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Resistance gene deployment strategies in cereal hybrids using marker-assisted selection: Gene pyramiding, three-way hybrids, and synthetic parent populations

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Summary

Marker-assisted selection (MAS) for resistance genes (R-genes), identified using molecular markers and quantitative trait loci (QTL) analysis, is now possible in many crops. MAS can be used to pyramid several R-genes into a single host genotype. However, this may not provide durable genetic resistance because the pathogen is exposed to a full homozygous pyramid during hybrid seed production and to a full heterozygous pyramid in the resultant hybrid. Alternative gene deployment strategies that generate genetic variability were analysed, for hybrid cereal cultivars of pearl millet, maize, sorghum and rice, using maintainer lines (B-lines) with two smaller complementary pyramids. An F_1 seed parent, produced on two such B-lines, can be used to produce a three-way hybrid. All target loci are heterozygous for resistance alleles in the F_1 seed parent, and the pathogen is exposed in the hybrid to a host population that is heterogeneous and heterozygous for alleles at the resistance loci targeted by MAS. Alternatively, single-cross hybrids can be made on seed parents that are maintained by two B-lines that differ for the complementary resistance gene pyramids. In a cross-pollinated crop, the B-lines are allowed to intermate to produce a synthetic B-line. In an inbreeding crop, the B-lines are equivalent to a two-component multiline variety. In inbreeding crops, because there is no intermating between the B-line components, the resultant synthetic seed parents have a higher frequency of genotypes with resistance alleles (R-alleles) at several resistance loci. However, in both cross-pollinated and inbreeding crops the genotypic structure in the hybrids is almost the same. All alternatives to a single-cross hybrid having a full pyramid produce hybrid cultivars having lower frequencies of resistance alleles. The frequency of genotypes having R-alleles at several loci increases greatly in both seed parent and hybrid when the overall frequency of R-alleles in the maintainer lines increases. This is simply done by adding a maintainer line that has a full pyramid or by the component lines having overlapping pyramids.

Introduction

Hybrid cereal cultivars often perform better, because of heterosis, than non-hybrid cultivars of comparable phenology. In farmers' fields, hybrids are also attractively uniform but this increases the vulnerability of popular cultivars to pests and diseases – strains able to overcome the resistance presented by a single host genotype are strongly favoured. This can result in widespread epidemics such as those of yellow corn leaf blight, caused by *Mycosphaerella zeae-maydis* Mukunya and Boothroyd, and Southern corn leaf blight, caused by race T of *Cochliobolus heterostrophus* Drechsler on maize (*Zea mays* L.), in the U.S.A. in 1969–1971 (Tatum, 1971; Ullstrup, 1972; Pring & Lonsdale, 1989). In pearl millet, *Pennisetum glaucum* (L.) R. Br., single-cross hybrids were plagued by epidemics of downy mildew in India during the

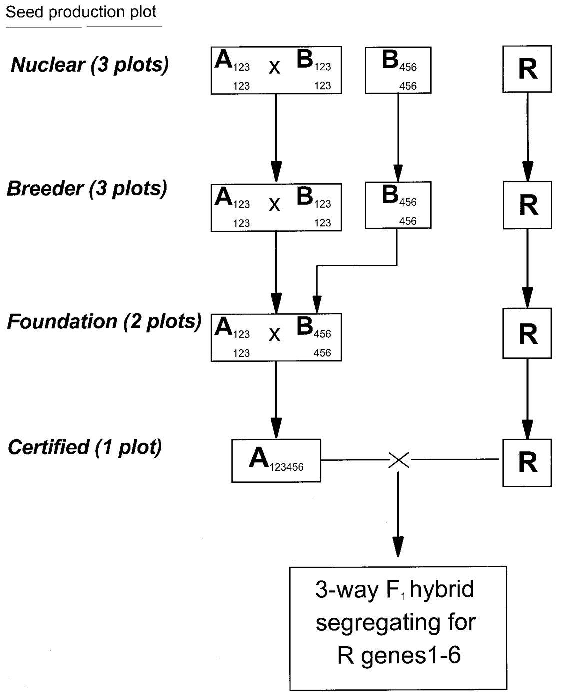

Figure 1. Production of a three-way hybrid using an A-line, and R-line and two nuclear inbred maintainer lines (B-lines) each having a pyramid at three of six resistance loci numbered 1 to 6. Homozygotes are indicated as e.g., 11, and heterozygotes as e.g., 1. From the foundation seed stage onwards seed production procedures exactly match those for a conventional single-cross hybrid. The full resistance gene pyramid, in a heterozygous condition, is present in the F_1 male-sterile line in the certified seed production plot.

1970s and 1980s (Dave, 1987) caused by *Sclerospora graminicola* (Sacc.) J. Schröt.

Recent advances (Paterson et al., 1991; Gale & Witcombe, 1992) have made possible the routine use of marker-assisted selection (MAS) to produce near-isogenic lines (NILs) and gene pyramids for disease and pest resistance. For example, in pearl millet, Jones et al. (1995) have identified a number of quantitative trait loci (QTL) that confer strain-specific, host-plant resistance to downy mildew. Similar results for pests and pathogens in other crops include rice blast disease caused by *Magnaporthe grisea* (Herbert) Barr (Yu et al., 1991). We consider the application of MAS to produce stable resistance against biotic stresses in hybrids of the cereals pearl millet, maize, sorghum (*Sorghum bicolor* Moench) and rice (*Oryza sativa* L.).

