

Gene Pyramiding to Increase the Sustainability of Vegetables under Biotic and Abiotic Stresses

Manish Chauhan¹, Shivangi Negi^{1}, Shilpa² and Priyanka Bijalwan³*
¹Department of Agriculture, Tula's Institute Dehradun (Uttarakhand), India.
²CSK HPKV Palampur, Kangra, (Himachal Pradesh), India.
³Himgiri Zee University (Uttarakhand), India.

(Corresponding author: Shivangi Negi*)

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ABSTRACT: Numerous ecological factors, including drought, extreme temperatures, too much salt, parasitic diseases and insect pest infestation, endanger sustainable vegetable production. Future vegetable production in many nations may be negatively impacted by these difficult environmental factors. In modernized agriculture, conventional crop-breeding techniques are insufficient on their own to sustainably supply the rising population's food need. The advancement of molecular genetics and associated technologies is a promising method for selecting new crop species. Gene pyramiding using marker-assisted selection (MAS) and other strategies have been applied to produce resilient/tolerant lines with high precision and rapid growth for agricultural sustainability. Gene stacking has not been used to its full potential in the majority of the major farmed crops for the development of biotic stress tolerance and quality enhancement. The focus of this review is on gene pyramiding techniques that are successfully used in contemporary agriculture to increase crop tolerance to abiotic and biotic stresses for long-term crop improvement. Overall, gene pyramiding has the potential to revolutionize vegetable production by enhancing crop quality, productivity and sustainability.

Keywords: Gene pyramiding, Marker Assisted Selection, Crop breeding, Vegetable, Stresses.

INTRODUCTION

The green revolution has markedly increased the yield and quality of essential food crops worldwide (Davis *et al.*, 2019). Yet, the traditional crop-breeding approach is insufficient to promote crop development at the rate necessary to fulfill the continually increasing human population's demand for food (Barrett, 2010; Atique *et al.*, 2018). According to the Food and Agriculture Organization, in order to meet the growing population's demand for food, global food production must increase by 70% by 2050. (Mwobobia *et al.*, 2020). So, in order to simultaneously improve numerous agronomic and nutritional traits in crop breeding, smart and rapid tools are needed to meet the growing food demand (Kage *et al.*, 2016).

Food grain yields are affected by a number of factors, and it is estimated that biotic and abiotic stresses globally cause annual losses of about 25% and 6-25%, respectively (Pandey *et al.*, 2017; Waddington *et al.*, 2010). Environmental pressures are significantly affecting agricultural production in many developing nations (Bowman *et al.*, 2013). Plant ailments and insect pests rank as the two most significant biotic stressors for food crops (Belsky and Joshi 2019). A challenge for sustainable agriculture is the wide variety of insect pests and pathogens that are present (Choudhary *et al.*, 2008). Chemical pesticides have been used to reduce biotic damage to crops for a while, but more recently, their use has been discouraged due to a number of negative

factors, including their high cost and the requirement for multiple applications, which most small-scale farmers cannot afford (Sharifzadeh *et al.*, 2018). Chemical pesticides are also a serious threat to both the environment and human health (Vincelli, 2016). Another issue is pest resistance to chemical pesticides.

The majority of crop-breeding techniques for biotic and abiotic stress resistance depend on the insertion of a single resistant gene into plants; as a result, crop resistance is transient (Kottapalli *et al.*, 2010; Steiner *et al.*, 2019). As a result, emphasis is now placed on the making of genotypes that can resist a variety of stresses by stacking numerous genes from various sources into a single plant (Suresh and Malathi 2013). In several studies, the pyramiding of various resistance genes has helped to explain how crop stress tolerance develops (Bai *et al.*, 2018; Ruengphayak *et al.*, 2015). For biotic stress resistance and crop quality enhancement in the majority of the major cultivated crops, this technique hasn't been fully applied (Rana *et al.*, 2019).

