival rates of 2.41 and 2.14%, resulting in identification of 327 highly tolerant lines, whereas none of the NPT BC progeny survived the stress. Promising donors that produced more ZDT plants in the Teqing background included TKM9, Hei-He-Ai-Hui, Jiangxi-Si-Miao, Khazar, Madhukar, Shwe-Thwe-Yin-Hye, Basmati 385, Iksan 438, Yu-Qiu-Gu, Tetep, Nipponbare, Co43, Rasi, and Yunhui, and those in the IR64 background were Bg304, BR24, FR13A and Gayabyeo.

3.3. Anaerobic germination (AG)

Screening of the 130 BC_2F_2 populations resulted in identification of 343 BC_2F_3 lines with significantly

Table 2
Summary results of BC populations for screening salinity tolerance

Details	BC_2F_2 screening				BC_2F_3 progeny testing			
	IR64	Teqing	NPT	Total	IR64	Teqing	NPT	Total
Number of populations screened	62	58	55	175	24	34	10	68
Single plant selections per BC population	4–12	4–13	1–14		0–43	0–49	0–11	
Total selected tolerant BC_2F_3 lines	490	428	374	1292	448	392	21	861
Selection intensity (%)	3.95	3.69	3.40	3.69				
Number of *indica* donors	47	47	42	136	20	27	7	54
Selected lines	369	345	289	1003	372	269	21	662
Selection intensity (%)	4.39	3.67	3.44	3.69				
Number of *japonica* donors	9	9	7	25	3	6	1	10
Selected lines	70	66	44	180	43	123	0	166
Selection intensity (%)	3.89	3.67	3.14	3.60				
Number of intermediate donors	4	1	3	8	1	1	2	4
Selected lines	35	5	19	59	33	0	0	33
Selection intensity (%)	4.38	2.50	3.16	3.69				

Table 3
Summary results of BC populations screened for zinc deficiency tolerance

Details	BC_2F_2 screening under moderate field stress				BC_2F_4 progeny testing under very severe field stress			
	IR64	Teqing	NPT	Total	IR64	Teqing	NPT	Total
Number of populations screened	51	42	36	129	11	21	10	42
Single plant selections per BC population	4–12	2–12	0–12	0–12	0–10	0–15	0	
Total selected BC_2F_3 lines	454	383	324	1161	72	255	0	327
Selection intensity (%)	7.42	7.60	7.50	7.50	2.14	2.41	0.00	2.01
Number of *indica* donors	40	33	25	98	9	17	9	35
Selected lines	370	320	237	927	63	200	0	263
Selection intensity (%)	7.77	8.08	7.90	7.88	2.07	2.31	0.00	1.91
Number of *japonica* donors	7	6	7	20	1	4	1	6
Selected lines	63	63	84	210	0	55	0	55
Selection intensity (%)	7.50	8.75	7.86	8.75	0.00	2.86	0.00	2.29
Number of intermediate donors	3	–	1	4	1	0	0	1
Selected lines	21	–	9	30	9	0	0	9
Selection intensity (%)	5.83	–	7.50	6.25	5.63	0.00	0.00	5.63

improved germination under anaerobic conditions. These included 215 NPT lines, 81 Teqing lines and 47 IR64 lines (Table 4). Generally, RPs differed in the number of AG plants selected from specific donors. Genetic backgrounds and the number of BC generations appeared to have significant effects on the number of high AG plants in the BC populations (Table 4). A comparison between 18 IR64 populations and 30 NPT populations in different BC generations (BC_2F_2, BC_3F_2 and BC_4F_2) from crosses between two RPs (IR64 and NPT) and 10 donors (Y134, TKM 9, Khazar, Gayabyeo, Shwe-Thwe-Yin-Hyv, Nan29-2, Babaomi, Jiangxi-Si-Miao, FR13A and OM1706), indicated that on average, the frequency of high AG plants increased with the advancement of BC generation (Table 5). In particular, the survival rate was much higher in the NPT populations than the IR64 populations. Eleven NPT BC_4F_3 lines with introgressions from TKM9, Y134, Shwe Thwe Yin Hyv, Babaomi and OM1706 showed 100% survival, significantly higher than NPT and the donors (Table 6).