MAS-based resistance gene (R-gene) deployment in alternatives to conventional hybrids and to single-cross hybrids on pure-line seed parents with single pyramids are first considered. Alternatives concentrated on the more complex case of seed parent, rather than restorer line, breeding. The genetic variation produced, and the difficulties of seed production procedures, were analysed for three types of seed parents and hybrids. In all cases, two near-isogenic maintainer lines (B-lines) having complementary partial pyramids for resistance genes were used for:

- three-way hybrids made on F_1 seed parents (Figure 1)
- single-cross hybrids in cross-pollinated crops produced on synthetic seed parents (defined in theory and methods)
- single-cross hybrids in inbreeding crops made on synthetic seed parents

Because R-gene frequencies are lower when complementary partial pyramids are used than for a single pyramid, alternatives that use three component B-lines and provide higher frequencies whilst maintaining genetic variability were also analysed.

Theory and methods

Of the strategies considered, three require explanation of theory and methods:

1. The genotypic structure of seed parents produced on a mixture of two near-isogenic B-lines (NIB-lines) having partial complementary pyramids that either predominantly outcross (maize and pearl millet) or predominantly self (rice and sorghum). Because the seed parents so produced are heterogeneous and heterozygous for the R-genes they are termed synthetic seed parents following the terminology of synthetic populations produced by intermating inbred lines.
2. The genotypic structure of synthetic seed parents produced on a mixture of three NIB-lines: two NIB-lines having partial complementary pyramids and a NIB-line having a full pyramid.
3. The genotypic structure of hybrids produced on synthetic and F_1 seed parents.

Modelling genotypic frequencies in synthetic seed parents where there is complete outcrossing of the NIB-lines

Genotypic frequencies were modelled in synthetic seed parents maintained by two NIB-lines, that intercross at random, differing for complementary partial pyramids where all loci independently segregate (e.g., for four unlinked loci, $R_1R_1R_2R_2r_3r_3r_4r_4$ and $r_1r_1r_2r_2R_3R_3R_4R_4$). These two NIB-lines are the nuclear maintainer lines that, upon intermating, produce

Table 1. Deriving genotypic frequencies for many unlinked loci for populations in Hardy-Weinberg equilibrium

Number of loci	Genotypic frequencies by expansion of	Explanation of terms
1	$(p+q)^2$	p = frequency of R_1 q = frequency of r_1
2	$(p+q)^2\ (r+s)^2$	r = frequency of R_2 s = frequency of r_2
3	$[(p+q)^2\ (r+s)^2](t+u)^2$	t = frequency of R_3 u = frequency of r_3
4	$\{[(p+q)^2\ (r+s)^2](t+u)^2\}(v+w)^2$	v = frequency of R_4 w = frequency of r_4

Table 2. Expected genotypic ratios, at Hardy-Weinberg equilibrium, for unlinked loci that are heterozygous or homozygous for R-alleles for 0 to 8 resistance loci

Number of of loci = j	Smallest perfect population	Number of loci with at least one resistance allele = i								
		0	1	2	3	4	5	6	7	8
		Genetic ratios								
0	1	1								
1	4	1	3							
2	16	1	6	9						
3	64	1	9	27	27					
4	256	1	12	54	108	81				
5	1024	1	15	90	270	405	243			
6	4096	1	18	135	540	1215	1458	729		
7	16384	1	21	189	945	2835	5103	5103	2187	
8	65536	1	24	252	1512	5670	13608	20412	17496	6561

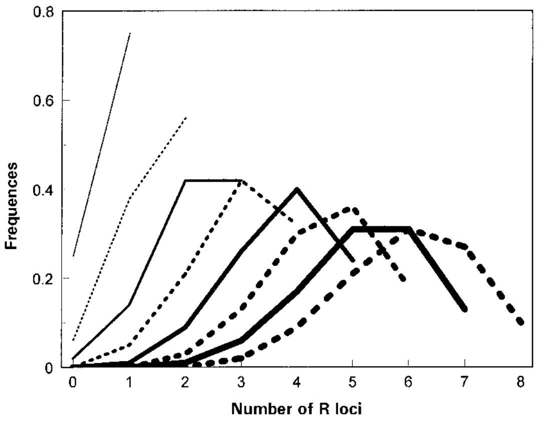

Figure 2. Expected frequency of genotypes, in a seed parent maintained by B-lines that random mate, according to the number of loci that have at least one resistance allele. One to eight resistance loci and equal allelic frequencies at each locus are considered. Solid lines odd number of loci, dotted lines even number of loci, with line thickness increasing with number of resistance loci.