In order to protect the yield potential of different crops under stressful growing conditions, advanced and innovative technologies have to be adopted due to the prevalence of crop stresses. The inability to conveniently screen for undesirable genes makes it difficult to improve crops using traditional breeding techniques because not only do some undesirable genes persist even after several backcross generations, but also some desired genes do. Modern molecular technologies have

made it possible to breed animals with greater accuracy, sophistication, and speed than in the past thanks to molecular markers (Kage *et al.*, 2016). Marker-assisted selection (MAS) involves the indirect selection of traits with the marker linked to the desired gene for the tagging of some significant agronomic traits that are otherwise difficult to mark for resistance against pathogens, diseases, and abiotic stresses and thus protects against losses in yield and quality characteristics.

Gene pyramiding was first proposed by Watson and Singh in 1953. A technique known as "gene pyramiding" is used to combine several desirable genes from various parents into a single genotype. Gene pyramiding programmes result in genotypes that contain all of the

target genes. A breeding technique called gene pyramiding brings together several genes with established effects on the traits being targeted. It is primarily used to improve current elite cultivars for a few undesirable traits for which large-scale beneficial genes have been discovered (Malav *et al.*, 2016).

PRINCIPLE

Pyramiding multiple genes is accomplished by mating complementary genes to the parental lines and selecting the desired recombinants from the populations of offspring.

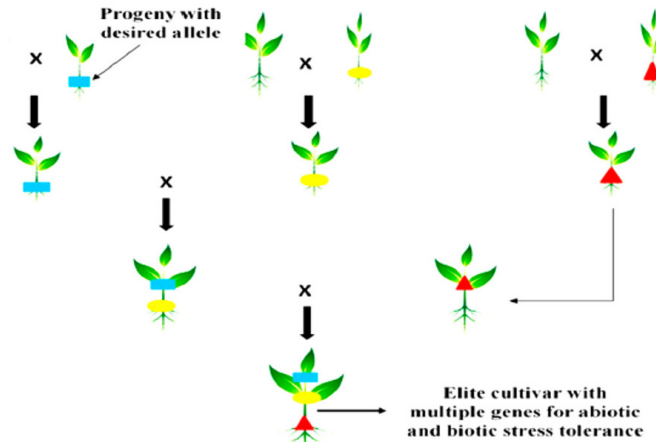
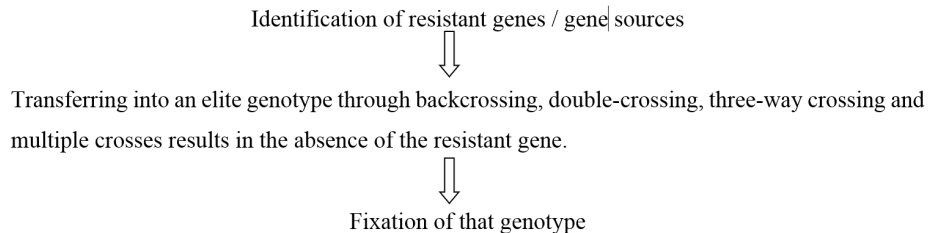


Fig. 1. An illustration of gene pyramiding for improved crop sustainability under biotic and abiotic stress (Dormatey *et al.*, 2020).

Strategy of gene pyramiding



FIXATION OF THE PYRAMIDED GENOTYPE

When character is transferred into line, working with asexually propagated crops has a major advantage over sexually propagated crops in that maintenance is simple.

1. Formation of haploids with doubled DNA from root genotype
2. Selfing the genotype of the root
3. Selfing the offspring after crossing the root genotype with a blank parent
4. Crossing a founding parent genotype with the root genotype

DISTINCT GENE PYRAMIDING SCHEME

In a gene pyramiding scheme, the objective is to combine genes found in multiple parents into a single genotype. The speed of the pyramiding process is accelerated by the use of DNA markers, which enables complete gene identification of the progeny at each generation. Gene pyramiding generally aims to create a homozygous

genotype for the advantageous alleles at all the loci, which is the ideal genotype (Malav *et al.*, 2016). It is possible to divide the gene pyramiding scheme into two parts. The first section, referred to as a pedigree, aims to combine all target genes into a single genotype known as the root genotype. The fixation step, which is the second stage, aims to convert the target genes into a homozygous state, creating the ideal genotype from a single genotype. An intermediate genotype is the term given to each node of the tree and it has two parents. Each of the varieties in this intermediate genotype can withstand. Pyramiding can also be improved by making a parent in the subsequent cross. The intermediate genotypes are not just any offspring of a particular cross; rather, they are a specific genotype chosen from the offspring that possesses all of the parental target genes. Even though the pedigree step of gene pyramiding is frequently used, fixation can be accomplished using a variety of techniques. One method for the fixation steps is to produce a population of doubled haploids from the

root genotype (Ahmed *et al.*, 2020). Here, a population of gametes with doubled genetic material is created from

the genotypes. As a result, there is a population of people who are fully homozygous, and among them, the

