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Standard Review

Quality pyramiding-An expansive range procedure for creating durable stress resistance in yields

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The development of molecular genetics and associated technology like MAS 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. The present review discusses the design parameters in a gene pyramiding scheme, potential application of gene pyramiding in crop plant improvement, and the prospect and challenges in integrating MAS based gene pyramiding with conventional plant breeding programmes.

Key words: Gene pyramiding, marker-assisted selection, durable resistance.

INTRODUCTION

Since the beginning of agriculture, humans have sought to improve crops by selecting for desired traits. Genetics have played an important part in this field. With the advent of genetic engineering and biotechnology, plant breeding has got a new dimension to produce crop verities with more desirable characters. Marker assisted selection (MAS) which involves indirect selection of traits

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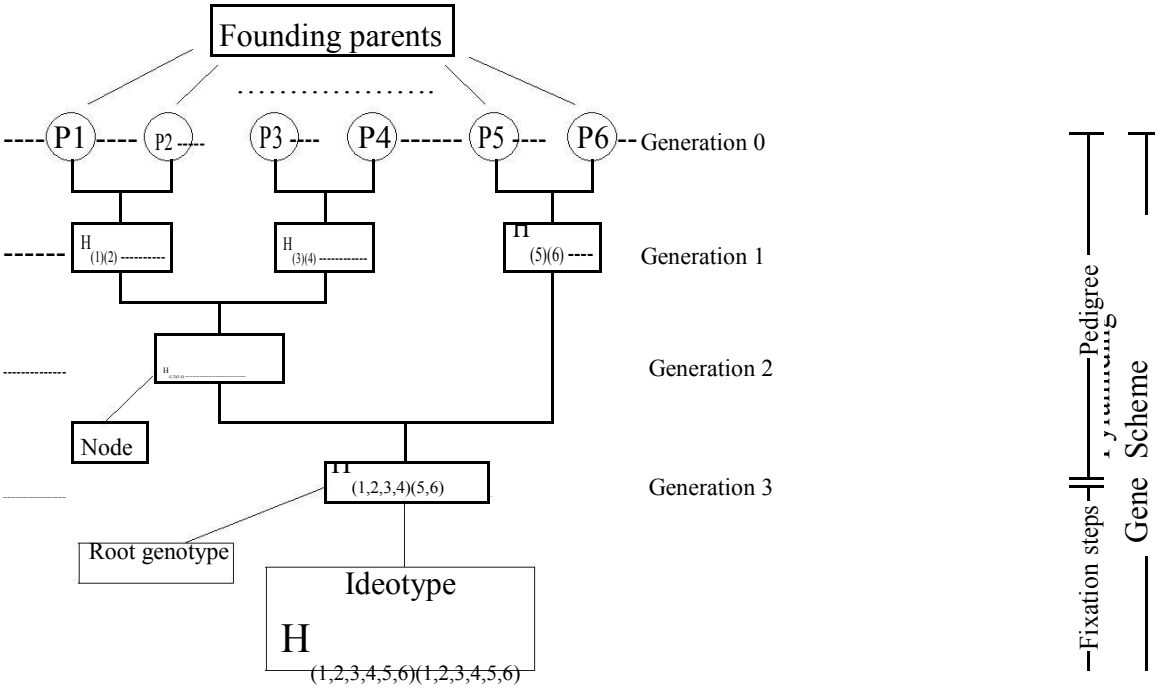

Figure 1. A distinct gene pyramiding scheme cumulating six target genes. (Hospital et al., 2004)

by selecting the marker linked to the gene of interest has become a reality with development and availability of an array of molecular markers and dense molecular genetic maps in crop plants. Molecular markers are especially advantageous for agronomic traits that are otherwise difficult to tag such as resistance to pathogens, insects and nematodes, tolerance to abiotic stresses, quality parameters and quantitative traits. Molecular markers studies using near isogenic lines (NILs), bulk segregant analysis or recombinant inbred lines (RILs) have accelerated the mapping of many genes in different plant species. Sequence tagged sites have been developed using RAPD and RFLP markers in tomato and rice and microsatellite markers in rice, wheat and cereals().In other words, there is now a large amount of research that addresses marker-aided selection in some form. However, although the process is now more efficient and sophisticated, it still mostly based on field selection and data analysis. Moreover, many MAS based improved traits have broken up in the past few years due to lack of durable resistance effect.