3.4. Submergence tolerance (SUBT)

Of 179 BC populations screened, BC progeny were recovered in most BC populations, giving a total of 1665 (approximately 9%) surviving BC_2F_2 plants (Table 4). On average, BC populations in the three genetic backgrounds did not differ much in the number of survivors, even though IR64 populations had a slightly higher survival rate (10.8%) than the others. Promising donors that gave similar numbers of SUBT plants in the three RPs included Cisedane, FR13A, IR50, Nan29-2, OM1706, Shwe-Thwe-Yin-Hyv, Tarom Molaii, TKM 9 and Y134 (Tables 8 and 9). Progeny testing confirmed that most selected lines had a significantly improved SUBT relative to their RPs.

3.5. Low temperature germination (LTG)

Of the 39 BC_4F_2 populations screened for LTG, an average of 5.31% of the seeds per population were able to germinate at 10 °C (Table 7). On average, *japonica* donors

Table 4
Summary results of BC populations screened for anaerobic germination and submergence tolerance

Details	Anaerobic germination				Submergence			
	IR64	Teqing	NPT	Total	IR64	Teqing	NPT	Total
Number of populations screened	47	47	36	130	60	57	62	179
Single plant selections per BC population	0–5	0–6	0–14		0–15	3–13	0–12	
Total selected BC_2F_3 lines	47	81	215	343	652	483	530	1665
Selection intensity (%)	0.52	0.93	3.11	1.32	1.08	0.85	0.85	0.93
Number of *indica* donors	37	38	29	104	47	50	50	147
Selected lines	31	67	77	175	538	431	451	1411
Selection intensity (%)	0.43	0.90	3.10	0.84	1.15	0.86	0.90	0.96
Number of *japonica* donors	6	8	5	19	8	7	7	22
Selected lines	11	14	31	56	83	52	63	198
Selection intensity (%)	0.91	1.03	3.10	1.47	1.00	0.74	0.90	0.90
Number of intermediate donors	3	0	1	4	4	0	3	7
Selected lines	5	0	7	12	40	0	16	56
Selection intensity (%)	0.83	0.00	3.50	1.50	1.04	0.00	0.53	0.80

Table 5
Comparison of different IR64 and NPT BC generations in screening for anaerobic germination (%)

	BC_2F_2 bulks		BC_3F_2 bulks		BC_4F_2 bulks	
	IR64	NPT	IR64	NPT	IR64	NPT
Number of populations screened	9	10	–	10	9	10
Surviving plants/population	0–28	0–50	–	33–78	17–78	97–162
Number of *indica* donors	7	8	–	8	7	8
Selected lines (*indica*)	36	158	–	452	296	1038
Selection intensity (%)	5.1	19.8	–	28.3	21.1	64.9
Number of *japonica* donors	2	2	–	2	2	2
Selected lines (*japonica*)	2	8	–	35	44	121
Selection intensity (%)	2.0	8.0	–	17.5	22.0	60.5
Mean selection intensity (%)	4.4	13.9		26.1	21.3	62.7

BC_2F_2, BC_3F_2 and BC_4F_2 bulks all had 200 seeds in 2 replications. For individual BC populations of 200 plants, a difference of 4% between two populations in selection intensity (survival rate) is statistically significant at $P < 0.05$ when the selection intensity is between 0.1 and 0.5.

produced significantly more LTG plants in the BC populations than the *indica* donors. LTG was as high as 62% in the NPT/Nan29-2 population, but only 6% in the Teqing/Nan29-2 population and zero in the IR64/Nan29-2 population, indicating a strong genetic background effect. In contrast, all three BC populations involving a Korean donor, Gayabyeo, showed relatively high LTG between 20 and 36%.