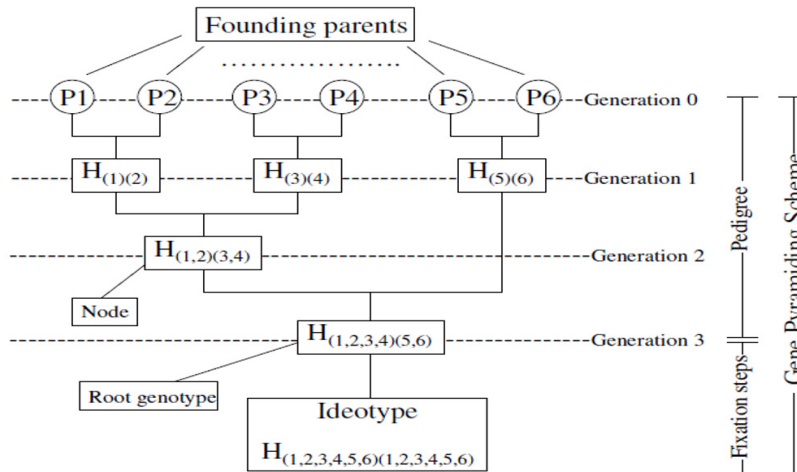


Fig. 2. A distinctive gene pyramiding approach with six target genes (Hospital *et al.*, 2004).

Gene pyramiding's effectiveness depends on
 (i) Number of genes to be transferred.
 (ii) Spacing between flanking markers and the target genes.
 (iii) Number of genotypes chosen during each breeding generation; and type of germplasm.

Types of gene pyramiding

1. Conventional technique: Serial gene pyramiding:

Genes are deployed in same plant one after other

- a) Pedigree breeding
- b) Backcross breeding
- c) Recurrent selection

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

- a) Marker assisted selection
- b) Transgenic method

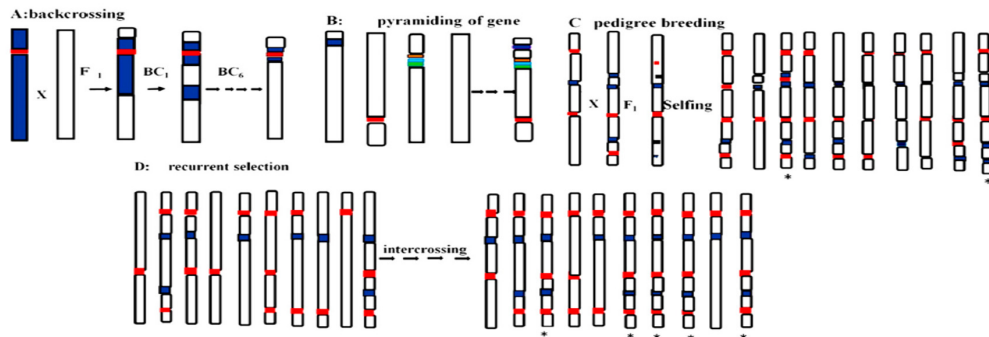


Fig. 3. Conventional techniques of plant breeding (Dormatey *et al.*, 2020).

Drawbacks of Conventional Methods

1. The main objective of gene pyramiding is to enhance qualitative traits like disease and insect resistance. This is related to the fact that the presence of target trait genes must be verified primarily at the individual level through phenotyping, and that an individual's phenotypic performance is only a reliable predictor of the genotype if genes have a significant impact on that performance and the phenotyping error is low.
2. Other factors influencing the success of gene pyramiding include the inheritance model of the genes for the target traits, linkage and/or pleiotropism between the target trait and other traits, in addition to the accuracy of phenotyping at the individual level.
3. For instance, two genotypes of allelic genes cannot be combined. Heterozygous people cannot be tested for a recessive gene's effects, necessitating progeny testing.