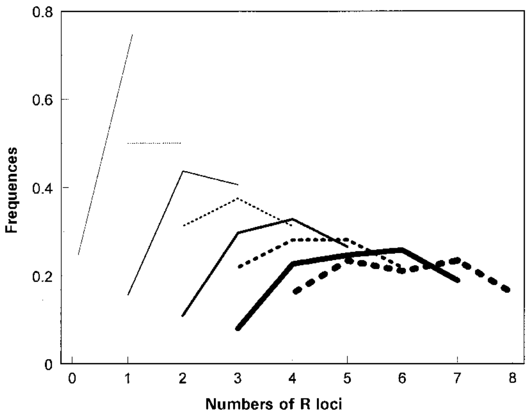

Figure 3. Expected frequency of genotypes, in a seed parent maintained by B-lines that only self pollinate, according to the number of loci that have at least one resistance allele. One to eight resistance loci and equal allelic frequencies at each locus are considered. Solid lines odd number of loci, dotted lines even number of loci, with line thickness increasing with number of resistance loci.

the synthetic maintainer population (B-population). Complete random mating amongst the B-lines is assumed, resulting in a Hardy-Weinberg equilibrium. The synthetic seed parent has to outcross to the maintainer population every generation and after several generations of outcrossing to the B-population it is assumed that the synthetic seed parent is in equilibrium with its maintainer.

Analyses were done on a spreadsheet to determine genotypic frequencies in synthetic seed parents at equilibrium. Diallels of male and female gametes in Hardy-Weinberg equilibrium are modelled for differing numbers of loci (Table 1). The genotypes produced in the diallel are classified by the number of loci having at least one R-allele (i.e., a phenotypic classification assuming complete dominance of resistance at all loci and no epistasis. The diallels produce genetic ratios that are summarised, for equal gene frequencies at each locus, in Tables 2 to 4 and Figure 2.

It is not difficult to extrapolate beyond 8 loci. Let $j_{0\rightarrow n}$ = the number of segregating resistance loci, and $i_{0\rightarrow j}$ = the number of these loci with at least one R-allele: the smallest perfect populations are then 2^{2j}, which equal the sums of the genetic ratios for the cases $j_{1\rightarrow 8}$ in Table 2. These genetic ratios can be obtained by multiplying 3^i by the appropriate term from Pascal's trianggle (e.g. 1:2:1 for j = 2). The progressions become clearer when the spreadsheets are summarised, as in Table 3 for j = 5 and Table 4 for j = 6: the rows of genetic ratios in Table 2 correspond to the column totals in Tables 3 and 4. The analysis can also be extended beyond 8 loci by continuing the progressions that can be seen, e.g., those commencing with 2 are 2, 8, 12, 8, 2 (Table 3) and 2, 10, 20, 20, 10, 2 (Table 4), where 10, 20, 20, 10 are obtained by addition from the previous ratios. Also the progressions 32. . .1 (Table 3) and 64. . .1 (Table 4) occur twice in each Table, once on the top line and once on the bottom line (diagonal) and can easily be extended.

Table 3. Expected genotypic ratios in the seed parent in equilibriun with its maintainer, a synthetic population at Hardy-Weinberg equilibrium, for the number of loci that are heterozygous or homozygous for 5, 4, 3, 2, 1 or 0 R-alleles, in the case of 5 unlinked resistance loci

Genotypes by number of loci with >0 R-alleles	Number of times genetic ratios occur[a]						Total[c]
	1	5	10	10	5	1	
	Genetic ratios[b]						
5	32	16	8	4	2	1	243
4		16	16	12	8	5	405
3			8	12	12	10	270
2				4	8	10	90
1					2	5	15
0						1	1

[a] The ratios of 1:5:10:10:5:1 on the top row are those of gametes carrying 5, 4, 3, 2, 1, or 0 R-alleles. These gametes produce the genetic ratios shown in the column below when intermated at random with gametes in Hardy-Weinberg equilibrium.

[b] The genetic ratios are multiples of simpler genetic ratios found in Bonanati's triangle e.g. 2:8:12:8:2 is a 1:4:6:4:1 ratio.

[c] The total is derived from the products of the gametic ratios and the genotypic frequencies for that class, e.g., for 5 loci having resistant alleles it is (1 × 32) + (5 × 16) + . . . + (1 × 1).

Table 4. Expected genotypic ratios in the seed parent in equilibriun with its maintainer, a synthetic population at Hardy-Weinberg equilibrium, for the number of loci that are heterozygous or homozygous for 6, 5, 4, 3, 2, 1 or 0 R-alleles, in the case of 6 unlinked resistance loci

Genotypes by number of loci with >0 R-alleles	Number of times genetic ratios occur							Total
	1	6	15	20	15	6	1	
	Genetic ratios							
6	64	32	16	8	4	2	1	729
5		32	32	24	16	10	6	1458
4			16	24	24	20	15	1215
3				8	16	20	20	540
2					4	10	15	135
1						2	6	18
0							1	1

Modelling genotypic frequencies in synthetic seed parents where there is complete selfing in the NIB-lines

In a self-pollinated crop, such as rice, no intermating will occur between the two NIB-lines so the maintainer is effectively a multiline. However, outcrossing by the seed parent (A-line) creates a synthetic seed-parent population. The two NIB-lines, with complementary partial pyramids, that maintain the seed parent, produce unrecombined gametes carrying one of the partial pyramids, (e.g., gametes $R_1R_2r_3r_4$ and $r_1r_2R_3R_4$ in the case of four loci). In contrast, because the A-line has to outbreed each generation, female gametes are produced after recombination. Further, as a result of outcrossing each generation with male gametes that are not in Hardy-Weinberg equilibrium, the female gametes are also not in equilibrium.