3.6. BPH resistance

Screening of the 193 BC_2F_2 populations resulted in identification of 909 resistant BC_2F_3 lines, giving an average selection intensity of 4.7% (Table 7). On average, significantly more BPH resistant plants were selected in the BC populations involving *indica* donors than those with *japonica* donors. The IR64 populations showed significantly higher frequency of resistant plants (10.19%) than the Teqing populations (3.81%) and the NPT populations (0.03%). Promising donors for both IR64 and Teqing included Jiang-Xi-Miao, Babaomi and TKM9, while BG300, C418, Lemont, Madhukar, MR167, OM1706, Shwe-Thwe-Yin-Hyv and Y134 were good donors only for IR64.

Table 6
Performance for anaerobic germination (AG) of 11 promising NPT BC_4F_3 lines and their parents

Recipient	Donor	Seedling height (cm)	AG at 10 d (%)	AG at 21 d (%)
NPT	Khazar (J)	33.5	20.0	90.0^{*}
NPT	Khazar (J)	32.5	75.0^{**}	95.0^{**}
NPT	FR13A (I)	37.7	30.0	95.0^{**}
NPT	TKM 9 (I)	37.7	95.0^{***}	100.0^{***}
NPT	TKM 9 (I)	36.2	60.0^{*}	100.0^{***}
NPT	TKM 9 (I)	37.9	95.0^{***}	100.0^{***}
NPT	Babaomi (I)	34.9	50.0	100.0^{***}
NPT	Babaomi (I)	36.7	50.0	100.0^{***}
NPT	OM1706 (I)	33.9	65.0^{*}	100.0^{***}
NPT	OM1706 (I)	36.1	80.0^{***}	100.0^{***}
Donors				
TKM 9 (I)	I	31.4	12.7	20.0
Khazar (J)	J	30.3	0.0	3.0
Babaomi (I)	I	26.3	0.0	5.0
Jiangxi-Si-Miao (I)	I	31.5	0.0	9.0
OM1706 (I)	I	29.1	2.0	18.0
IR64 (I)	I	26.5	2.0	20.0
NPT (J)	J	36.2	39.0	68.0

I = *indica* and J = *japonica*. All 10 ILs had significantly higher AG than the recurrent parent, NPT at $P < 0.001$. Asterisk (*, ** and ***) indicate that the differences between AG of the 11 BC_4F_3 lines and their recurrent parent, NPT, at the significance levels of $P < 0.05$, 0.01 and 0.001, respectively, based on Z tests.

4. Discussion

Several distinctive features make our BC breeding program unique. First, the number of donors and traits screened is greater than in any other similar study. The second feature was that the donors in the IRMBP were selected based on the geographic pattern of genetic diversity in rice germplasm collections worldwide (Li and Rutger, 2000). The parental lines are highly diverse at the molecular level, based on 101 SSR markers (Yu et al., 2003). Thus, these donors could be considered as a sample of the core collections of the rice primary gene pool. The third feature was that selection (screening) for tolerance to a particular stress was not based on the donor performance for any target trait, most of which are known to be complex and poorly understood genetically. We initiated the BC breeding program without first evaluating parental performance for target traits, though some donors were subsequently evaluated for performance during progeny testing. Many selected BC progeny for each target trait have been tested, and the results indicated that most selected BC progeny were indeed improved, though individual selected BC progeny did vary for the level of tolerance. Several important results were obtained regarding the amount of genetic variation for abiotic stress tolerance in the primary gene pool of rice and these suggest approaches for more efficient exploitation of this rich source of genetic diversity.

