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Rice variety Dhanrasi, an example of improving yield potential and disease resistance by introgressing gene(s) from wild species (*Oryza rufipogon*)

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Rice variety Dhanrasi (C 11-A-41) was developed by introgressing gene(s) for yield enhancement, resistance to blast and moderate resistance to bacterial blight and rice tungro disease from wild species (*Oryza rufipogon*). The *O. rufipogon* accession resistant to blast, bacterial blight and rice tungro disease was crossed with high-yielding line B 32-Sel-4. The F_1 (B 32-Sel-4 × *O. rufipogon*) was crossed with another high-yielding line B 127. Two cycles of selective intermating were followed in the F_2 generation and thereafter pedigree method of selection was continued. In the F_5 generation 32 lines were evaluated with the *indica* parents for yield, and also screened for blast and bacterial blight resistance. Eight lines were superior in yield to both the *indica* parents by 10.2–21.4%, indicating introgression of yield-enhancing gene(s) from *O. rufipogon*. Culture C 11-A-41 yielded the highest (6.48 t/ha) with yield superiority of 38.2 and 21.4% over its parents B 32-Sel-4 and B 127 respectively. The present results indicated that the gene(s) for yield enhancement, resistance to blast and moderate resistance to bacterial blight and rice tungro disease in C 11-A-41 were introgressed from *O. rufipogon*. Considering its yield superiority and multiple resistance to diseases, the culture (C 11-A-41) was released as variety Dhanrasi for cultivation in Andhra Pradesh, Tamil Nadu, Karnataka and Maharashtra in 2002.

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RICE productivity in irrigated and rainfed shallow lowland areas in India has remained almost stagnant for a long time after the release of varieties Salivahana and Pranava in 1988. Since then several breeding lines have been evaluated in these ecosystems under the All India Coordinated Rice Improvement Project (AICRIP) with Salivahana and Pranava as the national checks, but none could qualify for release in irrigated shallow lowlands in the southern region[1]. This showed that the yield improvement *per se* in the lines bred for these ecosystems specially in irrigated shallow lowland remain limited, though there has been considerable improvement in quality and pest resistance in varieties released at the state-level. The possible reason for low genetic gain in yield may be the narrow genetic base due to the fact that a small core of adapted germplasm has been used repeatedly in breeding programmes.

Wild species of *Oryza* are important reservoirs of genes for agronomically important traits such as resistance to biotic and abiotic stresses, improved quality characteristics and yield[2]. A number of major genes showing resistance to bacterial blight (*Xa21*, *Xa23*), blast (*Pi9*), rice tungro disease, brown plant hopper (*Bph10*, *bph12*, *Bph13*, *Bph14*, *Bph15* and *Bph18*) and grassy stunt virus have been introgressed from different wild species using the backcross breeding method[2]. Recent studies using molecular markers have demonstrated that wild relatives are also important sources of useful alleles for complex traits such as yield in crops like tomato[3] and rice[4]. Though several yield-enhancing quantitative trait loci (QTLs) have been identified from different species of wild rice such as *Oryza rufipogon*[5–7], *O. nivara*[8] and *O. glumaepatula*[9], no variety has been so far released having known yield-enhancing genes/QTLs introgressed from wild rice. Combining the favourable yield-enhancing genes/QTLs located on different chromosomes in one background following repeated backcross breeding method is a difficult task. In the present study we followed selective intermating in segregating generations to combine different favourable traits introgressed from wild species for enhanced yield potential and biotic stress resistance.