The number of generations for the A-line to reach a genotypic equilibrium was determined for 2 unlinked loci. In each generation, the male gametes are always R_1r_2 and r_1R_2 (i.e., R_1R_2 and r_1r_2 do not occur). In the founder (0) generation the seed parent was assumed to have allele frequencies equal to that of the maintainer

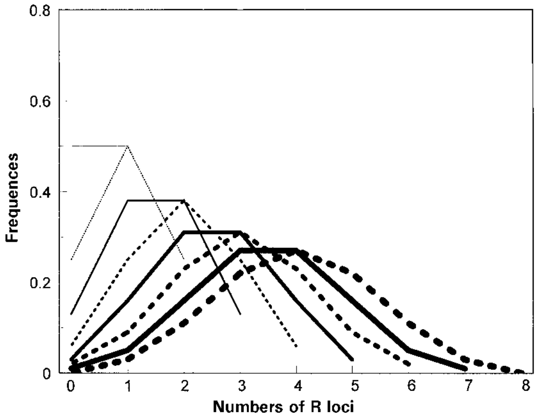

Figure 4. Expected frequency of genotypes in a hybrid according to the number of loci that have at least one resistance allele. The hybrid is produced on a seed parent maintained by random mating B-lines where, at each locus, there are equal allelic frequencies. One to eight resistance loci are considered. Solid lines odd number of loci, dotted lines even number of loci, with line thickness increasing with number of resistance loci.

line, as is the case with any A/B pair after repeated backcrossing, and the female gametes were initially assumed to be in Hardy-Weinberg equilibrium (i.e., equal frequencies of R_1R_2, R_1Rr_2, r_1R_2, and r_1r_2). The genotypic ratios resulting from these female gametes mating with unrecombined male gametes from the selfed NIB-lines were determined in the succeeding generations in a spreadsheet analysis. The equilibrium frequencies did not differ greatly from those obtained after only the first generation of crossing to unrecombined male gametes. For example, the frequency of the R_1R_2 genotypes is 0.25 in Hardy-Weinberg equilibrium (generation 0), 0.19 (generation 1) and 0.17 at equilibrium. Hence, all subsequent modelling for varying numbers of loci was simplified by analysing only generation 1 (Table 5 and Figure 3). To go beyond this generation is unnecessarily precise. The effects of non-random mating in commercial seed production plots can produce much larger errors than this simplification.

It is assumed that for even numbers of loci the loci having R-alleles are equally distributed between the two NIB-lines, and for odd numbers their distribution differs only by one. This assumption is only important when B-lines self. Inequality of distribution of R-alleles between the NIB-lines has no effect in the case considered above for cross-pollinated crops.

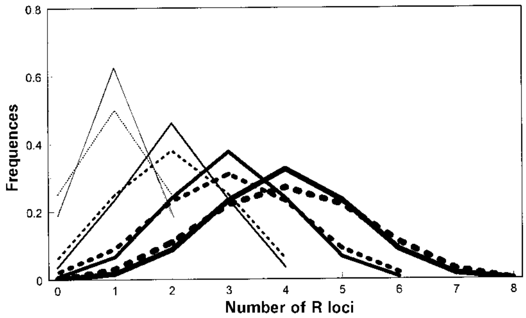

Figure 5. Expected frequency of genotypes in a hybrid according to the number of loci that have at least one resistance allele. Hybrids made on a seed parent maintained by B-lines that self (solid lines) and hybrids made on a seed parent maintained by B-lines that random mate (dotted lines). Cases of 2, 4, 6, and 8 resistance loci considered. Line thickness increases with number of resistance loci.

Prediction of genotypic frequencies in hybrids

In cross-pollinated crops, the genotypic frequencies in the hybrid are entirely dependent on the gene frequencies in the A-line because gametes are produced in Hardy-Weinberg equilibrium. It is assumed that the male parent (pollinator or restorer line) of the hybrid carries no R-alleles, so the genotypic ratios in the hybrid equal those of the gametes of the seed parent. The gametic ratios form a simple progression and are shown for 5 (Table 3) and 6 (Table 4) unlinked loci. Results of the analysis are presented in Figure 4. The genetic structure of synthetic seed parents on B-lines that self is very different to that where they cross pollinate. The hybrid structure was determined for inbreeding crops by deriving the gametic ratios produced by the seed parent generation 1. The results are presented in Figure 5.

Increasing the frequency of R-alleles by including maintainer lines with full pyramids

A third NIB-line having a complete pyramid can be added to the two NIB-lines having complementary partial pyramids. A B-line synthetic population can, for example, be made up of the following: 20% $R_1R_1R_2R_2$, 40% $R_1R_1r_2r_2$ and 40% $r_1r_1R_2R_2$. At equilibrium, this will result in a frequency of R-alleles at both loci of 0.6. This was modelled, using the structure outlined in Tables 1 to 3, for three gene frequencies (0.5, 0.6 and 0.7) where the NIB-lines random mate to produce a synthetic B-population. The gametic frequencies from the seed parent were used to determine the genetic structure of resultant hybrids when the restorer lines have no R-alleles. The res-

Table 5. Expected genotypic ratios at generation 1 in the synthetic seed parent, when maintained by self-pollinated maintainer lines, for loci that are heterozygous or homozygous for R-alleles for 1 to 8 unlinked resistance loci

