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Gene Pyramiding: An Overview

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Abstract

The development of molecular genetics and associated technology like marker assisted selection has led to the emergence of a new field in plant breeding-Gene pyramiding. Pyramiding entails stacking multiple genes leading to the simultaneous expression of more than one gene in a variety to develop durable resistance expression. Gene pyramiding is gaining considerable importance as it would improve the efficiency of plant breeding leading to the development of genetic stocks and precise development of broad spectrum resistance capabilities. The success of gene pyramiding depends upon several critical factors, including the number of genes to be transferred, the distance between the target genes and flanking markers, the number of genotype selected in each breeding generation, the nature of germplasm etc. Innovative tools such as DNA chips, micro arrays, SNPs are making rapid strides, aiming towards assessing the gene functions through genome wide experimental approaches. The power and efficiency of genotyping are expected to improve in the coming decades.

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Introduction

Watson and Singh (1953) first introduced the concept called gene pyramiding. Gene pyramiding is defined as a method aimed at assembling multiple desirable genes from multiple parents into a single genotype. The end product of a gene pyramiding program is a genotype with all of the target genes. Gene pyramiding is a breeding method aimed at assembling multiple genes with known effects on target traits. It is mainly used in improving existing elite cultivars for a few unsatisfactory traits, for which genes with large positive effects are identified.

Traditionally, the identification of the sources of useful gene is very slow and breeder's capability to trace the presence or absence of the target genes is limited. This limits the number of genes to be incorporated into elite cultivars at any times. The development of modern molecular and genomics technology has not only accelerated the discovery of favorable gene but also widened the sources of useful genes.

Objectives of gene pyramiding

1) Enhancing trait performance by combining two or more complementary genes,
2) Remedying deficits by introgressing genes from other sources,
3) Increasing the durability.

Types of gene pyramiding

1. Conventional technique: Serial gene pyramiding: Genes are deployed in same plant one after other (Fig. 1).
* Pedigree breeding
* Backcross breeding
* Recurrent selection