The *O. rufipogon* accession collected from lowlands in Moirang (Manipur, India) showed resistance to blast and rice tungro disease and moderate resistance to bacterial blight (Table 1). It was crossed with a high-yielding, medium-duration breeding line B 32-Sel-4. The F_1 was top-crossed with another high-yielding, late-duration breeding line B 127. Both the *indica* parents (B 32-Sel-4 and B 127) were susceptible to blast, bacterial blight and rice tungro disease. In the F_2 generation, 50 plants of three-way cross were selected with better agronomic traits and were intermated to develop 25 crosses. All the F_1 seeds were grown to maturity and plants with better agronomic traits were harvested. Progenies of the selected plants were grown and at flowering, 50 plants with desirable traits were again selected and intermated. The fresh 25 F_1 seeds were grown and selections against weedy traits were operated at maturity. Thereafter, single-plant pedigree selection was followed in segregating generations considering plant height, flowering duration, number of tillers/plant, panicle length, number of grains/panicle and grain yield/plant. The breeding strategy followed is given in below:

Breeding strategy followed

Indica parent	
(B 32-Sel-4)	× *O. rufipogon*
F_1 ×	B 127 (*indica* parent)
↓	
F_1	Selection against weedy traits
↓	
F_2	Fifty plants with good agronomic traits selected
↓	
	First cycle of intermating between selected plants
↓	
25 F_1s	Selection against weedy traits
↓	
F_2	Fifty plants selected with good agronomic traits
↓	
	Second cycle of intermating between selected plants
↓	
25 F_1s	Selection against weedy traits
↓	
F_2	Selection for superior agronomic traits
↓	
↓	Pedigree selection
↓	
F_5	Evaluation of progenies for yield and disease resistance

In the F_5 generation, 32 introgression lines with superior agronomic traits were evaluated with *indica* parents (B 32-Sel-4 and B 127) for yield and also screened for blast and bacterial blight resistance under natural and artificial infection. Twenty day old seedlings of the entries were planted at 20 × 15 cm spacing in three replications with plot size of 10 sq. m. Observations on days to flowering, plant height, number of ear-bearing tillers/plant, number of grains/panicle and yield/plot were recorded. All the entries along with checks IR64 (resistant) and CO 39 (susceptible) were grown in one line of 50 cm length and with a spacing of 5 cm row-to-row in order to screen for blast resistance. The susceptible check CO 39 was grown all around the nursery and also after every ten entries. Inoculum of local isolate with suspension of 10^5 spores/ml was prepared and spread twice after 15 and 20 days of sowing. Observations of blast reaction (0–9 scale)[10] on introgression lines were recorded when all the plants of CO 39 were dead. Introgression lines along with TN (1) and (Ajaya) as susceptible and resistant checks, respectively were also screened for bacterial blight resistance at tillering stage with freshly prepared inoculum of the isolate present in the Andamans. The disease reaction was recorded after 21 days of inoculation.

Three introgression lines C 23-Sel-R (IET 15355), C 28-6 (IET 15356) and C 11-A-41 (IET 15358) with higher yield and resistance to blast and moderate resistance to bacterial blight were evaluated in All-India Coordinated Trials for shallow lowlands, along with national checks Salivahna, Pranava and Swarna, and several other entries developed elsewhere in Andhra Pradesh, Tamil Nadu, Karnataka and Maharashtra during 1996–99 and in Orissa and Bihar during 1999. Replicated yield trials were conducted at eight locations in 1996, nine locations in 1997, nine locations in 1998 and ten locations in 1999, with a plot size of 10–12 sq. m and spacing of 20 × 15 cm (row to plant). Observations on flowering duration, plant height and yield (t/ha) were recorded. Entries were also screened at nine locations for blast and bacterial blight, ten locations for sheath blight and three locations for rice tungro disease in a national screening nursery (NSN-1 and NSN-2) during 1997–99 following standard techniques. The disease reaction was recorded in a 0–9 scale[10].