The challenge now is to develop new efficient marker assisted selection strategies aimed at plant improvement. Gene pyramiding holds greater prospects to attain durable resistance against biotic and abiotic stresses in crops. Different resistance genes often confer resistance to different isolates, races or biotypes. Combining their resistance broadens the number of races or isolates that a more than one character in a variety at the same time. In general, the development of pyramid lines is a long and costly affair in addition to the epistatic effect. However MAS based gene pyramiding could facilitate in pyramiding of genes effectively into a single genetic background. When hybrids crops are the goal, additional options for pyramiding different resistance gene combinations into different parents also exist.

A 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 n loci. The gene pyramiding scheme can be distinguished into two parts (Figure 1). 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. 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. By far this is the most accepted and efficient method to do the 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 (Figure 2). 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

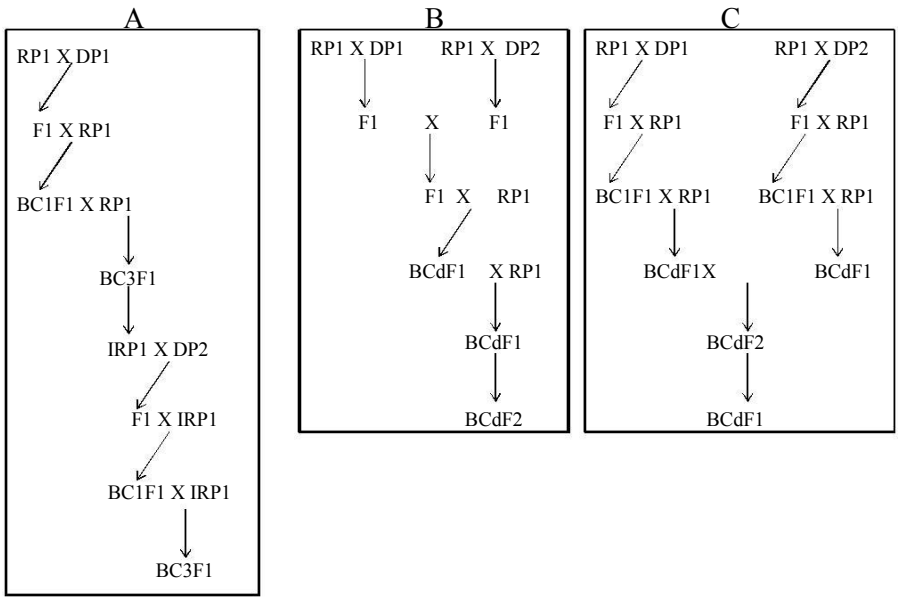

Figure 2. Different schemes of backcrossing for gene pyramiding. RP- Recurrent parent; DP- Donor parent; BC- Backcross; IRP- Improved recurrent parent. A. Stepwise transfer; B. Simultaneous transfer; C. Simultaneous and stepwise transfer.

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 (Weeden et al., 1992; Francia et al., 2005). When these entire selection criterions are maintained properly, only then a well acceptable MAB based gene pyramiding scheme can lead to durable crop improvement.

EFFICIENCY OF GENE PYRAMIDING

Computer simulations and theoretical calculations have provided powerful tools for analyzing the efficiency of gene pyramiding programmes. Three different gene pyramiding schemes, one based on a cascading pedigree, and two based on the order of crosses of the founding parents were evaluated to check the transmission probabilities of the target genes and the cumulated population size needed in each scheme (Ribaut and Hiosington, 1998; Ribaut et al., 2001; Hospital et. al 2004). The simulation was based on identical recombination fractions between adjacent loci and spaced about 20 cM. The major conclusions from this experiment are as follows:

A MAS based gene pyramiding scheme based on a cascading pedigree is less expensive as it spans five generations in general and requires the smallest cumulated population size of all the schemes. The average transmission probability is 0.9975.

Gene pyramiding scheme based on the crosses of founding parents spans four generations but the population size is somewhat higher. The average transmission probability is 0.9967.