Genotypes by number of loci with >0 R-alleles	Genetic ratios Number of loci with resistance alleles 0	 1	 2	 3	 4	 5	 6	 7	 8	Smallest perfect population size
1	1	3								4
2		1	1							2
3		5	14	13						32
4			5	6	5					16
5			7	19	21	17				64
6				7	9	9	7			32
7				41	116	126	132	97		512
8					41	60	54	60	41	256

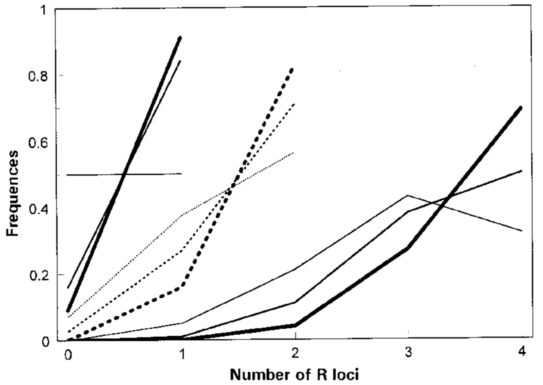

Figure 6. Expected frequency of genotypes, in a seed parent maintained by B-lines that random mate, according to the number of loci that have at least one resistance allele. 1, 2, or 4 resistance loci considered. The frequency of the resistance allele at each locus varies (0.5, 0.6 or 0.7). Cases of 1 and 4 resistance loci in solid lines, 2 in dotted. Line thickness increases with higher frequencies of resistance alleles.

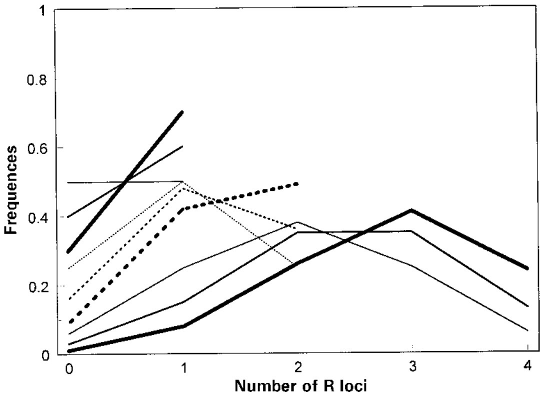

Figure 7. Expected frequency of genotypes in a hybrid according to the number of loci that have at least one resistance allele. Hybrid produced on a seed parent where, at each locus, the frequency of the resistance allele is 0.5, 0.6 or 0.7. 1, 2, or 4 resistance loci are considered. Cases of 1 and 4 resistance loci in solid lines, 2 in dotted. Line thickness increases with higher frequencies of resistance alleles.

ults of this analysis are presented for the seed parent (Figure 6) and hybrid (Figure 7).

Results and discussion

Alternative strategies to conventional single-cross hybrids

More variable hybrids can be used to avoid the rapid break down of disease resistance in genetically uniform single-cross hybrids. However, in maize the trend has been away from more complex hybrids that provide more variability (e.g., double-cross hybrids produced on four parental lines) towards single-cross hybrids. In rice, hybrid cultivars have, so far, been restricted to single-cross hybrids produced on inbred parental lines. In pearl millet topcross hybrids have recently been introduced as an alternative to single-cross hybrids. Topcross hybrids are produced on a genetically uniform male-sterile seed parent and a genetically heterogeneous and heterozygous pollen parent population (Witcombe et al., 1996; Talukdar et al., 1996, 1998). This combines most of the attributes of single-cross hybrids with genetically heterogeneous resistance that is expected to be durable. The topcross pollinator can be partially inbred and this enables the use of MAS methods. Inbred lines derived from the topcross pollinator can be improved by hybridisation to resistance sources and subsequent MAS. The inbred MAS products can then be introgressed into the topcross pollinator. Similarly, genetically heterogeneous, three-way hybrids produced on a male-sterile F_1 seed parent have recently been released to control rust in pearl millet (Hanna et al., 1997).

Seedparent or restorer line breeding

Should efforts be concentrated on deployment of R-genes in seed parents or restorer lines? Although a MAS programme is technically easier in a restorer line, than in a seed parent that consists of an A/B pair, economic considerations can make MAS more attractive in seed parents. Restorer lines are often used to produce only a single hybrid, whereas male-sterile lines, because they are more difficult to breed, tend to be used to produce many hybrids over an extended period. For example, in pearl millet MAS for resistance to a range of diverse *S. graminicola* populations is being carried out by the authors in 843B, the maintainer line of 843A that is widely used for both private and public sector hybrid production in India (Hash et al., 1997). Various putative downy mildew R-genes have been incorporated, individually, and in combination.

Single-cross hybrids based on one inbred seed parent

Producing single-cross hybrids with a single inbred B-line is the simplest way of deploying several resistance genes but all of the R-genes have to be employed in a single pyramid (Table 6). This carries the risk involved with exposing the pathogen to the complete pyramid of R-genes in both the hybrid seed production plots and in farmers' fields.

Although gene deployment is straightforward, the plant breeding procedures are more complex than those that employ smaller pyramids. Often, small pyramids can be obtained by selfing a single plant that is heterozygous at the desired loci and homozygous for the recurrent parent alleles elsewhere. Finding a single plant of the required heterozygous genotype for all of the loci in the pyramid is less likely, so further MAS is required to obtain the full pyramid by combining R-alleles found in two or more NILs.