Among the 32 introgression lines evaluated, eight were superior in yield to both of *indica* parents (B 32-Sel-4 and B 127), with the yield advantage of 10.2 to 21.4% (Table 2). Line C 11-A-41 yielded the highest (6.48 t/ha) with 38.2 and 21.4% higher yield than the parents B 32-Sel-4 (4.69 t/ha) and B 127 (5.34 t/ha) respectively. The other lines C 23-Sel-R (6.32 t/ha), C 28-6 (6.27 t/ha), C 28-16-2 (6.08 t/ha) and C 11-21-5 (6.01 t/ha) also showed significantly higher yield than the parents. Further, lines C 11-A-41, C 23-Sel-R, C 28-6 and C 11-21-5 were resistant to blast and moderately resistant to bacterial blight, while lines C 28-16-2 and C 15-5-4 were resistant to blast. Yield superiority of introgression lines indicated intro-

Table 1. Disease and pest reaction on parents (*Oryza rufipogon*, B 32-Sel-4 and B 127) of variety Dhanrasi

Disease and insect pest	Reaction		
	B 32-Sel-4	B 127	*Oryza rufipogon*
Artificial screening			
Blast			
• Against Andaman isolate	S	S	Immune
• Against DRR isolate	S	S	Immune
Bacterial blight	S	S	MR
Sheath blight	S	S	MR
Rice tungro disease	S	S	R

R, Resistance; MR, Moderate resistance; S, Susceptible.

Table 2. Yield performance of promising introgression lines derived from the cross *O. sativa* × *O. rufipogon*

Entry	Yield (t/ha)	Per cent increase in yield over		Plant height (cm)	Days to 50% lowering	No. of EBT/ sq. m	No. of grains/ panicle	Reaction to	
		B 32-Sel-4	B 127					Blast	BB
C 11-A-41	6.48	38.2	21.1	118	117	323	251	R	MR
C 23-Sel-R	6.32	34.8	18.4	108	107	332	237	R	MR
C 28-6	6.27	33.7	17.4	117	104	315	221	R	MR
C 28-16-2	6.08	29.6	13.9	112	119	293	236	R	S
C 11-21-5	6.01	28.1	12.6	103	110	318	189	R	MR
C 11-A-84	5.98	27.5	12.0	120	114	291	188	MR	S
C 15-5-4	5.92	26.2	10.9	127	91	306	157	MR	S
C 17-5-9	5.89	25.6	10.3	123	110	317	132	R	S
B 32-Sel-4	4.69			96	95	288	143	S	S
B 127	5.34			109	100	301	166	S	S
CD at 5%	0.23			6.75	3.2	8.92	18.9	–	–

R, Resistance; MR, Moderate resistance; S, Susceptible; BB, Bacterial blight; EBT, Ear-bearing tillers.

gression of yield-enhancing gene(s) from *O. rufipogon*. The present results support earlier findings indicating that the yield of cultivated rice can be improved by introgression of yield-enhancing QTLs from *O. rufipogon*[4–7]. Alleles *yld1-1* on chromosome 1 and *yld2-1* on chromosome 2 from a Malaysian accession of *O. rufipogon* were associated with yield improvement of 17–29% in Chinese hybrids[6]. Moncada *et al.*[5] reported two major yield-enhancing QTLs from *O. rufipogon*, *yld1-1* and *yld11-1* on chromosome 1 and 11 respectively, in the same region as *yld1-1* reported by Xiao[6] and *yld11-1* reported earlier[11,12] using different accessions of *O. rufipogon*. Thus it will be of interest to determine whether the *O. rufipogon* alleles associated with improved yield of variety Dhanrasi under shallow lowland conditions are the same or different from those reported earlier.

Yield improvement in the introgression lines was observed mainly due to increase in number of ear-bearing tillers and number of grains/panicle, which were more than both *indica* parents and *O. rufipogon*, indicating transgressive segregations. Earlier reports also indicated that increase in number of grains/panicle was the main factor for yield improvement in *O. rufipogon* derived populations[5–7]. Increase in the number of grains per unit land area, or sink size has been suggested as the primary determinant of grain-yield improvement in rice[13–15].