When gene pyramiding is carried out involving a larger number of target genes, each trait starts as a founding parent resulting in intermediate genotypes by subsequent crossing. It is based on a cascading pedigree and span one or two less generation in general.

QUALITATIVE IMPROVEMENT THROUGH GENE PYRAMIDING- SOME CASE STUDIES

Marker aided pyramiding of rice genes for BLB and blast disease

The successful effort on gene pyramiding in rice includes resistance to blight, blast, gall midge etc. Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *Oryzae* (Xoo) is one of the most destructive diseases of rice throughout the world and in some areas of Asia it is responsible for yield loss of more than 60%. The most efficient approach to overcome bacterial blight caused by *X. oryzae* is to produce resistant varieties; more than 25 BLB resistant genes have been identified and subsequently transferred into modern rice cultivars by cross breeding. However, the recent breakdowns of many resistant genes to BLB pathogens have significantly affected the rice production in many countries. One way to delay such a breakdown is

Table 1. Selected examples of MAS based gene pyramiding for important traits in major crops.

Crop	Trait	Pyramided genes	Reference
Rice	Blight resistance	*Xa4,xa5,xa13,Xa21*	Huang et al., 1997, Singh et al., 2001, Narayanan et al., 2002 Hittalmani et al., 2000
	Blast resistance	*Pi(2)t,Piz5,Pi(t)a*	Kumaravadivel et al., 2006
	Gallmidge resistance	*Gm1,Gm4*	
Wheat	Leaf rust resistance	*Lr41, Lr42, Lr43*	Cox et al., 1994 Liu et al., 2000
	Powdery mildew resistance	*Pm-1, Pm-2*	
Cotton	Insect pest resistance	*Cry 1Ac, Cry 2Ac*	Jackson et al., 2003, Gahan et al., 2005
Pea	Nodulation ability	*Sym9, Sym10*	Schneider et al., 2002 Werner et al., 2005 Zhu et al., 2006
Barley	Yellow mosaic virus resistance	*rym4, rym5, rym9, rym11*	
Soybean	Soybean mosaic virus resistance	*Rsv*1, *Rsv*3, *Rsv*4	

to pyramid multiple resistance genes in to rice varieties. It is practically difficult to transfer genes through conventionally gene transfer process due to vertifolia effect. International Rice Research Institute (IRRI) have successfully used the MAS based gene pyramiding to transfer four genes *Xa21,xa5,xa4* and *xa13* in elite rice cultivars (Huang et al., 1997). (Table 1) The pyramided lines showed a wider spectrum and a higher level of resistance than lines with only a single gene. Similarly, Sanchez, et al. 2000 successfully transferred three bacterial blight resistance genes into three susceptible rice lines possessing desirable agronomic characteristics via a marker-aided backcrossing procedure. In India, at Punjab Agricultural University (PAU), three BB resistance genes xa5, xa13 and Xa 21 were pyramided in PR106 (Singh et al., 2001) and Pusa 44 background and two of the PR1106 have been included in all India Coordinated testing during 2002. A similar work has also been successfully carried out in Central Rice Research Institute to pyramid three genes *xa5, xa13 and Xa21* in to elite rice cultivars Lalat and Tapaswini. All combinations of the three resistance genes were pyramided using STS markers.

Narayanan et al 2002 improved an elite indica rice line IR50 by pyramiding blast resistance gene Piz5 and bacterial blight resistance gene Xa21 through marker-assisted selection and genetic transformation. Ramalingam et al 2002 made four cross combinations of IRBB21 and successfully obtained improved lines pyramided with Xa21 and Wx (waxy) gene showing durable resistance to bacterial leaf blight and high amylose content.

Rice blast caused by the fungal pathogen *Magnaporthe grisea* is another devastating disease that provides constant challenge to rice production. The most effective way to reduce the crop yield is to breed for resistance to disease (Zeigler et al., 1994). Recently, many genes for qualitative blast resistance have been mapped using molecular markers and some of them have also been tried in MAS for blast resistance. Hittalmani et al. (2002) have successfully pyramided three genes, *Pi1, Piz5* and *Pita* in a susceptible rice variety, Co39 using RFLP and PCR based markers for durable blast resistance.