The most important conclusion of this study is that there are tremendous amounts of diversity in the primary gene

Table 7
Summary results of BC populations screened for low temperature germination and brown planthopper resistance

Details	Low temperature germination				Brown planthopper resistance			
	IR64	Teqing	NPT	Total	IR64	Teqing	NPT	Total
Number of populations screened	14	15	10	39	64	67	62	193
Single plant selections per BC population	0–21	0–18	0–31		0–22	0–22	0–2	
Total selected BC_2F_3 lines	79	77	51	207	652	255	2	909
Selection intensity (%)	5.64	5.13	5.10	5.31	10.19	3.81	0.03	4.71
Number of *indica* donors	1	1	0	2	49	60	49	158
Selected lines	0	3	0	3	565	221	2	788
Selection intensity (%)	0.00	3.0	0.00	1.5	11.53	3.68	0.04	4.99
Number of *japonica* donors	9	11	7	27	9	11	7	27
Selected lines	55	52	39	146	54	21	0	75
Selection intensity (%)	6.11	4.73	5.57	5.41	6.00	1.91	0.00	2.78
Number of intermediate donors	4	3	3	10	4	3	3	10
Selected lines	24	22	12	58	33	2	0	35
Selection intensity (%)	6.00	7.33	4.00	5.80	8.25	0.67	0.00	3.50

For individual BC populations of 100 plants, a difference of 2.5% between two populations in selection intensity (survival rate) is statistically significant at $P < 0.05$ when the selection intensity is <0.1.

pool of rice for all the traits we screened. This hidden diversity was reflected in at least two observations. The first was that BC progeny showing transgressive performance of the target traits over the parental lines were obtained in most BC populations for all abiotic stresses we screened, regardless of performance of their donors. Because the levels of different abiotic stresses applied in our ST, AG, SUBT, LTG and ZDT screening were very severe and typically killed the RPs and most donors, it is unlikely that the selected BC progenies survived by escaping the stress. This suggests the presence of genes for improved stress tolerance in the donors, which in some cases were not expressed in the donor phenotype. For example, while both IR64 and Teqing are susceptible to submergence and were killed by the stress, we were able to select 488 SUBT plants from these BC_4F_2 progeny with introgression from 11 non-submergence tolerant donors (Table 8). Similarly, results from 9 BC_2F_2 and 3 BC_3F_2 populations derived from the three RPs and three common donors clearly indicated that the number of surviving SUBT BC progeny was not correlated with the performance of the donors (Table 9). The NPT BC_3 population involving donor Khazar was peculiar in that the surviving rate was much higher than the other two BC_3 populations involving TKM9 and FR13A (Table 9). This was most likely due to sampling, because the BC_3 populations were not randomly generated, but were selected for some agronomic traits in the field. Thus, it was apparent that genes for the selected trait(s) are linked to major genes for SUBT, such as *Sub1* (Xu and Mackill, 1996) which alone was insufficient for the donor to survive the stress we applied. Furthermore, our results indicate that the sub-specific differentiation of *indica* and *japonica* within *O.*

Table 8
Genetic background effects of the recurrent parents on the number of surviving plants under 2-week submergence in 33 BC_4F_2 populations from crosses between three recurrent parents (RP) and 11 common donors during the dry season of 2003

Donor	RP	Survival (%)	*Z*-value	Donor	RP	Survival (%)	*Z*-value
BR24	IR64	8.00	3.61	Jhona349	IR64	5.00	2.73
BR24	Teqing	8.00	3.61	Jhona349	Teqing	5.67	3.09
BR24	NPT	0.00		Jhona349	NPT	0.00	
C418	IR64	7.33	3.19	Madhukar	IR64	7.33	3.60
C418	Teqing	10.00	4.35	Madhukar	Teqing	6.00	2.95
C418	NPT	0.00		Madhukar	NPT	0.00	
Chenhui448	IR64	9.33	4.14	SN89366	IR64	3.33	1.87
Chenhui448	Teqing	7.33	3.25	SN89366	Teqing	6.67	3.75
Chenhui448	NPT	0.00		SN89366	NPT	0.00	
FR13A	IR64	7.67	3.64	Y134	IR64	7.33	3.45
FR13A	Teqing	6.67	3.17	Y134	Teqing	7.33	3.45
FR13A	NPT	0.00		Y134	NPT	0.00	
IR50	IR64	6.67	3.13	Zihui 100	IR64	11.67	4.87
IR50	Teqing	8.00	3.76	Zihui 100	Teqing	7.33	3.06
IR50	NPT	0.00		Zihui 100	NPT	0.00	
IR72	IR64	8.00	0.45				
IR72	Teqing	8.00	0.45				
IR72	NPT	7.00					