The screening results of introgression lines for blast indicated that 14 lines and *O. rufipogon* were resistant (0–3 score), seven moderately resistant (score 5), and 11 lines with *indica* parents (B 32-Sel-4 and B 127) susceptible. Among the 14 resistant lines, three lines along with *O. rufipogon* were immune, six lines with score 1, five lines with IR64 having score 3. While for bacterial blight reaction, one line found to be resistant and six lines along with *O. rufipogon* moderately resistant. The rest of the lines and *indica* parents were susceptible. The results suggest introgression of resistance gene(s) for blast and moderate resistance for bacterial blight from *O. rufipogon*. Though *O. rufipogon* was moderately resistant to bacterial blight and *indica* parents were susceptible, the unexpected resistance in one line may perhaps be due to expression of some hidden gene from *O. rufipogon*. Introgression of blast and bacterial blight resistance genes from different wild species into cultivated rice has already been reported[16–18].

In multilocation testing under AICRIP trial in 1996 under shallow lowland conditions, culture C 11-A-41 (IET 15358) yielded the highest with mean yield of 5.28 t/ha

Table 3. Mean yield, range and per cent yield increase of variety Dhanrasi over high-yielding national check varieties

		Grain yield (t/ha)										
		National check variety						Most popular variety				
		Dhanrasi		Salivahana		Pranava		Swarna		Per cent yield increase of Dhanrasi over		
Year of testing	No. of locations	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Salivahana	Pranava	Swarna
1996	7	5.28	3.85–6.59	4.82	3.33–6.89	4.41	3.33–6.98	–	–	9.5	19.7	–
1997	6	4.48	3.59–5.63	3.75	2.10–5.39	–	–	4.06	3.13–5.15	19.5	–	10.3
1998	9	4.69	2.99–7.31	4.42	2.04–6.07	4.35	2.99–6.12	–	–	6.1	7.8	–
1999	10	4.50	2.67–6.10	4.12	2.60–5.54	–		3.52	1.93-5.31	9.2	–	27.8

–, Not evaluated.

Table 4. Location-wise yield performance of variety Dhanrasi in multilocation trials

		Grain yield (t/ha)						
			Check variety					
Location	Year	Dhanrasi (IET 15358)	Salivahana	Pranava	Swarna	Pooja	CD at 5%	CV (%)
Maruteru	1996	6.58	6.89	6.98	–	–	0.18	1.5
	1997	4.81	4.83	–	5.05	–	NS	28.0
	1998	7.31	6.07	6.12	–	–	NS	21.9
	1999	5.02	4.71	–	3.16	–	1.00	12.7
Ragolu	1996	5.88	5.39	5.37	–	–	0.73	8.5
	1997	4.97	4.09	–	4.37	–	0.42	5.7
R.C. Puram	1997	3.59	3.05	–	3.26	–	1.21	20.6
	1998	4.18	3.64	3.53	–	–	0.98	22.0
Aduthurai	1996	4.94	4.55	3.93	–	–	0.55	8.8
	1997	5.03	2.09	–	3.44	–	0.47	8.0
	1998	4.97	5.92	5.82	–	–	0.38	5.2
Coimbatore	1996	6.10	5.70	4.27	–	–	0.28	4.2
	1997	4.91	5.39	–	5.15	–	0.24	3.0
	1998	4.23	5.47	4.82	–	–	0.15	2.0
	1999	6.10	5.54	–	5.31	–	0.16	1.7
Mandya	1996	5.76	3.33	3.33	–	–	NS	45.6
Mangalore	1996	3.85	4.17	3.68	–	–	0.47	6.0
	1998	5.29	4.73	4.06	–	–	0.75	11.6
	1999	5.81	4.38	–	3.94	–	0.99	12.4
Sirsi	1996	3.85	3.72	3.33		–	0.62	9.0
	1997	3.53	3.07	–	3.12	–	NS	42.1
Mugad	1998	5.83	4.44	4.44	–	–	1.62	18.2
Karjat	1998	3.55	2.44	3.55	–	–	0.75	16.0
	1999	5.17	5.05	–	4.35	–	0.71	9.0
Sakoli	1998	3.83	3.86	3.85	–	–	0.39	8.7
	1999	3.20	3.05	–	2.51	–	0.60	13.1
Sindewahi	1998	2.99	3.18	2.94	–	–	0.63	15.8
	1999	2.40	2.28	–	1.92	–	0.36	10.2
Jeypore	1999	5.03	4.77	–		5.30	0.89	11.0
Bhubaneswar	1999	2.67	2.60	–	–	1.57	0.33	8.2
Ranchi	1999	4.60	3.83	–	–	3.53	0.33	4.9
Sabour	1999	5.03	5.00	–	–	4.55	0.51	7.2

–, Not evaluated; NS, Non-significant.

