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Gene Pyramiding and Its Role in Plant Breeding

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Genetic variation essential for greater chances of survival and flourishing, reduces the incidence of unfavorable inherited traits (Brenner et al., 2002). Plant breeders have also advantage of genetic variants to improve existing plants and create new varieties. Through cross breeding in plants leads to disease resistance in plants as resistance genes inherit in the F1 crosses. The other benefit of genetic variation is production of superior fruit, increased cold tolerance, or other desirable traits.

Gene Stacking

The little modification in the transformation concept comes by which the improvement of crop varieties has been made. The combination of two or more gene of interest in the genome of the host plant i.e. the created genetically modified organism carries two or more different genes and traits.

GM hybrid v/s Gene stacking events

In a genetically modified (GM) hybrid, the transgenic trait originates from the GM inbred parental line that was crossed with one or more non-transgenic elite inbred lines. In a GM staev, two or more transgenic traits are brought together by crossing GM inbred lines, each being different initial events. De schrijver et al. (2007) define “oneway GM stacked events” as stacked events where two transgenic traits are combined, while “three-way gm stacked events” contain three transgenic traits.

Gene Pyrimiding v/s Gene Staking

Gene pyramiding: assembling multiple desirable genes from multiple parents into a single genotype. Gene stacking: combination of two or more trans genes of interest in the genome of the host plant. Transgenic corn triple stacks, for instance containing a corn root worm (CRW) protection trait (*e.g.*, *Cry3B(b)1*), a corn stalk-boring insect control trait (*e.g.*, *Cry1A(b)*), and RR trait for herbicide tolerance.

Why gene stacking?

Why Gene Stacking Compared to mono-trait crop varieties, stacks offer broader agronomic enhancements that allow farmers to meet their needs under complex farming conditions. Biotech stacks are engineered to have better chances of overcoming the myriad of problems in the field such as insect pests, diseases, weeds, and environmental stresses so that farmers can increase their productivity. Strategy for gene stacking

i. Interactive procedure/ Sexual Hybridization
ii. Re-Transformation
iii. Co-Transformation
iv. Linked genes or multigene cassette transformation

Current scenario of gene stacking

It is estimated that a total of 77.7.4 million ha were planted to biotech stacks in 2017. This accounts for more than 41 percent of the 189.8 million ha of biotech crops planted worldwide. 10 The first stack that gained regulatory approval in 1995 was a dual hybrid cotton stack produced by crossing Bollgard™ cotton that expresses the Bt toxin cry1Ab and Roundup Ready™ cotton that produces the epsps enzyme conferring resistance to herbicide glyphosate. Following commercial success of this hybrid stack, developers sought to stack up more biotech traits into their crop portfolio to create multi-stack hybrids. The cotton triple stack which combines two Bt genes with the glyphosate resistance gene occupied more than 54 percent of the US cotton area in 2008. The recently released eight-gene maize stack known by its trade name SmartStax™ is the result of crossing four different biotech maize lines to combine two herbicide tolerance genes with six Bt genes. The resulting stack features dual modes of control for weeds, lepidopteran insects and coleopteran insects and allowed for the refuge requirement to be reduced from 20 percent to 5 percent in the US Corn Belt.11 The increasing number of biotech traits in recent stacks has set the trend for the next generation of biotech crops. Future stacks are likely to involve not only multiple pest resistances but the combination of these traits with engineered metabolic pathways and simultaneous introductions of multiple pathways through metabolic engineering (for example, pathways for beta carotene, ascorbate, folate and vitamin E synthesis) (Halpin, 2005; Naqvi et al., 2009)

Regulatory Approaches

Regulatory principles and procedures for approval and release of biotech stacks differ globally. In countries like the USA and Canada, no separate or additional regulatory approval is necessary for commercializing hybrid stacks that are products of crossing a number of already approved biotech lines. This policy is based on the argument that interactions between individual trait components in a stack that have been shown to pose no environmental or health hazard would not result in new or altered hazards. The US Environmental Protection Agency, however, may require separate safety review of a stack upon identification of a specific hazard associated with combined “plant incorporated protectants” or PIPs (eg. Bt insecticidal proteins), since combinations of PIPs may result in higher or altered toxicity. In Japan and European Union (EU) countries to the contrary, stacks are considered new events, even if individual events have market approval, and must pass through a separate regulatory approval process, including risk assessment of their safety, similar to mono-trait biotech events. Risk assessment is focused on the identification of additional risks that could arise from the combined genes. Possible risks are altered effects of interacting proteins on the target and non target organisms and increased invasiveness of the crop that may pose environmental risks.

Technological Challenges

For the developer, the choice between a large molecular stack or a complex hybrid stack will be based on the monetary cost and timeline for developing and registering a stack. In countries where a stack must pass a separate regulatory review, the one-shot molecular stacking may be more cost effective than the lengthy hybrid stacking. There are, however, technological concerns in molecular stacking which include the design of large multi-gene constructs, method of delivery into plant cells and the stability of expression of multiple genes. Molecular biologists are developing new genetic engineering approaches to address these concerns. Among the promising technologies include site-specific gene recombination systems in conjunction with the use of engineered DNA cutting enzymes and the artificial gene assembly known as minichromosome (Halpin, 2005; Que et al., 2010)

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