The durability of a full pyramid of R-genes cannot be predicted. If it is sufficiently large for the pathogen populations to lack any genotype with all the corresponding virulence alleles, and sexual or parasexual recombination between different strains of the pathogen do not take place, then the pathogen should never evolve a virulent genotype. However, if all of the deployed genes are overcome at once, yet more resistance loci will have to be found and deployed. The risk depends on the genetic variability present in the pest or pathogen. Rarely is sufficient information available, although for some pests and diseases much is known (e.g., gall midge (Mohan et al., 1997) and blast (Ziegler et al., 1997) in rice). For other diseases, such as downy mildew in pearl millet, the obligate nature of the parasite that makes maintenance of individual isolates exceedingly difficult, and its rapid evolution due to allogamy, render such studies impracticable. Only QTL analysis has permitted identification of R-genes effective against different pathogen populations (Jones et al., 1995).

Because of the risk of simultaneous loss of resistance alleles at several loci, other methods that may lead to more durability are considered.

Hybrids produced from synthetic seed parents where the nuclear lines have no gene pyramiding

Synthetics could be made from nuclear lines each having resistance at a single locus (Table 6). For n-line synthetic seed parents produced on random mating B-line components, the frequency of individual resistance alleles present in the seed parent synthetic is inversely proportional to n. The frequency of plants having all loci without a resistance allele is high in the seed parent, and unacceptably high in its hybrid (Table 6). For example, with three nuclear inbreds the frequency of any resistance allele in the seed parent is 0.33, and in its hybrid is only 0.17. There is also a high proportion of plants that have all loci without R-alleles.

Three-way hybrids produced on an F_1 male-sterile line

Three-way hybrids (i.e., hybrids produced between an F_1 male-sterile line and an inbred restorer line), can be employed in cereal crops including pearl millet, maize, sorghum and rice. In pearl millet, Wilson et al. (1993) and Hanna et al. (1997) employed F_1 seed parents produced on two contrasting maintainer lines (B-lines) and one male-sterile line (A-line). Only in maize can the use of cytoplasmic male-sterility for the

Table 6. Some options for deployment of resistance alleles in hybrid seed production

Option	Seed parent	Hybrids	Comments
1. Single-cross hybrid produced on one inbred seed parent	Full pyramid. Homozygous. Uniform. Each R-allele frequency = 1.0	Full pyramid. Heterozygous. Uniform. Each R-allele frequency = 0.5	Full pyramid required. Exposes pathogen to full uniform pyramid at hybrid seed production stage and in the hybrid itself. Seed multiplication scheme equals that for single-cross hybrid. Minimum opportunity for segregation distortion or random drift. Frequency of pyramid in hybrid = 1.0.
2. Single-cross hybrid produced on Syn2 synthetic seed parent derived from n nuclear inbreds homozygous for resistance alleles at different single loci, and selfing occurs in the Syn0 of the seed parent maintainer	Full and partial pyramids. Homozygous and heterozygous. Heterogeneous. Each R-allele frequency = 1/n	Full and partial pyramids. Heterozygous. Heterogeneous. Each R-allele frequency = 1/(2n)	No pyramids required. Never exposes pathogen to full uniform pyramid. Seed multiplication scheme less complex than in option 3 above. Random drift and segregation distortion occur. Frequency of pyramids in hybrid depends on number of loci and is lower than in all above options.
3. Three-way hybrid produced on hybrid seed parent	Full pyramid. Heterozygous. Uniform. Each R-allele frequency = 0.5	Full pyramid. Heterozygous. Heterogeneous Each R-allele frequency = 0.25	Two smaller pyramids required. Exposes pathogen to full uniform pyramid only in the hybrid seed production plot. Simple seed multiplication scheme. Limited opportunity for segregation distortion or random drift. Frequency of pyramids high in hybrid.
4. Single-cross hybrid produced on synthetic Syn2 seed parent derived from two nuclear inbreds when no selfing is assumed in the Syn0 of the seed parent maintainer	Full and partial pyramids. Heterozygous. Heterogeneous. Each R-allele frequency = 0.5	Segregating. Heterozygous. Heterogeneous. Each R-allele frequency = 0.25	Two smaller pyramids required. Exposes pathogen to full uniform pyramid only in Syn1 seed parent. Seed multiplication scheme more complex. Random drift and segregation distortion occur. Frequency of pyramids in hybrid depends on number of loci, but is lower than in options 1 or 2 above.

production of F_1 seed be avoided by detasseling the female parent.

In a conventional three-way hybrid, the genetic differences between the two B-lines results in segregation in the commercial hybrid and a reduction in uniformity for agronomically important traits (e.g., flowering time and plant height). However, for R-gene deployment, NIB-lines differ only at resistance loci so segregation in the commercial hybrid is theoretically limited to disease resistance (Figure 1, Table 6). The two B-lines have complementary, partial pyramids of R-alleles at three loci, resulting in an F_1 seed parent having resistance alleles at all six loci (Figure 1). The hybrid will segregate for resistance at all these loci, giving a predictable degree of R-gene heterogeneity.

For producers of foundation stage parental seed, and certified hybrid seed, the three-way hybrid seed production does not differ at all from that used to produce a single-cross hybrid (Figure 1). For the plant breeder, nucleus seed and breeder seed production is more complex than for single-cross hybrid seed production.

