Stacking disease QTLs with drought tolerance in rice

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The objective of our project is to demonstrate the feasibility of combining traits essential for drought-prone environments. For upland rice in India and Indonesia, germplasm with improved drought tolerance must have blast resistance to have any chance of adoption. Thus, selection for drought tolerance should be done in conjunction with selection for disease resistance. We accumulated different disease QTL (dQTLs) from Moroberekan into Vandana, a drought-tolerant variety and evaluated the advanced backcross progenies in blast hotspots in India and the Philippines. Selected intermated F_6 lines showing resistance to blast and agronomically similar to Vandana were evaluated for yield performance under managed drought. Six candidate genes (CGs) co-localizing with known dQTLs – aldose reductase, chitinase, oxalate oxidase (OXO), oxalate oxidase-like proteins (OXLP), peroxidase and thaumatin, including several SSR markers were significantly associated with resistance to blast. We identified IR78221-19-6-33 and IR78222-20-7-148-2B as the two best lines containing the CG alleles from Moroberekan. These two lines showed agronomic similarity to Vandana but with added blast resistance. In Indonesia, two lines (BC_2F_5-168 and BC_2F_5-49) derived from Way Rarem/Oryzica Llanos 5 were found to show resistance to blast and perform well under drought. Our data, though preliminary, did not show obvious antagonistic effects in combining blast resistance with drought tolerance. To further extend the use of defense CGs to other cereals, we identified maize BAC clones containing orthologs for these CGs, which may also be candidate genes for resistance to fungal pathogens in maize.

Promising Vandana/Moroberekan lines showing resistance to blast and tolerance to drought

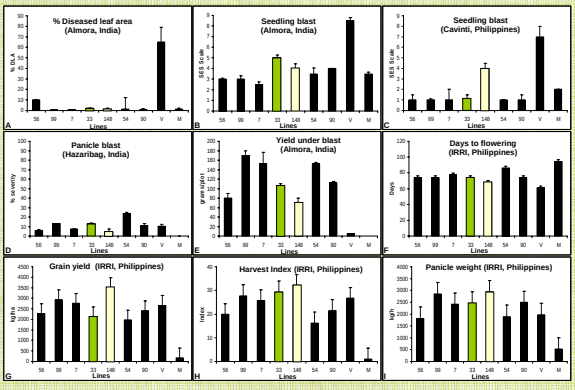

Figure 1. Performance of promising Vandana/Moroberekan intermated advanced backcross lines with resistance to blast and tolerance to drought. We identified Vandana/Moroberekan lines with different candidate defense gene alleles from Moroberekan but with high similarity to Vandana. A-D) Resistance to blast was determined by screening advanced lines in blast hotspots in India and the Philippines (2004-2005). E) Yield under blast was measured in Almora, India. F-I) Advance lines were evaluated for phenotypic similarity to Vandana and drought tolerance at IRRI (2004 wet season, with low rainfall at reproductive stage). Lines 33 and 48 performed well under reproductive drought stress. These are also candidates for agronomic /testing in station trials in India. Lines: 56 - IR78221-19-6-56-B; 99 – IR78221-19-6-99-B; 7 – IR78221-6-7-B; 33 – IR78221-19-6-33-B; 148 – IR78222-7-148-2-B; IR78221-19-4-54-B; 90 – IR78221-19-6-90; V – Vandana; and M - Moroberekan

Mapping of QTLs for resistance to blast and drought tolerance

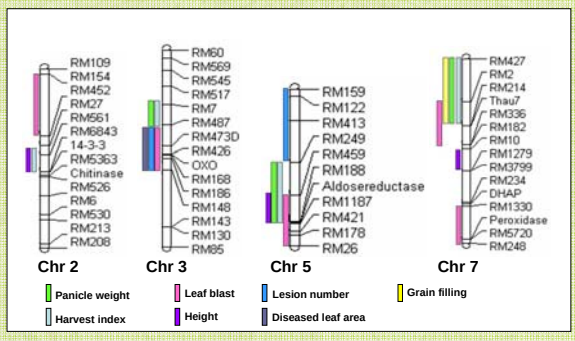

Figure 2. QTL map for Vandana/Moroberekan intermated advanced backcross lines. We used candidate gene-based PCR markers, SSRs co-localizing with candidate genes and randomly selected SSRs to conduct a genome scan of the Vandana/Moroberekan lines. Aldose reductase, chitinase, oxalate oxidase, oxalate oxidase-like proteins, peroxidase and thaumatin were located in QTLs identified by interval mapping using WinQTL Cartographer.

Table 1. Candidate gene alleles[1] contributed by Moroberekan in blast-resistant, drought tolerant Vandana/Moroberekan lines

Candidate Gene	IR78221-19-6-56-B	IR78221-19-6-99-B	IR78221-19-6-7-B	IR78221-19-6-33-B	IR78222-20-7-148-2-B	IR78221-19-4-54-B	IR78221-19-6-90-B	Vandana	Moroberekan
Chitinase	+	-	-	-	+	+	-	-	+
Oxalate Oxidase	+	+	+	+	+	+	-/+	-	+
Aldose reductase	-	-	-	-	-	-	-	-	+
Thaumatin (Chr6)	-	-	+	-	-	-	-	-	+
HSP90	-	-	-	-	+	-	-	-	+
Thaumatin (Chr7)	-	-	-	-	-	+	-	-	+
Deoxyphosphohept onate aldolase	-	-	-	-	-/+	-	-	-	+
Peroxidase	+	+	+	+	-	+	+	-	+
Oxalate Oxidase-like	+	-	+	-/+	+	+	+	-	+
PR1	-	-	-	-	+	-	-	-	+
Aldose reductase	-	-	-	-	+	-	-	-	+
Seedling blast (Philippines) Scale 0-9	1	1	1	1	2	1	1	7	0.3
Seedling blast (Almora, India) Scale 0-9	3	3	2	4	4	4.5	4	8.5	1.5
Yield under blast (g/plot)	80	170	153.5	105	70	152.5	130	5	nd
Grain yield (kg/ha)	2285.02	2941.76	2765.05	2116.98	3506.4	1979.39	2416.5	2360.34	181.63

[1] Candidate gene allele from Moroberekan: (+); candidate gene allele from Vandana: (-).