2. Molecular technique Simultaneous gene pyramiding:
Genes are deployed at a time in a single plant.

* Marker assisted selection
* Transgenic method

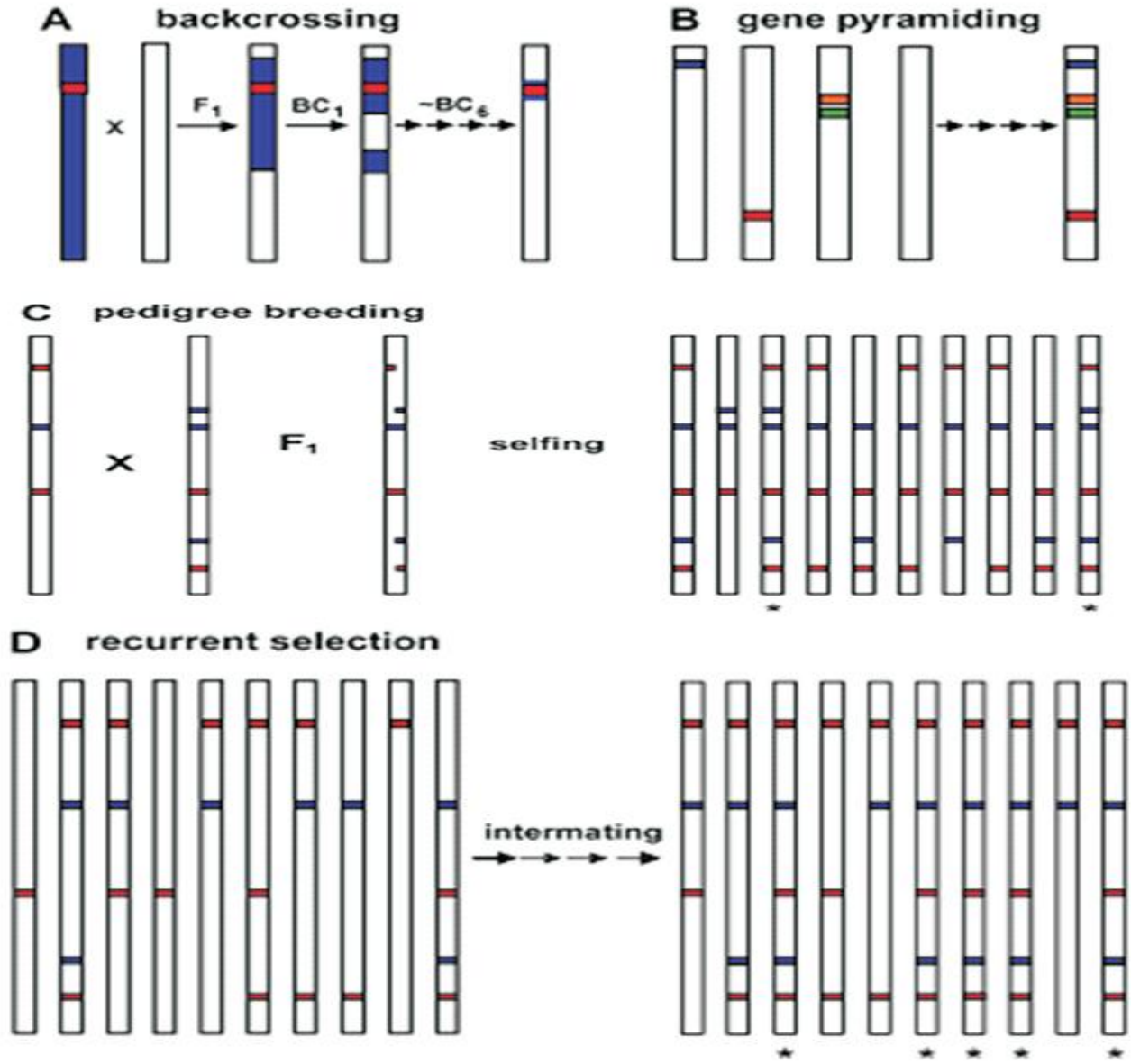

Fig. 1: Conventional techniques of plant breeding.

Disadvantages of conventional methods

1. Gene pyramiding is mainly used to improve qualitative traits such as disease and insect resistance. This is associated with the fact that the presence of target trait genes must be confirmed by phenotyping mostly at the individual level and that individual phenotypic performance is a good indicator of the genotype only if genes have a major effect on phenotypic performance and the error of phenotyping is minimal.
2. In addition to the reliability of phenotyping at individual level other factors influencing the success of gene pyramiding are the inheritance model of the genes for the target traits, linkage and/or pleiotropism between the target trait and other traits.
3. For instance, allelic genes cannot be combined in the same genotype. The effect conferred by a recessive gene cannot be evaluated on heterozygous individuals and progeny testing is required.

4. If the target gene is tightly linked to genes with large negative effects on other traits, these undesirable genes may be transferred together with the target gene into the recipient line and result in reduced performance of other traits (linkage drag).

Therefore, any improvement in the knowledge of the trait genetics (inheritance, genetic relationship, etc.) and techniques for inferring genotype-phenotype relationship will be useful.

Molecular technique

Marker assisted selection (Fig. 2)

(1) Use of DNA markers that are tightly-linked to target loci as a substitute for / to assist phenotypic screening
(2) A marker is a "genetic tag"
(3) Use of molecular markers for indirect selection of different traits
(4) Speeding up the process of conventional breeding
(5) Facilitating the improvement of traits

