Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(4): 1862-1865
Received: 21-05-2018
Accepted: 25-06-2018

Sandeep Kumar Singh
Department of Plant Breeding and Genetics, OUAT, College of Agriculture, Bhubaneswar, Odisha, India

Jyoti Prakash Sahoo
Departments of Agricultural Biotechnology, College of Agriculture, OUAT, Bhubaneswar, Odisha, India

Epsita Swain
Department of Agricultural Sciences, MITS Institute of Professional Science, Rayagada, Odisha, India

Correspondence
Sandeep Kumar Singh
Department of Plant Breeding and Genetics, OUAT, College of Agriculture, Bhubaneswar, Odisha, India

A review on gene stacking in crop plant

Sandeep Kumar Singh, Jyoti Prakash Sahoo and Epsita Swain

Abstract
The desired variability can be achieved by gene stacking. Gene stacking is the process of addition of two or more gene of interest into a single plant. The new evolved trait is known as stacked trait and the crop is known as biotech stacked or simply stacked. In this review we are trying to explain the gene stacking principle, need of the biotech stacked and with its different method of gene stacking.

Keywords: gene stacking, golden rice, blue rose, DFR, co-transformation

Introduction
Variability is the foremost important requirement the for any crop improvement programme. The source of variability may be a local variety of that crop, or wild variety, weedy species or obsolete variety. If there is no desired variability present in these variety then breeder has to create the desired variability by two way, i.e. either he goes for inter specific or inter-generic cross by conventional hybridization method or producing the transgenic plant with the help of gene cloning. Gene stacking is a type of gene cloning that refers to the process of combining two or more genes of interest into a single plant. The emerging combined traits from this process are called stacked traits. A genetically engineered crop variety that bears stacked traits is called a biotech stack or simply stack. An example of a stack is a plant transformed with two or more genes that code for *Bacillus thuringiensis* (*Bt)* proteins having different modes of action. It is a hybrid plant expressing both insect resistance and herbicide tolerance genes derived from two parent plants.

Need of gene stacking
In comparison 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 numerous of problems in the field such as insect pests, diseases, weeds, and environmental stresses so that farmers can increase their productivity. Gene stacking boost up and simplifies pest management for biotech crops as demonstrated by multiple insect resistances based on Bt gene technology. It has shown that the resistance conferred by a single Bt gene has the potential to break down as the target insect pest mutates and adapts to defeat the Bt trait. To prevent or delay the emergence of resistance to the *Bt* gene, require a refuge or an area planted to a non-*Bt* variety alongside the *Bt* crop. Generally a refuge is about 20 percent of the total crop area for a mono-*Bt* trait variety (Fig. 1). While the refuge strategy reduces the chance for the insect pest to overcome the *Bt* trait. The next generation of *Bt* crops with multiple modes of action for insect control were then developed by stacking several classes of Bt genes. This approach has reduced the potential of resistance breakdown though it is more difficult for the pest to tackle multiple insecticidal proteins. This greater durable resistance of *Bt* stacks allow a lower refuge area requirement that somehow limits yield (N.P. Storer *et al.* 2012) [10].

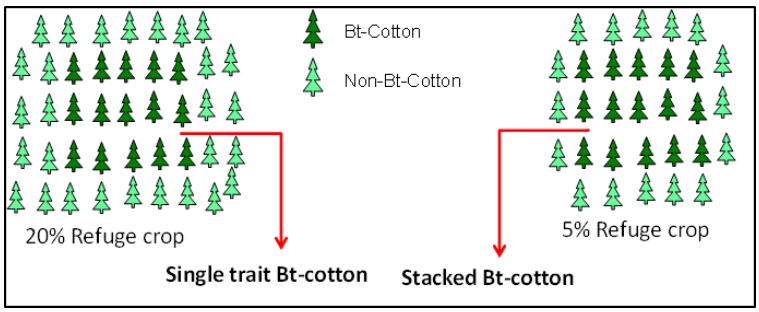

Fig 1: Showing the performance of single Bt-cotton vs Stacked Bt-cotton at field level

