The Pharma Innovation Journal 2022; SP-11(6): 1090-1094

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(6): 1090-1094

www.thepharmajournal.com
Received: 26-03-2022
Accepted: 30-04-2022

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A review on gene pyramiding to develop disease resistant crop variety

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Abstract
The advancement of molecular genetics and related technologies such as marker assisted selection has resulted in the establishment of a new discipline in plant breeding known as gene pyramiding. Pyramiding is the stacking of various genes that leads in the simultaneous expression of more than one gene in a variety to promote long-lasting resistance expression. Pathogens cause significant crop loss, thus specific genes from various crops are incorporated to develop resistance against these disease causing pathogens. Pyramiding of genes is critical for broad spectrum resistance to diseases and ensuring disease resistance durability. The feasibility of gene pyramiding is dependent on several essential criteria, including the number of genes to be transferred, the distance between the target genes and flanking markers, the number of genotypes chosen in each breeding generation, the germplasm's nature. The gene pyramiding strategy is clearly efficient in increasing the genetic basis of resistance, and it may be used successfully in combination with all other management strategies.

Keywords: Gene pyramiding, marker assisted selection, marker assisted gene pyramiding, disease resistance

Introduction
Gene pyramiding, which tries to combine multiple desirable genes into a single genotype and it is a typical breeding approach for primarily self-pollinated crops. Traditionally gene pyramiding has been used to improve an existing elite cultivar by introducing a few significantly effective genes from other sources, because the presence of the target genes must be verified by phenotyping, which is only effective for major genes. (Ye and Smith 2010) [55]. Traditional crop breeding methods rely on traditional procedures and natural processes that need extensive field trials. Recurrent selection, progressive hybridization, backcross breeding, and pedigree crossing are examples of these processes (Dormatey *et al*., 2020; Janila *et al*., 2016) [14, 19]. However, tracing the presence of targeted genes is challenging, restricting the selection of desirable offspring. (Malav *et al*., 2016) [31]. Plant breeders employ MAS to transfer genes from pyramided lines into the desired crop in order to increase the proportion of better genotypes in the desired population (Das and Rao, 2015; Magar *et al*., 2014; Pradhan *et al*., 2015; Shamsudin *et al*., 2016) [11, 30, 40, 45]. Mostly crop-breeding procedures for biotic resistance are based on the insertion of a single resistant gene into plants, therefore crop resistance is only temporary. As a result, the generation of genotypes with resistance to many stresses by the pyramiding of several genes from diverse sources into a single plant is presently being addressed. Several studies have used the pyramiding of several resistance genes to explain crop stress tolerance development. (Dormatey *et al*., 2020) [14]. Pyramiding multiple genes is accomplished in principle by crossing parental lines with complimentary desirable genes and choosing the appropriate recombinants from the offspring population. (Allard, 1999) [1]. Breeders aim to combine as many desirable alleles as possible in a single breeding cycle since breeding is a time-consuming procedure (from crossing to the generation of near-homozygous breeding lines). When the number of genes to be assembled is known, the purpose of gene pyramiding is to create near-homozygous breeding lines that are fully homozygous for the desired alleles of the target genes utilising the fewest number of selection generations and the lowest genotyping and phenotyping costs. This implies that while designing and comparing pyramiding systems, the total cost and time are the two primary parameters. The total number of plants to be tested for phenotypic performance and genotypic status can be used to approximate the phenotyping and genotyping costs. (Ye and Smith, 2008) [54].

Objectives of Gene Pyramiding

1. Combining two or more complimentary genes to improve trait performance
2. Improving deficits by introducing genes from other sources
3. Improving durability

Types of Gene Pyramiding

Conventional Breeding Methods

Gene pyramiding is a crop-breeding strategy that may be used in both traditional and enhanced molecular breeding programmes to introduce new lines. In comparison to contemporary and advanced technologies, the traditional crop breeding approach generates new crop varieties by applying old procedures and ordinary natural processes. (Floros *et al*., 2010; Su *et al*., 2019; Zhang *et al*., 2018) [15, 48, 58].