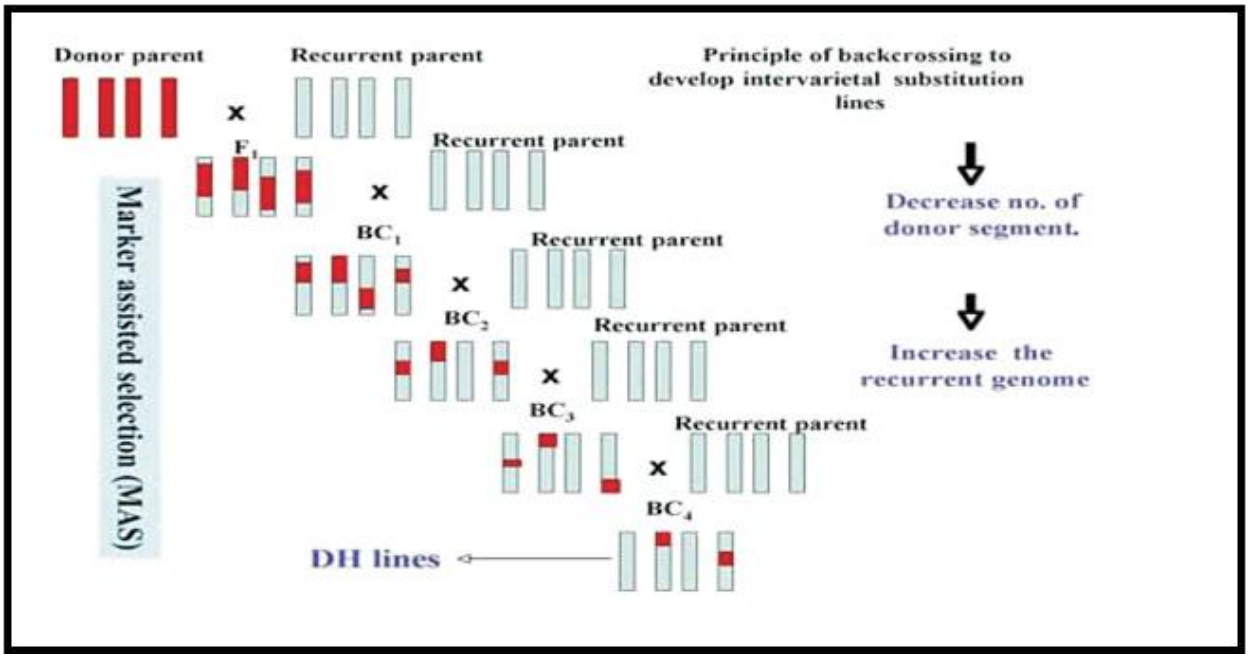

Fig. 2: Marker assisted selection.

Marker assisted gene pyramiding

Marker-assisted selection (MAS) is a method of rapidly incorporating valuable traits into new cultivars. Molecular markers, or DNA tags, that have been shown to be linked to traits of interest are particularly useful for incorporating genes that are highly affected by the environment, genes for resistance to diseases and pests, and to accumulate multiple genes for resistance to specific diseases and pests within the same cultivar – a process called gene pyramiding. One of the first wheat cultivars to be developed using MAS was the soft winter wheat cultivar "Madsen", released in 1986 by the USDA-Agricultural Research Service (ARS) and Washington State University. "Madsen" was developed using the isozyme marker from the endopeptidase protein, EpD1b, to incorporate a gene for resistance to eyespot (*Tapesia yallunde*) (Allan et al., 1989). Since 1990, detailed molecular maps of wheat have been constructed that include more than 3,000 molecular markers and several important traits have been associated with DNA markers. Additional markers can be developed from the 8,000 expressed sequence tags (ESTs) that have been mapped in wheat [Maps and references are available online (USDA-ARS 2005)].

Distinct gene pyramiding scheme

In a gene pyramiding scheme, strategy is to cumulate into a single genotype, genes that have been identified in multiple parents. The use of DNA markers, which permits complete gene identification of the progeny at each generation, increases the speed of pyramiding process. In general, the gene pyramiding aims at the derivation of an ideal genotype that is homozygous for the favorable alleles at all the loci. The gene pyramiding scheme can be distinguished into two parts (Fig. 3). The

first part is called a pedigree, which aims at cumulating of all target genes in a single genotype called the root genotype. The second part is called the fixation step which aims at fixing the target genes into a homozygous state i.e. to derive the ideal genotype from the one single genotype. Each node of the tree is called an intermediate genotype and has two parents. Each of this intermediate genotype variety can resist. Moreover, pyramiding can also improve becomes a parent in the next cross. The intermediate genotypes are not just an arbitrary offspring of a given cross but it is a particular genotype selected from among the offspring in which all parental target genes are present.