In weed management also the *Bt* gene stacking is used. Weed resistance to commercial herbicides has been catalogued for different herbicidal modes of action (International Survey of Herbicide Resistant Weeds. 2017). To catch up in countering weed resistance, biotech seed developers have stacked up genes to widen the herbicidal mode of actions. For example, this is done by combining the glyphosate resistance gene *epsps* with the *pat* gene conferring resistance to herbicide glufosinate and/or with the *dmo* gene conferring resistance to herbicide dicamba. The stack rose with modified flower colour was produced by stacking two genes in the anthocyanin biosynthetic pathway (Fig.4) that altered the flower pigmentation process, yielding the biotech stack rose flowers with novel shades of blue (Y. Tanaka *et al.* 2009) [11]. It is especially useful in metabolic engineering of plants since most metabolic processes and biochemical pathways involve numerous genes interacting with each other (S. Naqvi *et al.* 2009) [8]. For example, the entire pathway for provitamin A (beta carotene) biosynthesis was engineered in the rice endosperm by stacking 2 genes into rice (Fig.3) (P. Beyer, I. Potrykus, *et al* (2000) [12].

Different Process of gene stacking

There are mainly two types of gene stacking process *viz:* hybrid stacking and molecular stacking out of which hybrid stacking is easiest and earliest method of developing stacks.

In hybrid stacking (Fig.2) plants containing several transgenes can be produced by crossing parents with different transgenes until all the required genes are present in the progeny. Development of multi stack hybrid occurs via iterative hybridization.