1. Back cross breeding
2. Pedigree breeding
3. Recurrent selection

Molecular Techniques in Breeding Programs

Crop breeding has progressed significantly in recent years, and with the introduction of current molecular tools, precision breeding is now achievable in the shortest time period. Innovative molecular breeding technologies, namely MAS and gene transformation, are being employed to enhance crop varieties.

1. Molecular Marker-Assisted Selection
2. Marker-Assisted Backcrossing
3. Marker-Assisted Recurrent Selection

Marker Assisted Gene Pyramiding in Developing Disease Resistance Varieties:

Gene stacking or pyramiding is a useful technique for transferring multiple desired genes or QTLs from different parents into a single genotype in the shortest period of time (two to three generations), as opposed to conventional breeding, which requires at least six generations to recover 99.2 percent of the recurrent parent genome. (Suresh and Malathi, 2013; Hasan *et al*., 2015) [49, 16]. It tries to accumulate many resistance genes with known effects on a target trait and to provide long-term resistance to various stresses. (Das *et al*., 2017) [12] and Recent developments in molecular marker technology have made this feasible. (Perumalsamy *et al*., 2010; Arunakumari *et al*., 2016; Van Ooijen 2006) [38, 5, 52]. Plant scientists have successfully used this strategy to pyramid resistance genes or QTLs against biotic stresses using closely related markers. such as late blight (*Phytophthora infestans* L.), bacterial blight (*Xanthomonas campestris* L.), gall midge (*Contarinia quinquenotata* L.), mosaic viruses, powdery mildew (*Podosphaera xanthii* L.),

Marker-assisted gene pyramiding also makes it easier to find QTL allele-linked markers that have comparable phenotypic expression. Thus, pyramiding of many genes or QTLs is suggested as a feasible strategy for improving quantitative and qualitative traits in plants. (Moose and mumm, 2008; Chukwu *et al*., 2019; Richards, 2006) [35, 9, 44]. Marker-assisted selection allows for the simultaneous monitoring of several traits, whereas traditional breeding requires separate field experiments to screen for specific qualities. (Rai *et al*., 2018) [42]. Furthermore, MAS enables cost-effective gene stacking by choosing the suitable plants at an early stage of growth, reducing field area, germplasm maintenance costs, and agronomic input costs for field trials. When many genes conferring resistance to similar stresses are combined, the markers become powerful and useful for distinguishing between plants with desirable genes and those with undesirable genes. (Kumar *et al*., 2018) [24]. It can be concluded without a doubt that marker-assisted gene pyramiding is a rapid, competent, cost-effective, and simple strategy used in plant breeding to pyramid genes of interest to develop different stress tolerances in crops. (Moose and Mumm, 2008; Collard *et al*., 2005; Kumar *et al*., 2018 [24], Angeles-Shim *et al*., 2020) [35]. The pyramiding of multiple QTL and their cumulative effect has been reported in many crop plants such as wheat, barley, and soybean (Li *et al*., 2010) [27].

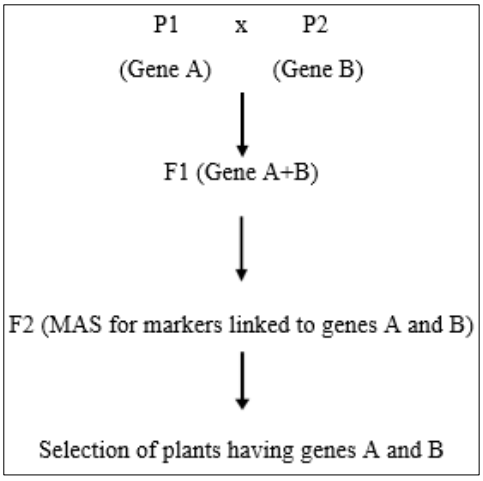

Fig 1: A simple scheme for marker assisted pyramiding of two different traits (R. Muthurajan and P. Balasubramanian, 2010) [36]