Approximately 300 seeds per population were screened. The significance levels of $Z_{0.05}$, $Z_{0.01}$ and $Z_{0.001}$ = 1.65, 2.58 and 3.10.

Table 9
The number of surviving plants and selection intensity (%) of 9 BC_2F_2 and 3 BC_3F_2 populations (300 seeds per population) under two-week submergence under field conditions

	Donors			
Recipient	TKM9 (SS) [a]	Khazar (SS)	FR13A (T)	Total
IR64 (BC_2F_2)	12 (4.8)	8 (3.2)	14 (5.6)	34
Teqing (BC_2F_2)	10 (4.0)	9 (3.6)	8 (3.2)	27
NPT (BC_2F_2)	6 (2.4)	6 (2.4)	6 (2.4)	18
NPT (BC_3F_2)	10 (0.7)	71 (3.7)*	2 (0.2)	83
Total	38	94*	30	162

[a] SS and T indicate the high susceptibility and high tolerance of the donors and the population size was 250 for all BC_2F_2 populations, 1475 for the NPT/TKM9 BC_3F_2 population, 1900 for the NPT/Khazar BC_3F_2 population, and 820 for the NPT/FR13A BC_3F_2 population.

* Indicated significantly higher frequency ($P < 0.001$) in pairwise comparisons based on Z tests. Note that BC_3F_2 populations were not random populations but selected for certain agronomic traits under the normal conditions.

sativa does not seem to have broad implications regarding the useful genetic variation for most traits we screened in this study, except that *indica* lines tended to be good donors for BPH resistance, and *japonica* parents tended to be better donors for LTG.

The second observation was the fact that it was quite common to identify BC progeny with extreme phenotypes (tolerances). For instance, many BC progeny survived the severe zinc deficiency stress that virtually eliminated the best ZDT check, Madhukar (a landrace showing the highest ZDT in over 9000 germplasm accessions screened; A. Ismail, unpublished data). For ST, many of the selected BC progeny showed better tolerance than the tolerant checks, Pokkali and Bicol. Similarly, while some *japonica* accessions can germinate at 10 °C (Bertin et al., 1996), we have not seen a report that any *indica* rice cultivars can germinate at 10 °C, the low temperature threshold for growth of *indica* rice. But some IR64 and Teqing BC_4F_2 progeny were able to germinate at 10 °C, even though it took them 19 days to do so. It was surprising that 10 NPT BC progeny showed nearly 100% AG, significantly better than the RP, NPT (Table 6). This is the first report of this high level of AG being developed by breeding. Most cereals are unable to germinate under anoxic conditions (Kennedy et al., 1992; Perata and Alphi, 1993) and rice germplasm accessions showing such extremely high tolerance to anaerobic conditions during germination are very few (A. Ismail, personal communication). In this regard, AG is a very important trait for direct-seeded rice and can play an important role in seedling establishment under flash flooding. It is intriguing to note that the genes governing AG are expressed more strongly in the NPT genetic background and more advanced BC generations, suggesting that the trait is governed by relatively fewer genes of large effects.