Asian rice Gall Midge, *Orseolia oryzae* (Wood-mason) is a serious dipteran pest in major rice growing areas, causing an annual yield loss of US $550 million in Asia (Herdt, 1991). In India, the pest is widely distributed and is considered a major constraint to rice production (Bentur et al., 2003). Since no effective chemical control measure for gall midge is available, growing resistance varieties is a viable strategy which is not only economical, but is also ecologically, a friendly approach. On a similar note with BLB and Blast, extensive research has been undergone which have resulted in many mapped genes resistant to Gall Midge (Kumar et al., 2005). Katiyar et al. (2001) successfully did the genetic analysis and pyramiding of two gall midge resistance genes Gm2 and Gm6t in rice. Kumaravadivel et al. (2006) are in a process of pyramiding two dominant resistant genes *Gm1* and *Gm4* into the locally popular varieties of Tamil nadu. A similar work is also in progress Central Rice Research institute, Cuttack to pyramid *Gm1* and *Gm4* gene into popular cultivars like Swarna and Tapaswini. Recently, the Govt of India through Indian Council of Agricultural Research have started an extensive network project on gene pyramiding to produce multiple biotic stress resistance rice cultivars.

Molecular marker-facilitated gene pyramiding for powdery mildew resistance in wheat

The fungal pathogen *Blumeria graminis f. sp. tritici* is the

causal agent of the powdery mildew disease in wheat (*Triticum aestivum* L.). Resistance to this pathogen is mediated by the Pm genes (Chen et al 2005). Since race-specific resistance is restricted to pathogens that carry the matching avirulence (avr)-gene, this type of resistance can be overcome in the field. For breeders, it is therefore desirable to create plants with more broad-spectrum and long-lasting resistance features. One strategy to achieve this goal is to combine different resistance genes by classical breeding. However, this is a time-consuming approach. MAS based gene pyramiding provides a more rapid tool to introduce new disease resistance specificities into crop plants. Liu et al. (2000), have underwent a gene pyramiding approach in which three powdery mildew resistance gene combinations, *Pm2 + Pm4a, Pm2 + Pm21, Pm4a + Pm21* were successfully integrated into an elite wheat cultivar 'Yang158'. Double homozygotes were selected from a small F_2 population with the help of molecular markers. As the parents were near-isogenic lines (NILs) of 'Yang158', the progenies showed good uniformity in morphological and other non-resistance agronomic traits. The present work illustrates the bright prospects for the utilization of molecular markers in breeding for host resistance.

Gene pyramiding of rust-resistance genes Lr41, Lr42 and Lr43 in common wheat

Leaf rust is one of the most important diseases of wheat worldwide, particularly in the Great Plains region of the USA. Gene has been advocated as a long-term strategy for the control of this disease in the recent times. Cox et al. (1993) has successfully pyramided three leaf rust-resistance genes *Lr41*, *Lr42* and *Lr43* into the common wheat (*Triticum aestivum* L.). Here, In order to diversify the genetic base of resistance in hard red winter wheat (*T. aestivum* L.) to leaf rust (caused by *Puccinia recondita* Rob. ex Desm.), five genes for resistance were transferred from the diploid goatgrass *Triticum. tauschii* (Coss.) Schmal. to hexaploid wheat lines. One of the derived lines, KS90WGRC10, had a very low infection type when inoculated with 23 cultures of *P. recondita*. The others, KS91WGRC11, KS92WGRC16, U1865, and U1866, had low to intermediate infection types with three cultures. Their infection types varied similarly to those of lines carrying previously transferred alleles of *Lr21*. WGRC10 carries a completely dominant gene, *Lr41*, on chromosome ID that segregates independently of any other *T. tauschii*-derived leaf rust-resistance genes. WGRC11 carries the partially dominant gene, *Lr42*, also on ID, which is linked to *Lr21* with a recombination value of 0.286 +- 0.023. WGRC16 carries a partially dominant gene, *Lr43* that segregates independently of all known genes for seedling resistance from T. tauschii; its chromosome location is not known. The genes carried by U1865 and U1866 are allelic to *Lr21*. WGRC10, WGRC11, and WGRC16 have been released as germplasm by the Wheat Genetics Resource Center.