The greatest disadvantage of this method is the heterozygous pyramid of all of the R-genes at the hybrid seed multiplication stage. However, the area devoted to hybrid seed multiplication is far less than that for hybrid cultivation. Because this seed production crop has a higher value, it is more economic to protect this R-gene pyramid with intensive disease control measures. If the pest or pathogen overcomes the full pyramid in the seed production plots, its spread to areas of hybrid cultivation will be limited if, as is typical, seed production is in a different season and location than the main crop.

Producing hybrids on synthetic seed parents

Rather than a single pyramid of R-loci, a number of NIB-lines – produced by MAS – can be used that differ only for their R-genes. Two nuclear NIB-lines with complementary partial pyramids are considered that allow alleles at all unlinked resistance loci to segregate with equal allelic frequencies. If the smallest possible pyramids are used then, when the resistance loci number is even, each nuclear inbred has a half pyramid while for odd numbers the size of the pyramids differ by a single locus. However, when maintainer lines random mate the same result will be obtained in the seed parent regardless of the distribution of the resistance alleles between the two nuclear inbreds (e.g., a nuclear inbred with one locus homozygous for resistance and the other with seven produces the same result in the seed parent as when both have four).

The genotypic structure of the male-sterile line maintained by the B-lines will differ according to the degree of self pollination in the B-lines. The two most extreme cases are considered, complete random mating among the B-lines (closest to pearl millet and maize) to form a synthetic B-line population, and complete self pollination in the B-lines (closest to rice and sorghum) that will comprise a multiline. The genotypic frequency of the maintainer line gametes will depend on the degree of selfing.

Expected genotypic frequencies in the seed parent when the B-lines random mate

The expected genotypic distributions are shown for 1 to 8 resistance loci in Figure 2. For example, for one R-gene locus a simple Mendelian ratio of 1:3 is obtained with one plant having no resistance allele to every three that have one. Mendelian inheritance for two independently segregating R-gene loci produces 9/16 of plants with at least one resistant allele across the two loci, 6/16 with at least one resistance allele at one locus, and 1/16 with no resistance alleles. In general terms, once more than a single locus is considered, the frequency of genotypes having no resistant alleles is very low and effectively equals zero once there are more than three resistance loci. The frequency of genotypes having the full R-gene pyramid progressively declines as the number of R-gene loci increases. Despite this, when the number of loci are high there is a greater proportion of the plants with large pyramids (e.g., in the case of eight loci, a high proportion of the plants have pyramids of resistance alleles at five to seven loci). Overall, there is a satisfactory frequency of resistance pyramids even when there is a small number of resistance loci.

Resistance gene frequencies could change unexpectedly during the generations needed to produce a synthetic seed parent because of genetic drift or distorted segregation patterns among male gametes (Busso et al., 1995). However, if the population sizes of the early synthetic generations are reasonably large these effects will be unimportant.

Expected genotypic frequencies in the seed parent when the B-lines self

In autogamous crops, multiline varieties – mixtures of near isogenic lines (NILs) differing for disease resistance genes (R-genes) – were successfully used to control disease (Browning & Frey, 1969). However, without marker-assisted selection (MAS), NILs and R-gene pyramids were expensive to produce. lt is now economically practicable to use multiline inbred maintainer and restorer lines in rice using MAS. lt can be used, for example, for bacterial blight disease where QTLs of large effect have been identified (Yoshimura et al, 1995).

Although multiline maintainer lines are as simple to maintain as a multiline self-pollinated cultivar, the male-sterile lines produced on them are genetically more complex, because they have to outcross each generation to the maintainer line. The absence of genotypes in the synthetic seed parent with no resistance alleles, and the genotypic variability (Figure 3) means that a synthetic seed parent produced on multiline maintainer lines is an attractive option in rice for obtaining durable disease resistance in hybrid seed production plots.

In rice selfing would be almost 100%, whereas in other cereals, such as maize, self pollination would be much lower. Selfing reduces opportunities for recombination so extreme recombinants are less frequent and intermediate genotypes (e.g., those of the parents) are more common. This effect can be seen by comparing Figure 2 (random mating in the B-lines) with Figure 3 (only selfing in the B-lines). Synthetic maintainer lines can be produced with intermediate levels of self pollination, producing genotype frequencies between those in Figure 2 and Figure 3.

Selfing in the maintainer lines in allogamous crops will move the seed parent synthetic population closer to the genetic structure of the seed parent found in rice. However, in an allogamous crop, pure multiline maintainer lines would be too expensive to maintain beyond the nuclear seed production stage because of the need to prevent intercrossing between the component lines during the seed multiplication generations.

Increasing the frequency of R-alleles: The use of overlapping pyramids

When only two nuclear inbreds are used with complementary partial pyramids then the seed parent will have equal allele frequencies at any locus. However, if the number of nuclear lines is increased it is possible to produce any frequency of R-alleles in the seed parent. We have considered the addition of a nuclear inbred with a complete pyramid (see Theory and methods). An increased frequency of R-alleles can also be obtained with overlapping but incomplete pyramids. For example, an equal mixture of 4 B-lines:

$R_1R_1 \quad r_2r_2 \quad R_3R_3 \quad R_4R_4$
$R_1R_1 \quad R_2R_2 \quad r_3r_3 \quad R_4R_4$
$R_1R_1 \quad R_2R_2 \quad R_3R_3 \quad r_4r_4$
$r_1r_1 \quad R_2R_2 \quad R_3R_3 \quad R_4R_4$

will result in an A-line with alleles at a frequency of 0.75. If the B-lines always self-pollinate, all B-line gametes will have three R-alleles.