Our third conclusion is that the selection efficiency for abiotic stress tolerances in BC populations, defined as the number of superior plants identified per BC population, is highly dependent upon genetic background. In this study, common population size of 100–250 plants were used for target trait screening of the BC populations. Under the highly stringent stress conditions for most target traits (the average selection intensity was less than 10%), this range of population sizes allowed identification of surviving plants in most BC populations and a difference of 2.5% in selection intensity was statistically significant based on Z tests (Larsen and Marx, 1981). We observed that for ST, SUBT, ZDT and BPH resistance, selection was more efficient in the IR64 and Teqing populations (*indica*) than the NPT populations (*japonica*), whereas the opposite was true for AG. Because both IR64 and Teqing had better ST, ZDT and BPH resistance than NPT, it was not surprising that there were higher survival rates in the IR64 and Teqing BC progenies under the same stress conditions. Thus, the overall level of stress tolerance and the number of surviving plants in a specific BC population under stresses were highly dependent upon both RP and donor combinations.

The differences between the recurrent genetic backgrounds were at least partially attributable to the differences between the RPs for trait-enhancing alleles they carry. For instance, the AG tolerance level was NPT $\gg$ IR64 > Teq- Teqing, and the same ranking as for the frequencies of resistant plants in their BC populations under the same selection pressure. There were exceptions, however. Unexpectedly, one donor, Babaomi, that produced high frequencies of BPH resistant progeny in the IR64 and Teqing backgrounds, gave only a 2% survival rate in the NPT background. This implies that the BPH resistance genes in Babaomi are largely complementary to those in IR64 and Teqing, but are largely the same as those in NPT. Similarly, while Jiangxi Miao, Babaomi and TKM9 gave relatively similar frequencies of between 12 and 22% of BPH resistant progeny in the IR64 and Teqing backgrounds, other donors (Bg300, C418, Lemont, Madhukar, MR167, OM1706, Shwe Thwe Yin Hyv and Y134) gave much higher BPH resistant progeny in the IR64 background than the Teqing background. These results indicate that the genetic basis of BPH resistance is quite complex, being governed by many genes/QTL, and that a variety could be a good donor of a stress tolerance for one RP but not for another.

Our fourth conclusion is that selection efficiency was affected to a large extent by the level of stress applied. We found that high levels of stress could significantly increase the accuracy of selection and reduce the number of total selected plants to a manageable size. For instance, the EC 12 dsm^{-1} normally used for screening for ST at the seedling stage was too low to identify ST plants in our BC populations since over 80% of plants in many BC populations were little affected by this level of stress. Instead, the EC 24 dsm^{-1} level was more suitable for screening BC progeny from more susceptible recurrent parents such as NPT, and the EC 30 dsm^{-1} was better for those with a moderate level of ST (IR64 and Teqing). Thus,

Table 10
Performance of 22 promising IR64 introgression lines (ILs) with significantly improved salinity tolerance under the saline (EC 16–18) field condition at Ioillo, Philippines

ILs	Donor		Seed set (%)	Spikelets/ panicle	Filled grain weight (g)	1000-grain weight	Salinity damage score	
	Name	Origin					Seedling	Maturity
SAT2	Y134	China (I)	84.3	119.8	21.26	21.05	5	5
SAT4	Yue-Xiang-Zan	China (I)	89.0	48.9	9.91	22.78	4	7
SAT5	Zhong413	China (I)	96.0	48.1	8.57	19.66	4	5
SAT9	TKM9	India (I)	89.3	42.1	7.88	20.96	4	3–5
SAT17	TKM 9	India (I)	88.0	35.9	6.10	21.03	4	5
SAT36	STYH	Myanmar (I)	87.9	43.8	7.63	19.82	5	3
SAT39	Bg300	Sri Lanka (I)	86.4	47.1	7.52	18.48	5	3
SAT42	OM997	Vietnam (I)	93.0	35.1	6.31	19.91	5	3–5
SAT43	M401	USA (J)	86.4	40.4	6.79	19.46	5	3–5
SAT50	M401	USA (J)	87.9	41.2	7.97	22.02	4	5
SAT51	M401	USA (J)	81.0	39.7	5.63	17.70	5	3
SAT55	PMPM	Thailand (I)	82.9	34.5	5.53	19.34	5	1–3
SAT56	PMPM	Thailand (I)	81.8	42.9	6.37	18.15	4	1–3
SAT57	PMPM	Thailand (I)	82.0	34.4	5.51	19.54	4	1–3
SAT58	PMPM	Thailand (I)	87.1	41.7	6.97	19.20	3	1–3
SAT59	PMPM	Thailand (I)	83.9	38.6	6.78	20.93	3	1–3
SAT60	PMPM	Thailand (I)	86.7	39.1	7.41	21.86	3	3
SAT61	PMPM	Thailand (I)	88.6	35.9	6.97	21.92	5	3
SAT62	PMPM	Thailand (I)	88.3	37.7	7.10	21.32	4	3–5
SAT63	PMPM	Thailand (I)	85.1	48.9	7.72	18.56	5	3–5
SAT85	93072	China (I)	88.0	41.1	7.20	21.69	5	1–3
SAT87	93072	China (I)	81.1	79.7	12.06	18.67	5	1–3