Gene Pyramiding for Disease Resistance

Disease resistance is a very complicated multigenic characteristic, single gene changes usually result in inadequate and/or limited disease resistance. (Neuhans *et al*., 1991; Anand *et al*., 2003) [37, 2]. Furthermore, there is always the chance of resistance reversal due to the development of resistant pathogen strains. (Mew *et al*., 1992) [33]. Thus, genetic engineering of crop plants with (i) a set of genes that encode/control interdependent or synergistic disease-resistance subcomponents in order to achieve effective resistance to a certain disease and (ii) To achieve broad-spectrum disease resistance, a combination of genes providing resistance against different diseases would be more reasonable. A well-planned genetic engineering method incorporating the balanced expression of transgenes with distinct mechanisms of action will provide increased and long-lasting resistance to many diseases at the same time. Genes linked to disease resistance are readily available. (Song *et al*., 1995) [46] or genes involved in different defense mechanisms (Huang *et al*., 1994; Velazhahan *et al*., 1998; Takakura *et al*., 2000) [18, 53, 50] and the availability of effective transformation tools and strategies (Christou 1997; Chen *et al*., 1998; Kim *et al*., 2003) [8, 7, 21] has made it easier to pyramid genes implicated with disease resistance in agricultural plants.

The feasibility of multiple gene transformation or gene pyramiding, is highly dependent on the appropriate choice of genotypes/varieties to be altered in a certain crop and the genes to be transformed together. Selection of appropriate genes expressing proteins involved in pathogen identification and/or subsequent activation of signalling pathways leading to

defensive response activation (Van Loon and Van Strien, 1999) [51] as well as genes with direct antibacterial activity (Broglie *et al*., 1991; Yun *et al*., 1998) [6, 56] It is expected that their degradative interaction with pathogen cell wall and cell membrane may increase the possibility of producing crop plants with particular or broad-spectrum resistance to diseases. Plants detect pathogen signals (elicitors), which trigger host-pathogen interactions. Recognition of pathogen elicitors causes several defence responses in hosts, including the formation of defence chemicals known as phytoalexins, pathogenesis-related (PR) proteins, reactive oxygen species evolution, and hypersensitive cell death. During the plant's hypersensitive reaction, PR-proteins and other defense-related chemicals concentrate in plant cells (HR) (Heath, 2000) [17] against pathogen attack. PR-proteins are categorised based on their amino acid sequences, serological connections, and metabolic roles (Van Loon and Van Strien, 1999) [51], and some members of the PR-protein category have received extensive research. (Muthukrishnan *et al.*, 2001) [34]. It has been shown that constitutive and over-expression of PR-proteins in transgenic plants increased resistance to a number of diseases. Endochitinases (EC 3.2.1.14) from the PR-3, 4, 8, and 11 subgroups of PR-proteins (Van Loon and Van Strien, 1999) [51] catalyze the hydrolysis of b-1, 4 linkages between N-acetyl-glucosamine units of chitin, a major polysaccharide component of fungal cell-wall (Broglie *et al*., 1991) [6]. A few chitinase cDNAs have isolated from rice (Huang *et al*., 1994; Takakura *et al*. 2000) [18, 50] and have been employed to transform rice cultivars to engineer sheath blight resistance (Lin *et al*., 1995; Datta *et al.*, 2001; Kumar *et al*., 2003; Kalpana *et al*., 2006) [28, 13, 22, 20]. The expression of genes encoding this protein in plants such as apple, broccoli, carrot, cucumber, peanut, sorghum, strawberry, tobacco, wheat, bent grass, rice, and silver birch gave diverse levels of increased resistance to several fungal diseases (Punja, 2006). Thaumatin-like Proteins (TLP) are PR proteins that cause lysis of fungal cell membranes, resulting in pathogen death via altering cell membrane permeability. (Yun *et al*., 1998) [56]. Examples of thaumatin like proteins include thaumatin (*Thaumatococcus daniellii*), Osmotin (*Nicotiana tabacum*), Zeamatin (*Zea mays*) and permeatin (cereal). Several members of this protein family have antifungal effects and are classified as PR-protein group 5. (PR5). Disease resistance is a complicated attribute governed by several gene groups. As a result, constitutive expression of a single PR-protein transgenic like tlp is unlikely to give significant disease resistance. One of the key reasons for the reported failures is the minimal and narrow range disease resistance imparted by a single PR-protein. However, co-expression of many PR-protein genes, such as chitinase (EC 3.2.1.14) and b-1, 3-glucanase (EC 3.2.1.39), has been demonstrated to be substantially more effective against a variety of fungal diseases.