Stripe rust is another of the most devastating diseases of wheat worldwide. Santra et al. (2006) have successfully pyramided two single, dominant genes *Yr5* and *Yr15*, which independently confer complete resistance to all stripe rust races found in North America. The cereal cyst nematode (CCN) *Heterodera avenae* is a significant pathogen of wheat. The wild grass *Aegilops variabilis* Accession No.1 has been found to be resistant to pathotypes of CCN; at least two genes transferred to wheat, designated as *CreX* and *CreY*, are involved in the resistance response. Barloy et al. (2006) pyramided the two CCN resistance genes in a wheat background through marker-assisted selection. The completely linked RAPD marker of *Rkn-mn1* (*CreY*), $OpY16\text{-}_{1065}$, previously obtained, was converted into a SCAR. All these dominant markers were used to incorporate in the same genotype the two *Ae. variabilis* chromosome segments carrying the two genes for resistance. CCN bioassays with the Ha12 pathotype showed that the level of resistance of the pyramided line was significantly higher than that of *CreX* and *CreY* single introgression lines, but lower than that of *A. variabilis*.

Gene pyramiding as a Bt resistance management strategy in cotton

Reports on the emergence of insect resistance to *Bacillus thuringiensis* delta endotoxins have raised doubts on the sustainability of Bt- toxin based pest management technologies. Corporate industry has responded to this challenge with innovations that include gene pyramiding among others. Pyramiding entails stacking multiple genes leading to the simultaneous expression of more than one toxin in a transgenic variety. Recently gene pyramiding has been hailed as a lasting Bt resistance management strategy (Jackson et al., 2003, Shelton et al., 2002). The strategy of Bt gene pyramiding rests on three core assumptions (Gahan et al., 2005) . The first assumption is that insects resistant to only one toxin can be effectively controlled by a second toxin produced in the same plant.

This assumption forms the basis for the Bollgard® II cotton variety which has two toxins namely, Cry 1Ac and Cry 2Ac. The Cry 1Ac toxin controls tobacco budworm and pink bollworm while the Cry 2Ac toxin controls corn earworm (Jackson et al., 2003; Ferry et al., 2004; Purcell et al., 2004). The second assumption is that strains resistant to two toxins with independent actions can not emerge through selection pressure with one toxin alone. The third assumption underlying the strategy of Bt gene pyramiding is that a single gene will not confer resistance to two toxins that are immunologically distinct and that have different binding targets (Gahan et al., 2005).

Second generation pyramided dual- Bt gene cottons

Bollgard II® (Cry 1Ac + Cry 2Ab) and WideStrike™ (Cry1Ac + Cry 1F) express two Bt endotoxins and were introduced successfully by Monsanto in USA and India in order to raise the level of control for *H. zea*, which was not satisfactorily controlled by the Cry 1Ac toxin alone (Jackson et al., 2003; Ferry et al., 2004; Bates et al., 2005; Gahan et al., 2005). The Cry 1Ac and 2Ab toxins have different binding sites in the larval midgut and are considered to be a good combination to deploy in delaying resistance evolution. This is due to the fact that a species cannot easily evolve resistance to both toxins because that would require two simultaneous, independent mutations in genes encoding the receptors (Jackson et al., 2003). Future pest management practices will have to rely on the introduction of transgenic cottons that express other insecticidal toxins in addition to the Cry toxins (Ferry et al., 2004; Wu and Guo, 2005). Biological pest control using parasitoids and predators, cultural practices and other pest management tactics are all essential tactics in preserving the efficacy of Bt based products. But gene pyramiding approaches have definitely proven as effective method in broadening the scope and mode of action of toxins thereby providing growers with more options in their overall resistance management efforts (Manyangarirwa et al 2006).