The recurrent parent, IR64, and all donors had a salinity damage score of 9 at both seedling and final stages of evaluation, and none of them survived the stress. Grain yield was the mean grain weight per plant harvested from 10 plants in the field plot. PMPM = Pahk maw peun meuang, STYH = Shwe-Thwe-Yin-Hyv; I and J are *indica* and *japonica*.

it is necessary to adjust the level and timing of stresses based on the performance of RPs in BC breeding programs. Applying an appropriate level of stress in breeding for different types of stress tolerances remains a challenge. It is essential to always include two types of checks, the RPs and a tolerant one, with BC populations in screening in order to make accurate selection. We realize that screening for tolerances to some stresses at the seedling stage such as ST and BPH resistance may not always result in the corresponding tolerances at the reproductive stage (Mishra et al., 2001). However, 2-year progeny testing of the selected ST ILs at Iloilo of Philippines during 2002 and 2003 wet seasons identified 22 promising lines showing a high level of ST at both the seedling and reproductive stages (Table 10).

Fifth, selection efficiency for different target traits may vary in different BC generations. Much higher numbers of surviving plants were identified in BC_2 populations than in BC_3 populations for ST and ZDT. This decreased selection efficiency with advancement of BC generations has been reported recently for tolerances to salinity, drought and low temperature for seed germination in tomato (Foolad et al., 2003). This is not surprising since the number of QTLs from donors in random BC_3 populations is expected to be only half of that in BC_2 populations. However, the opposite was true for AG and SUBT in this study. It was reported that a major gene, *Sub1* is involved in the genetic control of SUBT (Xu and Mackill, 1996). Thus, our results suggest that AG may also be controlled by genes of large effects.

Finally, it is generally believed that the levels of tolerances/resistances to abiotic and biotic stresses tend to follow the order of wild species > landraces > modern cultivars, and the reverse is true for yield potential under modern cultivation, as a result of domestication and long-term artificial selection. While the presence of beneficial genetic variation for yield discovered in wild species of tomato (Tanksley et al., 1996) and rice (Xiao et al., 1998) opens a new opportunity to break the yield plateau of modern crops, our results have challenged the general belief that resistances/tolerances to biotic and abiotic stresses are more easily discovered in wild species than the cultivated types themselves. The wide presence and random distribution of stress tolerance genes in the primary gene pool of rice is certainly good news for plant breeders. The identification of many stress tolerant progeny in advanced BC populations, achieved in this study, means that introgression of genes from diverse donors into elite genetic backgrounds through BC breeding and efficient selection can exploit existing diversity for genetic improvement of complex phenotypes. Understanding the genetics of this 'hidden' diversity that underlies tolerances to abiotic and biotic stresses remains a challenging but promising task for plant scientists to in the years to come.

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