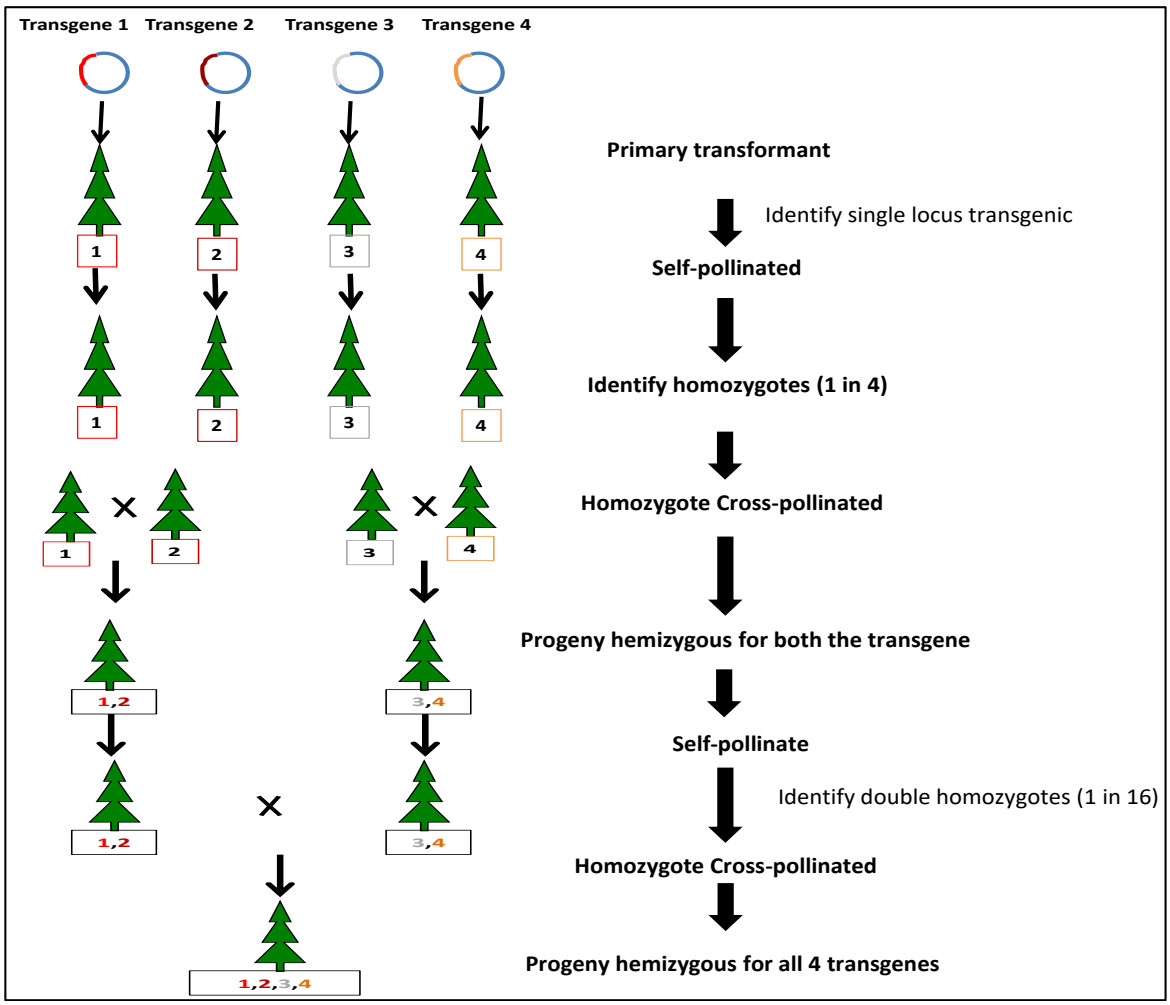

Fig 2: Schematic diagram to understand the hybrid gene stacking process

An early example of the power of this strategy was the production of secretory IgA antibodies in plants by cross-breeding of tobacco to combine, in one plant, four genes encoding different immunoglobulin polypeptides (Ma *et al.* 1995) [6].

Two genes for a bacterial organic mercury detoxification pathway (mercuric reductase, merA, and organomercurial lyase, mer B) were combined by crossing in *Arabidopsis*, and plants expressing both genes were able to grow on 50-fold higher methyl mercury concentrations than wild-type plants (Bizily *et al.* 2000) [1].

There are some limitations like transgenes not linked & can segregate; obtaining homozygous plants for all transgenes is a difficult task. Breeding cost will be increase; variety of selectable markers needed in the re-transformation; marker removal slow, multistep process; labour intensive and time consuming process.

Some stacks developed through this hybrid stacking are: In maize-Agrisure ™, Viptera™ 3220; In Cotton- Roundup Ready™, Flex Bollguard™ II (examples are taken from the GM approval database 2017).

Another way of developing gene stacking is molecular stacking, where the gene construct are inserted simultaneously or sequentially into the target plant by standard delivering system such as Agrobacterium mediated or by biolistic method (C. Halpin 2005, Q. Que *et al.* 2010) [3, 9]. Co-

transformation and Re-transformation may be two approaches of molecular gene stacking process.

Co-transformation

It can be done by either single plasmid co-transformation of linked transgene, where genes to be introduced are linked as single piece of DNA with each gene having its own promoter or by multiple plasmid co-transformation of unlinked transgene, which consist of several plasmid or discrete fragment DNA (if biolistic), each caring a different transgene (including a promoter), that are transformed together via *Agrobacterium* mediated transformation or biolistic method into the plant. Example: Maize-Knock out™, Naturgard™, Bt Xtra™ (examples are taken from the GM approval database 2017).

Production of golden rice (Fig.3) is a good example of co-transformation, where the entire pathway for provitamin A (beta carotene) biosynthesis was engineered in the rice endosperm by stacking 2 genes into rice (From Golden rice project, Golden rice humanitarian board). Out of which one gene was PSY gene (a plant phytoene synthase) from plant Daffodil and other was CRT-I gene (a bacterial phytoene synthase) from bacterium *Pantoea ananatis.* The PSY gene utilizes the endogenous (GGPP) Geranyl Geranyl di phosphate to form Phytoene, which is a colourless carotene. The CRT-I gene encode a bacterial carotene desaturation that introduce conjugation by adding 4 double bonds. The combined action of PSY and CRT-I gene yielded the lycopene which is a red colour compound found in tomato but not in rice. Instead of lycopene golden rice have α/β-carotenes and after oxygenation that produce variable amounts of Xanthophyll like lutein and zeaxanthin.