4. If the target gene is closely linked to genes that have major adverse impacts on other traits, these undesirable genes might be transferred into the recipient line along with the target gene and lead to a decline in the performance of other traits (linkage drag).

ADVANCES IN MOLECULAR TECHNIQUES FOR BREEDING PROGRAMS

Crop breeding has significantly advanced in recent years, and with the development of modern molecular tools, precision breeding is now possible in the shortest amount of time. Crop varieties are being improved through the use of cutting-edge molecular breeding techniques, which primarily involve MAS and gene transformation. In breeding programmes, single nucleotide polymorphisms and insertion deletion polymorphisms are a good source of MAS because they are widely distributed throughout the plant genome (Hayashi *et al.*,

2004). Building inter- and intra-specific maps has been made easier by the availability of polymorphic markers, linkage maps, and QTLs for various quantitative and qualitative traits. Resistance to numerous plant diseases has been linked to quantitative trait loci. It has not been widely used to link QTLs to traits related to yield (Ahmed *et al.*, 2020). By stacking multiple traits into a variety using contemporary breeding techniques, these can be used as a marker's linkage with key important genes for the selection and improvement of crops.

Molecular technique

Marker assisted selection

1. Use of DNA markers closely linked to target loci in place of or in addition to phenotypic screening.
2. As a "genetic tag" a marker.

3. Using molecular markers to select different traits indirectly.
4. Speeding up the conventional breeding process.
5. Assisting with trait improvement.

MAS in gene pyramiding: Why?

1. For traits that are purely inherited but challenging to phenotypically measure.
- Disease resistance in heterozygous situations, recessive genes can also be assessed.
3. Limiting linkage drag
4. Multiple specific gene selection without phenotyping is possible.

Gene pyramiding has several benefits, including:

1. It is frequently used to combine different disease resistance genes for particular pathogen races.
2. Pyramiding is extremely challenging to accomplish using traditional techniques.

Gene pyramiding to improve crop quality and combat biotic and abiotic stresses

CROP	TRAITS	PYRAMIDED GENES	REFERENCES
Biotic stress tolerance			
Potato	Late blight resistance	Rpi-phu 1, Rpi-zrc	Sliwka <i>et al.</i> , 2012
Cotton	Bacterial blight/sheath resistance	Chi11, t1p, Xa21	Maruthasalam <i>et al.</i> , 2007
	Bollworm resistance	Cry1Ac, Cry2Ab	Jackson <i>et al.</i> , 2004
	Weed and pathogen resistance	ptxD/Phi	Pandey <i>et al.</i> , 2017
	Insect pest resistance	Cry1Ac, Cry2Ac	Puspito <i>et al.</i> , 2015
Wheat	Leaf and stem rust resistance	SrCad, Sr33, Lr34, Fhb	Zhang <i>et al.</i> , 2019
	Cereal cyst nematode resistance	CreX, CreY, CRISPR-Cas9	Ali <i>et al.</i> , 2019
	Aphid resistance	Gn2, Gn4	Liu <i>et al.</i> , 2000
Rice	Gall midge resistance	Gm1, Gm2, Gm4	Das and Rao, 2015
	Blast resistance	Pi(2)t, Pi25, Pi(t)a, Xa4, Xa5, Xa13, Xa21	Joseph <i>et al.</i> , 2004; Singh <i>et al.</i> , 2001
	BPH resistance	Bph1, Bph2	Liu <i>et al.</i> , 2016
	Blight resistance	Xa5, Xa13, Xa21	Narayanan <i>et al.</i> , 2002
	Bacterial, sheath blight, stem borer	Xa12, Rc7, Cry1AB1, Cry14c	Datta <i>et al.</i> , 2002
Soybean	Mosaic virus resistance	Rsv1, Rsv3, Rsv4	Zhu <i>et al.</i> , 2012
Tomato	Leaf curl/spotted virus	Ty-1, Ty-3, Sw-5	Verlaan <i>et al.</i> , 2013
Barley	Mosaic virus resistance	rym4, rym5, rym9, rym11	Werner <i>et al.</i> , 2005
	Strip rust resistance	3 QTL	Castro <i>et al.</i> , 2003
Com	Com borer resistance	Cry1Ie, Cry1Ac	Jiang <i>et al.</i> , 2016
Chickpea	Lepidopteran resistance	Cry1Ac, Cry1Ab	Mehrotra <i>et al.</i> , 2011
Pea	Nodulation ability	Sym9, Sym10	Schneider <i>et al.</i> , 2002
Broccoli	Diamond back moth resistance	Cry1 Ac, Cry1c	Cao <i>et al.</i> , 2002
Pepper	Root-knot nematode resistance	Me1, Me2	Djian <i>et al.</i> , 2014