Table 2. Molecular markers associated with resistance to blast

Marker	Marker type	Seedling blast (Scale 0-9)	Diseased leaf area (% DLA)	Lesion number
AR[b]	Gene-based	4.89*	ns	ns
CHI[b]	Gene-based	4.54**	3.84*	7.19**
POX[b]	Gene-based	11.48**	5.06*	ns
OsOXO1[b] RM168[c]	Gene-based SSR	11.20*** 34.75****	16.10**** 10.18**	11.08*** 10.61**
RM25[c]	SSR	10.08**	6.38*	ns
RM331[c]	SSR	3.73*	ns	ns
RM5720[c]	SSR	10.91**	ns	ns
RM473D	SSR	20.69****	10.18**	8.47**
RM178	SSR	7.43**	7.63**	36.71****
RM452	SSR	ns	ns	20.74****
RM421	SSR	28.83****	6.70*	6.17*
RM2	SSR	25.07****	5.44*	5.31*

[a]Value is the percent phenotypic contribution of a specific allele to diseased leaf area variation in RI lines at a given location. Level of significance: *, **, ***and **** indicate *P* <0.05, 0.01, 0.001 and 0.0001, respectively; ns = no significant association at *P* < 0.05. [b]Gene based markers for AR – aldose reductase; CHI – chitinase; POX – peroxidase; OsOXO1 – oxalate oxidase. [c]These markers co-localize with candidate genes: RM168 – OXO; RM25 and RM331 – OXLP and RM5720 – peroxidase.

SSR markers co-localizing with CGs were used to determine CG allele contribution of Way Rarem and Oryzica Llanos 5 to Way Rarem/Oryzica Llanos 5 advanced backcross lines. CG-based markers were assayed using agarose-TILLING method. Two lines were selected for good agronomic trait and resistance to blast in Indonesia – BC_2F_5-168 and BC_2F_5-49. For more details about this population, please see poster 2.18.

Comparing candidate gene orthologs in rice and maize

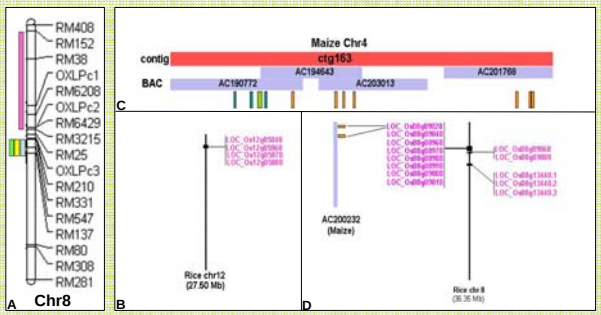

Figure 4. Organization of oxalate oxidase-like proteins in rice and maize. A)A dQTL for resistance to rice blast have been mapped in rice chromosome 8 and candidate gene mapping mapped an oxalate oxidase-like protein (OXLP) in this region. Twelve OXLPs were identified in the physical map of chromosome 8. In addition, 4 OXLPs are located in chromosome 12 (B). BLAST search for maize BAC clones using rice OXLPs as queries identified five BAC clones with OXLP sequences. B) Four BAC clones lie in tandem in ctg163 of maize chromosome 4, which is syntenic to rice chromosome 8. The box ▯ represents full-length OXLP sequences (~217 to 229 amino acids) while ▯ represents truncated OXLPs (~157 to 167 aa). These sequences were highly similar to LOC_Os12g05860 and LOC_08g08990. ▯ is an OXLP with a retrotransposon insertion. D) In rice, there are 12 OXLPs in chromosome 8 that span ~2.7 mb. An unmapped maize BAC clone has two OXLP proteins that are highly similar to LOC_Os08g09020.

Impact

- Using the candidate gene approach, defense response genes from Moroberekan were introgressed into Vandana, a drought tolerant variety. We have identified advanced backcross lines that performed better than Vandana in blast hotspots in India and the Philippines. These lines showed better yield under blast condition than Vandana and Moroberekan. These lines also performed well under reproductive drought stress with harvest index, grain yield, and panicle weight better than Vandana. Promising lines were identified that contain dQTLs from Moroberekan, are resistant to blast and show agronomic similarity to Vandana, Two lines, **IR78221-19-6-33** and **IR78222-20-7-148-2B**, are already selected for agronomic and yield testing by plant breeders in station trials in India.
- PCR markers have been developed for candidate defense response genes as well as SSRs co-localizing with these CGs have been identified and are available. These markers were used to identify CGs in an advanced backcross population of Way Rarem/Oryzica Llanos 5. Using the same approach, BC_2F_5-168 and BC_2F_5-49 were identified to have good agronomic trait and blast resistance.
- To extend the use of these candidate genes in other cereals, we identified orthologs of these genes in maize which may also be candidate genes for fungal resistance in maize as well sources of gene-based markers for breeding for resistance in maize.

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