Pyramiding resistance genes against the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2)

Barley Yellow Mosaic Virus disease caused by different strains of BaYMV and BaMMV is a major threat to winter barley cultivation in Europe. Pyramiding of resistance genes has been effectively used as a promising strategy to avoid the selection of new virus strains and to create more durable resistances by Werner et al. (2005). For pyramiding of resistance genes rym4, rym5, rym9 and rym11, located on chromosomes 3H and 4H of barley, two different strategies have been developed. These strategies are based on doubled haploid lines (DHs) and marker assisted selection procedures. On the one hand F1 derived DH-plants of single crosses were screened by molecular markers for genotypes being homozygous recessive for both resistance genes. These genotypes were crossed to lines carrying one resistance gene in common and an additional third gene, leading to a DH-population of which 25% carry three resistance genes, 50% have two resistance genes and 25% possess a single resistance gene homozygous recessively. Alternatively, F1 plants having one resistance gene in common were directly inter-crossed [e.g. (rym4 · rym9) · (rym4 · rym11)] and about 100 seeds were produced per combination. Within these complex cross progenies plants were identified by markers being homozygous at the common resistance locus and heterozygous at the others. From such plants, theoretically present at a frequency of 6.25%, DH-lines were produced, which were screened for the presence of genotypes carrying three or two recessive resistance genes in a homozygous state.

Gene pyramiding for soybean mosaic virus resistance using microsatellite markers

Gene pyramiding has been used as an effective approach to achieve multiple and durable resistance to various strains of *Soybean Mosaic Virus* (SMV) in soybean [*Glycine max* (L.) Merr.]. Zhu et al. (2006) have successfully pyramided three genes *Rsv* 1, *Rsv*3, and *Rsv*4 for SMV resistance with the aid of microsatellite markers in order to develop new soybean lines containing multiple resistance genes. A population of 84 lines derived from J05 (*Rsv*1, *Rsv*3) x V94-5152 (*Rsv*4) were developed, and six specific SSR markers were identified for SMV resistance genes. Two SSR markers Sat154 and Satt510 were used for selecting lines having the *Rsv*1 gene, Satt560 and Satt726 for *Rsv*3, and Sat_254 and Satt542 for *Rsv* 4. These SSR markers allowed for identification and selection of specific lines and individual plants containing different genes and for distinction of the homozygous and heterozygous lines or individual plants for all three resistance loci. Individual plants with homozygous alleles at three genetic loci (*Rsv*1*Rsv*1, *Rsv3Rsv* 3 and *Rsv*4*Rsv* 4) have been identified and new soybean germplasm is expected to be released with three genes combined for SMV resistance.

POLYGENIC TRAIT IMPROVEMENT BY GENE PYRAMIDING- A STEP FORWARD

Many economically important traits such as yield, quality and tolerance to abiotic stresses are of a quantitative nature. Genetic variations affecting such traits are controlled by a relatively large number of loci each of which can make a small positive or negative contribution to the final phenotypic value of the traits. These loci are termed QTLs. Molecular markers provide the opportunity to manipulate QTLs as Mendelian entities. Several QTLs for traits of economic importance like rice blast resistance (Wang et al., 1994) black mold resistance in tomato (Robert et al., 2001), flour colour in wheat (Parker et al., 2000), have been tagged with molecular markers. There have been some successful uses of MAS for polygenic traits in plants (Lande et al 1990; Johnson and Mumm 1996; Schneider et al. 1997; Stuber et al. 1998; Tanksley et al. 1996; Yousef and Juvik 2001). However, the improvement of polygenic traits through MAS raises more questions. Infact, no experiment has clearly demonstrated whether using DNA markers for quantitative trait improvement is superior to conventional breeding selection (Beavis 1998). This is because of the complexity of the process as several genes are involved

in the expression of polygenic traits and generally have smaller individual effects on the plant phenotype. This implies that several regions (QTL) must be manipulated at the same time in order to have a significant impact, and that the effect of individual regions is not easily identified. This warrants repetitions of field test to characterize accurately the effects of QTLs and to evaluate their stability across environments. Even in presence of these constraints we still believe that solutions exist for it. Recently, progressive work have been carried out for quantitative trait improvement through MAS and subsequently gene pyramiding. MAS for polygenic traits has been integrated with varying levels of success into various breeding methods such as recurrent selection (Yousef and Juvik 2001), selection for a target genotype (Stuber et al., 1998), and introgression of exotic germplasm into elite lines using advanced backcrossed inbred selection (Tanksley et al., 1996; Tanksley and McCouch, 1997). However, polygenic traits like yield present additional complexity because, unlike oligogenic disease resistance, selection for yield is usually conducted exclusively in crosses between elite lines from a restricted germplasm pool. So QTLs mapped in one population will have little relevance to those mapped in other populations. So, it is better that marker-QTL linkage estimates will have to be updated regularly to account for recombination occurring between many linked QTLs as well as between QTL and markers (Holland, 2004).