ACHIEVEMENTS

Gene pyramiding in vegetable crops for disease resistance				
Trait pyramided	Gene/QTL	Donor parent	Type of linked marker	Reference
Meloidogyne hapla of potato	R _{Mh-tar} and R _{Mh-ehca}	<i>S. tarijense</i> <i>S. chacoense</i>	AFLP	Tan <i>et al.</i> , 2008
Late blight of potato	Rpi-mcd1, Rpi-ber	<i>S. microdontum</i> <i>S. berthaultii</i>	CAPS, SCAR	Tan <i>et al.</i> , 2010
TYLCV (Monopartite and bipartite) in tomato	Ty-2, Ty-3	LA3473, CLN2585D, CA4, GC171, TY-172	CAPS, SCAR, SSR	Prasanna <i>et al.</i> , 2014
TYLCV & TOSPO in tomato	Ty-1, Ty-3, Sw-5	LA2779, LA1969	CAPS, RAPD, SCAR,	Consuegra <i>et al.</i> , 2015
Late blight, TYLCV, Bacterial wilt, Fusarium wilt, Gray leaf spot, Tobacco mosaic virus in tomato	Bwr-12, Ty-2, Ty-3, Tm2, Ph-2, Ph-3, Sm	CLN2777G, G2-6-20-15B, LBR -11	CAPS, SACR	Hanson <i>et al.</i> , 2016

3. Take into account how difficult it is to phenotype a single plant for various types of seedling resistance.
4. It's crucial to build "durable" disease resistance against various races.

5. Eliminates extensive phenotyping.
6. Primarily used to enhance existing elite cultivar.
7. controls linkage drag
8. Shortens the time spent breeding.

FUTURE CHALLENGES

There are several future challenges that may arise in the process of gene pyramiding in vegetable production:

1. Identification and selection of suitable genes: The success of gene pyramiding depends on identifying and selecting the most desirable genes that provide the desired traits. This requires extensive research and testing to ensure that the genes are effective and safe to use.

2. Development of efficient gene transfer methods: Gene transfer is the process of introducing the selected genes into the crop variety. While there are several methods available for gene transfer, including traditional breeding, biolistics, and Agrobacterium-mediated transformation, these methods can be time-consuming and costly.

3. Regulatory issues: Gene pyramiding is a highly regulated process, and strict regulations are in place to ensure that the genetically modified crops are safe for human consumption and the environment. Regulatory processes can be complex and time-consuming, and compliance with these regulations can be a significant challenge.

CONCLUSION

A key technique for crop improvement is gene pyramiding. In order to have a reasonable chance of obtaining the desired genotype, breeders must take into account the minimum population size that must be evaluated when using pyramiding. By lowering the number of generations that breeders must test to make sure they have the desired gene combination, molecular marker genotyping can speed up the gene pyramiding process.

FURTHER OUTLOOK

With the development of techniques for marker analysis and the identification of potential genes for economic traits, use of MAS is also anticipated. The application of MAS will be heavily influenced by the anticipated economics. MAS is a compelling alternative for some traits that are expensive or logistically challenging to evaluate. In order to create more enduring and clean transgene technologies that can streamline the process of regulatory approval and reassure consumers about the safety and stability of GM products, it will be necessary to refine the current technique. It would be better to design a vector system that can transfer multiple genes in a single transfer for both nuclear and plastid transformation.

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