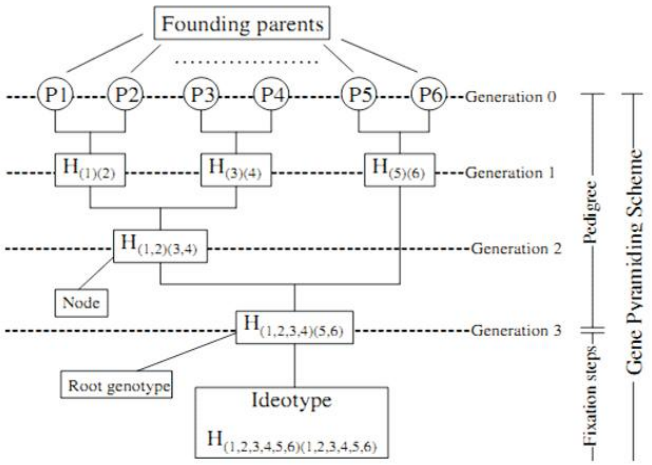

Fig. 3: A distinct gene pyramiding scheme cumulating six target genes (Hospital et al., 2004).

Although the pedigree step may be common, several different procedures can be used to undergo fixation in gene pyramiding. Generation of a population of doubled haploids from the root genotype is a possible procedure for the fixation steps. Here, a population of gametes is obtained from the genotypes and their genetic material is doubled. This leads to a population of fully homozygous individuals, among which the ideotype can be found. Using this process, the ideal genotype can be obtained in just one additional generation after the root genotype is obtained. However, producing large population of doubled haploid is difficult and cumbersome in certain plant species. A possible alternative to this method is to self the root genotype directly to obtain the ideal genotype. However, selfing the root genotype will result in the breakage of linkage between the desired alleles and it will be difficult to derive this breaks as the linkage phase is rarely visible in selfed populations. As a result, it may span too many generations thereby stretching the gene pyramiding scheme. Another alternative to all this methods would be to obtain a genotype carrying all favorable alleles in coupling by crossing the root genotype with a parent containing none of the favorable alleles. This confirms that the linkage phase of the offspring is known and the genotype can be derived without any mixing. The ideal genotype will be reached within two generations after the root genotype. However, instead of crossing with a blank parent, a more simplified method would be to cross the root genotype with one of the founding parents. In such programs, the linkage will still be known, and the selection will be for genotypes that are homozygous for the target gene brought by the founding parent but heterozygous for other regions. The desired genes need not be fixed subsequently, thereby increasing the probability of getting the ideal genotype. This is called as marker assisted backcross gene pyramiding.

Marker assisted backcrossing

Breeders transfer a target allele from a donor variety to a popular cultivar by a repetitive process called backcrossing; which, unfortunately, is slow and uncertain. Breeding a plant that has the desired donor allele but otherwise looks just like the popular cultivar usually takes four years or longer. Worse, the augmented variety may look just like the popular cultivar, but it inevitably retains stray chromosome segments from the donor. Consequently, to a greater or lesser extent, it will fail to perform exactly like the popular cultivar, thus limiting its appeal to farmers. Marker assisted breeding tackles both problems by allowing breeders to identify young plants with the desired trait and by facilitating the removal of stray donor genes from intermediate backcrosses. The result, in about two years, is an improved variety exactly like the popular cultivar except that it possesses the transferred advantageous gene. In principle, this technique can be applied to the breeding of any crop or farm animal. So far, however, breeders of trees and rice have dominated the field. Because markers allow breeders to select immature plants, the time saved in breeding slow-growing trees is immense. In the case of rice, the crop's relatively advanced state of genetic mapping has facilitated the application of molecular marker techniques. Markers are effective aids to selection in backcrossing in three ways. First, markers can aid selection on target alleles whose effects are difficult to observe phenotypically. Examples include recessive genes, multiple disease resistance gene pyramids combined in one genotype (where they can epistatically mask each other's effects), alleles that are not expressed in the selection environments (e.g., genes conferring resistance to a disease that is not regularly

present in environments), etc. Second, markers can be used to select for rare progeny in which recombination near the target gene have produced chromosomes that contain the target allele and as little possible surrounding DNA from the donor parent. Third, markers can be used to select rare progeny that are the result of recombination near the target gene, thus minimizing the effects of linkage drag.