Genetic enhancement, through AB-QTL strategy have been undergone by pyramiding various traits of agronomic importance, including fruit quality and black mould resistance in tomato were accomplished using wild relatives (Robert et. al 2001) . A broad spectrum project is under progress at CIMMYT to pyramid major QTLs for durable physiological expression in maize.

Conclusion

With MAS based gene pyramiding, it is now possible for the breeder to conduct many rounds of selections in a year. Gene pyramiding with marker technology can integrate into existing plant breeding programmes all over the world to allow researchers to access, transfer and combine genes at a rate and with a precision not previously possible. However, lot of problems still persists in this field. Some of the difficulties encountered have to do with the need to have better scoring methods, larger population sizes, multiple replications and environments, appropriate quantitative genetic analysis, various genetic backgrounds and independent verification through advanced generations (Young et al., 1999). However taking into account the number of ongoing experiments and the explosion of new molecular technology, it is not surprising that new or improved selection schemes are being developed and applied as in case of maize (Ribaut et al., 2001). This will help breeders get around problems related to larger breeding populations, replications in diverse environments, and speed up the development of advanced lines. Furthermore improved scoring methods and screening techniques can be developed and implemented, and much better choices about target traits can be made.

New technological developments such as automation, allele-specific diagnostics and diversity array technology (Jaccoud et al., 2001) will make MAS based gene pyramiding more powerful and effective. The main problem in front us to find out the most suitable way to use the genome information for biological intrigues including MAS based gene pyramiding. Any development in plant breeding is measured in terms of the contribution made to improvement in food production. Therefore plant breeders must be convinced on the advantages of MAS based gene pyramiding to implement it successfully in breeding programs. Recent success in MAS based gene pyramiding indicates that success was met because a good choice of target traits was made, information on the mode of inheritance was available, protocols to integrate MAS based gene pyramiding technology into breeding programs were developed with a multidisciplinary effort.

We have no doubt that MAS based gene pyramiding has the potential to increase the rate of genetic gain when used in conjunction with traditional breeding and the adoption of MAS by cereal breeders in Australia and the subsequent commercialization of pyramided lines of cultivars bred is testimony to this. The feasibility of gene pyramiding has been demonstrated, especially for pyramiding disease resistance genes, not only at one place but at several institutes in India like Punjab Agricultural University (PAU), Central Rice Research Institute (CRRI), University of Agricultural Sciences (Bangalore), Indira Gandhi Agricultural University (IGAU), and Tamil Nadu Agricultural University (TNAU). This was achieved more or less independent of plant breeders and mostly in well adapted varieties. Plant breeders simultaneously came up with new varieties that may be higher yielding, and hence the pyramided lines did not find their way to the farmers` fields even though they yield at par with the recurrent parents. The big question lies ahead is how to make MAS based gene pyramiding operational in the developing world to get maximum benefit from it. Some possible options are;

Since MAS is expensive and breeding programmes are mostly funded by the local governments, the national governments can start some MAS based gene pyramiding projects with committed funding. In India the Indian Council of Agricultural Research (ICAR) has already taken the initiative and MAS based gene pyramiding projects are successfully undergoing in rice, maize, wheat etc. This has been an integral part of the breeding programme and not just any other backcross programme.

Breeders are not much excited about gene pyramiding

for simply inherited traits, and not many QTL (especially the productivity related ones) with tightly linked markers are available. This will take some more time, especially the productivity related QTL from the wild species germplasm, to become available to breeders. However, with development and access to reliable PCR based markers like SSPs and SNPs in several crop plants, efficiency of pyramiding large populations or breeding materials has significantly increased. QTL pyramiding requires using better scoring methods, appropriate quantitative genetic analysis, and independent verifications through parallel populations. Appropriate DNA markers should be used at a definite stage to maximize the efficiency of MAS.

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