(Table 3). Yield superiority over Salivahana and Pranava was 9.5 and 19.7% respectively. It was superior to the highest yielding check at Ragolu, Aduthurai and Coimbatore (Table 4). It showed 7.6 and 20.1% higher yield than the best check in the Tamil Nadu and Karnataka respectively. The other two lines, IET 15356 (C 28-6) and IET

Table 5. Multilocation screening of variety Dhanrasi (IET 15358) for different diseases

			Mean disease score in 0–9 scale					
				Checks				
Disease	Year	No. of locations	Dhanrasi IET15358	HR12 S to blast	TN1 (S check)	IR64 R to blast	Ajaya R to BB	Vikramarya R to RTD
Blast	1997	7	1.4	5.5	4.2	1.4	–	–
	1998	9	2.1	6.9	5.8	3.8	–	–
	1999	7	3.0	7.8	5.3	2.3	–	–
Mean			2.2	6.7	5.1	2.5	–	–
Neck blast	1997	3	3.3	7.7	6.0	6.0	–	–
	1998	3	3.0	7.0	5.0	5.0	–	–
Mean			3.0	7.4	5.5	5.5	–	–
BB	1997	6	4.8	6.0	7.8	6.0	2.6	–
	1998	8	5.4	6.0	7.0	5.4	4.0	–
	1999	9	3.4	6.6	7.3	5.6	3.4	–
Mean			4.5	6.2	7.4	5.7	3.3	–
Sheath blight	1997	10	4.9	5.6	6.5	4.9	6.0	5.2
	1998	10	5.6	7.3	7.6	6.7	6.8	6.1
	1999	11	5.4	7.0	7.8	7.0	6.8	6.5
Mean			5.3	6.6	7.3	6.2	6.5	5.9
Sheath rot	1998	6	3.7	5.4	7.7	6.5	5.8	5.0
	1999	3	1.5	6.0	5.0	4.3	4.5	4.5
Mean			2.6	5.7	6.4	5.4	5.2	4.8
RTD	1997	1	3.0	–	9.0	–	–	3.0
	1999	3	4.3	5.0	6.0	–	–	3.0
Mean			3.7	5.0	7.5	–	–	3.0

Score 0–3, Resistance; 3.1–5.0, Moderate resistance; 5.1–6.0, Moderate susceptible; 6.1–9.0, Susceptible; R, Resistance; S, Susceptible; BB, Bacterial blight; RTD, Rice tungro disease.

15355 (C 23-Sel-R) were inferior to the highest yielding check (Salivahana). During 1997, yield data from Mugad, Ponnampet and Bapatla were not considered due to low experimental mean and high C.V. while at Maruteru and Sirsi C.V. was high resulting in non-significant differences. Regarding overall mean yield, the culture C 11-A-41 ranked first (4.48 t/ha) with yield superiority of 19.5 and 10.3% over checks Salivahana and Swarna respectively. It yielded higher than the best check at Aduthurai, Ragolu, R.C. Puram and Sirsi (Table 4). Its yield ranged from 3.59 t/ha to 5.63 t/ha (Table 3). Yield superiority of IET 15358 over checks Salivahana and Swarna was 11.8 and 5.4% in Andhra Pradesh and 32.9 and 15.6% in Tamil Nadu respectively. In 1998, IET 15358 was evaluated at nine locations in four States. Considering locations it ranked third with mean yield of 4.69 t/ha, ranging from 2.99 to 7.31 t/ha, which was 6.1 and 7.8% higher than Salivahana and Pranava respectively. In Andhra Pradesh, Karnataka and Maharashtra, its yield was higher than the best check (Salivahana) by 18.3, 21.1 and 9.5% respectively.