In general, the marker assisted backcross based gene pyramiding can be performed in three strategies (Fig. 4). In the first method, the recurrent parent (RP1) is crossed with donor parent (DP1) to produce the F1 hybrid and backcrossed up to third backcross generation (BC3) to produce the improved recurrent parent (IRP1). This improved recurrent parent is then crossed with other donor parent (DP2) to pyramid multiple genes. This strategy is less acceptable as it is time taking but pyramiding is very precise as it involve one gene at one time.

In the second strategy, the recurrent parent (RP1) is crossed with donor parents (DP1, DP2, etc.) to get the F1 hybrids which are then intercrossed to produce improved F1 (IF1). This improved F1 is then backcrossed with the recurrent parent to get the improved recurrent parent (IRP). As such, the pyramiding is done in the pedigree step itself. However, when the donor parents are different, this method is less likely to be used because there is chance that the pyramided gene may be lost in the process.

The third strategy is an amalgamation of the first two which involve simultaneous crossing of recurrent parent (RP1) with many donor parents and then backcrossing them up to the BC3 generation. The backcross populations with the individual gene are then intercrossed with each other to get the pyramided lines. This is the most acceptable way as in this method not only time is reduced but fixation of genes is fully assured. Marker assisted backcrossing to be effective, depends upon several factors, including the distance between the closest markers and the target gene, the number of target genes to be transferred, the genetic base of the trait, the number of individuals that can be analyzed and the genetic background in which the target gene has to be transferred, the type of molecular marker(s) used, and available technical facilities. When these entire selection criterions are maintained properly, only then a well acceptable MAB based gene pyramiding scheme can lead to durable crop improvement.