Table 1: Shows the successfully pyramided genes with their traits of some important crop plants

Crop	traits	Pyramided genes	References
wheat	stem rust, leaf rust and powdery mildew disease	Sr24/Lr24, Sr26 and Sr36/Pm6	Kumaran *et al*., 2021 [26]
	Stripe rust	Yr15, Yr62, Yr65 and Yr26+Yr48, Yr30+Yr64, Yr30+Yr48	Liu *et al*., 2020 [29]
	stem rust, leaf rust and powdery mildew disease	Sr2/Lr27/Yr30, Sr24/Lr24 and Sr36/ Pm6	Aravindh *et al*., 2020 [4]
	Leaf and stem rust resistance	SrCad, Sr33, Lr34, Fhb	Zhang *et al*., 2019 [57]
Rice	Bacterial blight, blast Sheath blight	xa5, xa13, and Xa21 Pi54 qSBR7-1, qSBR11-1, and qSBR11-2	Ramalingam *et al*., 2020 [43]
	Rice blast	Pi-1	Kumar *et al*., 2019 [25]
	Bacterial blight Sheath blight	chi11, tlp, Xa21	Maruthasalam *et al*., 2017 [32]
Tomato	Tomato leaf curl virus	Ty-1/Ty-3, Ty-2, ty-5, and ty-6	Prabhandakavi *et al*., 2021 [39]
	Tomato leaf curl virus Blast resistance	Ty-1, Ty-2 and Ty-3, Ph-2 and Ph-3	Kumar *et al*., 2019 [25]
Potato	Potato late blight	Rpi-rzc1 and Rpi-phu1	Stefańczyk *et al*., 2020 [47]

Future Prospects

1. Need to have better scoring methods, appropriate quantitative genetic analysis, various genetic back grounds and independent verification through advanced generations
2. Mapping of disease resistance gene in major crops.
3. Identify the new resources of desirable resistant genes.
4. It is crucial to create appropriate transformation and regeneration methods that are genotype dependent.

Conclusion

Gene pyramiding is an essential germplasm improvement approach. Breeders must consider the minimal population size that must be examined in order to have a reasonable chance of attaining the desired genotype during pyramiding. Molecular markers genotyping can facilitate the gene pyramiding process by reducing the number of generations that breeders must evaluate to ensure they have gene combinations. It is recommended that breeders and researchers rely more on the identification and use of resistance genes from related species in order to sustain long-term resistance breeding. Gene pyramiding using marker technology may be integrated into existing plant breeding programmes all around the world, allowing researchers to access, transfer, and combine genes at a rate and with precision previously unattainable. Even if other genes do not function in favour of the pyramided line, it can utilize any of the inserted genes to escape, tolerate, or resist stress. Based on the current theoretical and practical information, it is evident that the gene pyramiding method is effective in extending the genetic basis of resistance, and it can be successfully used in an integrated manner with all other management measures.

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