During 1999, IET 15358 with a mean yield of 4.5 t/ha (range 2.67 to 6.1 t/ha) showed 9.2 and 27.8% higher yield than Salivahana and Swarna respectively. It was superior to the highest yielding check variety Salivahana in Tamil Nadu, Andhra Pradesh and Karnataka, with yield increase of 10.1, 6.6 and 32.6% respectively. It was also evaluated against newly bred cultures and check varieties Swarna and Pooja under rainfed, shallow lowland conditions in the eastern and western regions of the country. Its mean yield was 3.53, 3.85 and 4.54 t/ha in of Maharashtra, Orissa and Bihar respectively. It showed yield improvement of 11.9 and 30.5% over Pooja and 4.3 and 8.6% over highest yielding check Salivahana in the Orissa and Bihar respectively. While in Maharashtra it showed 6.7 and 20.5% higher yield than the check varieties Salivahana and Swarna respectively. These results indicate that yield-enhancing gene(s) introgressed from *O. rufipogon* in Dhanrasi were stable over the years as well as locations. Moncada[5] also observed that *O. rufipogon* introgressions at the *yld1-1* and *yld2-1* loci would be advantageous in different genetic backgrounds and environments due to their stable effect under low and high input conditions.

The results of multilocation screening during 1997, 1998 and 1999 for blast, bacterial blight, rice tungro disease, sheath blight, brown spot and sheath rot indicated that the culture IET 15358 was resistant to leaf and neck blast and moderately resistant to bacterial blight, brown spot and rice tungro disease (Table 5). Since both *indica* parents of IET 15358 were susceptible, the resistance reaction of IET 15358 over the years as well as locations indicated that the genes for broad-spectrum resistance to blast and moderate resistance to bacterial blight and rice tungro disease introgressed from *O. rufipogon*. Genes introgressed from wild species into cultivated rice such as blast resistance

(*Pi9*) from *O. minuta*[16], bacterial blight resistance from *O. longistaminata*[17] and *O. rufipogon*[18], and rice tungro disease resistance from *O. rufipogon* showed broad-spectrum resistance to different lineages of the rice diseases[2]. The breakdown of resistance in modern, high-yielding varieties after a few years of cultivation was attributed to fast-changing pathogens. Introgression of such novel genes from wild species is considered a better approach for introducing stability in resistance against diseases/pests.

The present findings suggest that rice gene pools can be broadened to improve several traits, including yield and disease/insect pest resistance by introgressing the novel genetic variations from diverse primary and secondary gene pools through wide hybridization. The present investigation also indicates that for introgression of multiple genes of agronomic importance from closely related wild species into cultivated rice, a better approach would be one or two backcrosses followed by selective intermating in segregating generations to break the undesirable linkages for superior recombinants. Such a strategy may be useful for introgressing complex traits such as yield from wild rice. Considering its yield superiority over national checks with resistance/tolerance to multiple diseases, the introgression line C 11-A-41 (IET 15358) released as variety Dhanrasi in 2002 for cultivation in irrigated shallow lowlands of Andhra Pradesh, Tamil Nadu and Karnataka and in rainfed lowlands of Maharashtra, is an example utilizing wild rice for improvement in yield and multiple disease resistance.

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Arcellaceans and pollen/spores of a late Harappan settlement near Porbandar, west coast of India: Implications for palaeoecology and environmental monitoring

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Archaeological sites have been a storehouse of information on various aspects of the past, including climatic conditions and hydrological characteristics in a particular time bracket. Recent excavation near Porbandar has brought to light a late Bronze Age settlement close to Porbandar creek. The archaeological artefacts have close similarities with those of other Harappan settlements in the Saurashtra region. Soil samples were collected and analysed for pollen content. The samples show shallow riverine depositional environment, which was influenced by relative sea-level rise and fall corres-

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