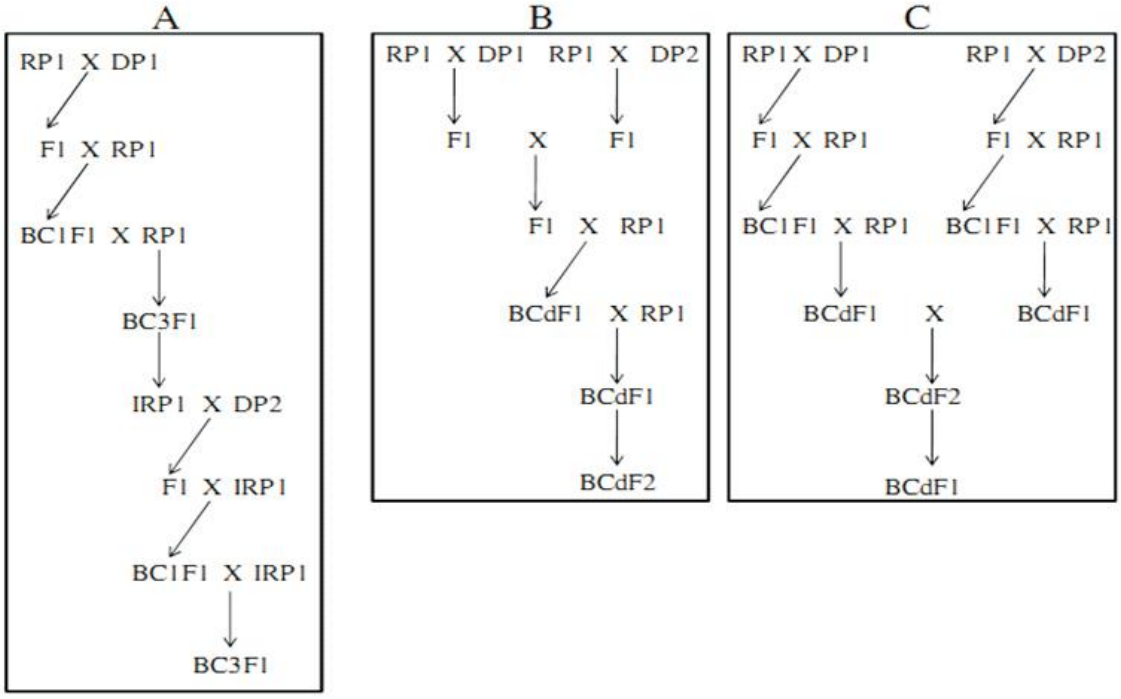

Fig. 4: Different schemes of backcrossing for gene pyramiding. RP- Recurrent parent; DP- Donor parent; BC- Backcross; IRP- Improved recurrent parent. A. Stepwise transfer; B. Simultaneous transfer; C. Simultaneous and stepwise transfer.

Advantages gene pyramiding

1. Widely used for combining multiple disease resistance genes for specific races of a pathogen
2. Pyramiding is extremely difficult to achieve using conventional methods
3. Consider - phenotyping a single plant for multiple forms of seedling resistance – almost impossible
4. Important to develop 'durable' disease resistance against different races
5. Main used to improve existing elite cultivar
6. Eliminates extensive phenotyping
7. Control linkage drag
8. Reduces breeding duration

Main factors affecting gene pyramiding

1. Characteristics of the target traits/genes
2. Reproductive characteristics
3. A breeder's capability to identify the 'desired' genotypes
4. Operating capital

Success stories

Table 1 below shows the successfully pyramided genes with their traits of some important crop plants, rice, wheat, cotton, pea, barley, broccoli, soyabean and chickpea.

Table 1. Successfully pyramided genes and their traits of crop plants.

Crop	Traits	Pyramided Genes	Reference
Rice	Blight resistance	*Xa4, xa5, xa13, Xa21* *Xa 5, Xa13* and *Xa21*	Singh et al. (2001), Narayanan et al. (2002), Joseph et al. (2004)
	Blast resistance	*Pi(2)t, Piz5, Pi(t)a*	Hittalmani et al. (2000)
	Gall-midge resistance	*Gm2,Gm6* *Gm1, Gm4*	Katiyar et al. (2001) Kumaravadivel et al. (2006)
	BPH resistance	*Bph1* and *Bph2*	Sharma et al. (2004)
	Multiple resistance	Bacterial blight *(Xa21)* Sheath b light *(RC7)* Yellow stem borer *Bt* fusion gene *(cry1AB /cry1Ac)*	Datta et al. (2002)
Wheat	Leaf rust resistance	*Lr41, L r42, Lr43*	Cox et al. (1994)
	Powdery mildew resistance	*Pm2 + Pm4a; Pm2 +Pm21;* *Pm4a+ Pm21*	Liu et al. (2000)
	Aphid resistance	*Gn2* and *Gn4*	
	Cereal cyst nematode	*CreX and CreY*	Dominiques et al. (2007)
Cotton	Insect pest resistance	*Cry 1Ac, Cry 2Ac*	Gahan et al. (2005)
	Bacterial blight and Sheath blight	*chi11, tlp and Xa21*	Maruthasalam et al. (2007)
Pea	Nodulation ability	*Sym9, Sym10*	Schneider et al. (2002)
Barley	Yellow mosaic virus resistance	*rym4, rym5, rym9,rym11*	Werner et al. (2005)
	Stripe rust resistance	3 QTL	Castro et al. (2003)
Broccoli	Diamond back moths resistance	*cry1 A c + cry1c*	Cao et al. (2002)
Soyabean	Soybean mosaic virus resistance	*Rsv1, Rsv3, Rsv4*	Zhu et al. (2006)
	Lepidopteran resistance	*cry1Ac +corn ear worm QTL*	Walker et al. (2002)
Chickpea	Lepidopteran resistance	*cry1Ac + cry1Ab*	Meenakshi et al. (2011)

Conclusion

Gene pyramiding is an important strategy for crop improvement. Pyramiding requires that breeders consider the minimum population size that must be evaluated to have a reasonable chance of obtaining the desired genotype. Molecular marker genotyping can facilitate the gene pyramiding process by reducing the number of generations that breeders must evaluate to ensure they have the desired gene combination.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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