A relatively small increase in R-allele frequency causes a large reduction in the proportion of genotypes with a small number of loci with R-alleles (Figure 6).

Genotypic structure of hybrids

In cross-pollinated crops, the genotypic structure of hybrids produced on a synthetic seed parent is equivalent to the genotype distribution of the gametes of the seed parent, assuming the restorer line lacks R-alleles at the target loci and so contributes nothing to the genetic variance. The proportion of plants in the hybrid having no loci with R-alleles is higher (Figure 4) than in the seed parent. However, with three R-genes this is only a little more than 10% of the population, and with four R-genes a little more than 5%. In practice, the pollen (restorer) parent is likely to have R-alleles at some of the loci targeted in the MAS programme, as well as at other loci, and the seed parent will be homozygous for R-alleles at non-targeted loci. Hence the absence of R-alleles at the targeted loci in a small proportion of the hybrids is not a serious disadvantage.

Synthetic seed parents in inbreeding crops have a higher frequency of genotypes with R-alleles at several loci than in cross-pollinated crops (compare Figure 2 and Figure 3) as a result of being maintained by the unrecombined gametes of the B-lines. However, there is little difference in the hybrids produced on synthetic seed parents where the B-lines either random mate or self (Figure 5).

Adding a NIL with a full pyramid to the B-line components had a large effect on the seed parent genotypic frequencies (Figure 6) and this is reflected in the hybrids produced on them (Figure 7).

Comparison of seed production for three-way hybrids and hybrids on synthetic seed parents

Seed multiplication procedures for hybrids produced on synthetic seed parents may be more difficult for the breeder than the three-way hybrid system that only uses inbred lines. This is because:

- A-line maintenance is slightly more complex as the A-line has to be maintained using a synthetic B-line,
- the A-line breeder seed distributed for foundation seed production should be an advanced synthetic generation (to bring R-alleles up to the frequency expected of repeated backcrossing to the B-line), and
- an early synthetic generation of the B-line needs to be distributed as breeder seed to minimise the effects of random drift and segregation.

The demands on resources are hardly greater than in the conventional system, but synthetic seed parents require different procedures than those normally used. However, as in the case of the three-way hybrid produced on an F_1 male-sterile line, the seed producer is supplied with breeder seed of the equivalents of an A-line, a B-line, and a pollinator line and follows exactly the same procedures as in single-cross hybrid seed production.

Conclusions

Because of the risk of losing many useful R-genes at the same time if a full pyramid is employed, alternative methods of R-gene deployment should be considered.

Of the alternatives, three-way hybrids offer the most predictable degree of genetic heterogeneity for resistance alleles in the F_1 hybrid cultivar grown by farmers. It also has the attraction that its seed multiplication process is very straightforward from the viewpoints of both the breeder and seed producer. The seed multiplication chain for such three-way hybrids is simple, requiring one A-line, two B-lines each containing a different set of pyramided R-genes (only one of these B-lines must be multiplied in breeder seed quantities), and the pollen parent. In the case of maize, two inbred parents of the seed parent F_1 – each with a different set of R-genes – and a pollen parent are all that is required. One disadvantage is that the maximum pyramiding of R-alleles occurs in the vulnerable hybrid seed multiplication stage, but chemical protection of the uniform pyramid at this stage would be much more economical and environmentally friendly than that of a similar pyramid in a single-cross hybrid cultivar sown on a much larger area.

Stability and durability of disease resistance in hybrids produced on synthetic seed parents may be greater than that of hybrids produced on seed parents in which all of the component resistance factors have been pyramided. Hybrids produced on synthetic seed parents should provide farmers with many of the benefits of the additional R-genes, with a reduced risk of reducing the useful life of those genes. Hybrids can be produced using a relatively simple seed production system, without ever exposing the pathogen to a uniform host having the full pyramid of R-genes. A satisfactorily high frequency of complex pyramids is obtained in the hybrids produced on synthetic seed parents. Synthetic parents can evolve in complexity as more near-isogenic B-lines become available from MAS programmes.

In predominantly self-pollinated crops synthetic parents are an attractive option. Their maintainer lines are equivalent to multilines. Seed parents maintained by them are genetically more complex, have a higher frequency of intermediate genotypes than a true synthetic, and no genotypes with no R-alleles.

When a line with a full pyramid is available then using it as an additional nuclear B-line inbred is desirable. Genetic variability is preserved in both the seed parent and the hybrid, but the frequency of the larger R-gene pyramids is increased.

The use of partial pyramids rather than full pyramids is easier and faster because R-genes can be deployed before a more complex pyramid of R-genes has been produced. Synthetic populations can evolve in complexity by adding more complex products of MAS over time. However, the initial synthetic seed parent should be sufficiently complex to provide adequate disease resistance. This proviso may be less important when MAS is used in maintenance breeding of an existing seed parent that already has some degree of resistance, particularly when the hybrid will inherit some resistance from its pollinator.

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