The main limitation of this co-transformation are difficulty to assemble complex plasmids with multiple gene cassettes; Problem of Gene silencing if same promoter is used with each transgene; high copy number integrating; undesirable incorporation of a complex T-DNA molecules from multiple sources.

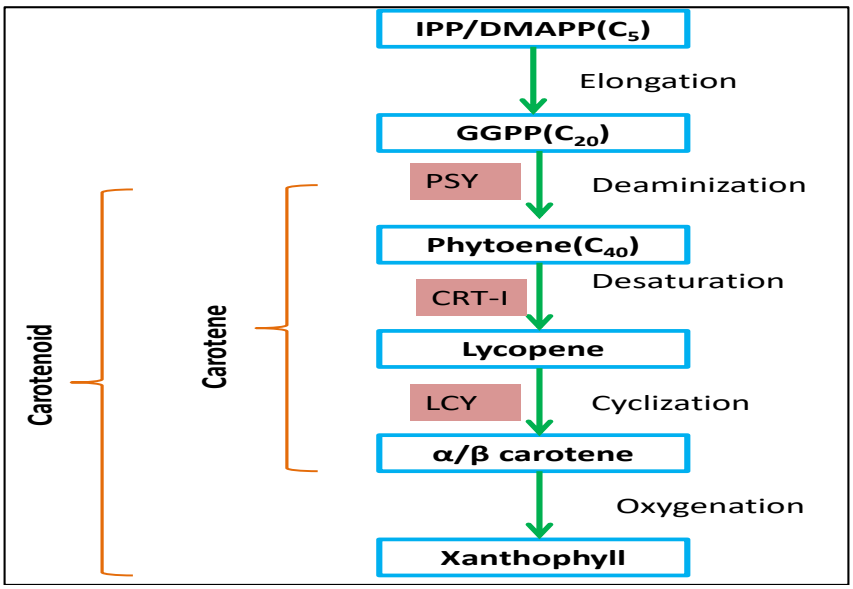

Fig 3: Schematic diagram of production of golden rice

Transgenes derived from different sources typically integrate at different locations in plant genome, which may lead to various expression patterns and possible segregation of the transgenes in the offspring.

Re-transformation

In this process a plant harbouring a transgene is transferred again with other gene. It is a multi-trait or combined trait event with separate inserts. The GM plant produced by iterative event with separate inserts transformation with vectors containing different transgenes/traits. The transgenic inserts are integrated in multiple loci. Multiple transgenes either harboured within different t-DNA in single *Agrobacterium* strain or harboured separately within different strain. The production blue shaded rose in collaboration of Australian Florigene Company with Japanese company Suntory is a good example of it. Generally the flower colour of wild roses is mainly due to the pelargonidin and/or cyanidin based anthocyanins, which are the two main endogenous flavonoid expressed in rose petals. Blue coloured flower contain the delphinidin based anthocyanin and roses don't have the pigments. Thus, they lack violet/blue varieties. This is ascribed to their deficiency of flavonoid 3',5'-hydroxylase (F3'5'H), a key enzyme in the synthesis of delphinidin (Fig. 4) (Holton and Tanaka 1994) [11]. So scientist first transferred the F3'5'H gene isolated from pansy flower to rose. But due to the presence of the endogenous DFR (a crucial protein for pigmentation in rose) the modified flower produces the original colour of rose with blue shades. So it required to downregulate the rose DFR gene and overexpression of F3'5'H gene. Researchers then use RNAi technology to depress the colour production by the rose DFR protein. Then a new iris DFR protein gene was re-transferred from iris to produce the true blue rose (Yukihisa Katsumoto *et al.* 2007) [13].

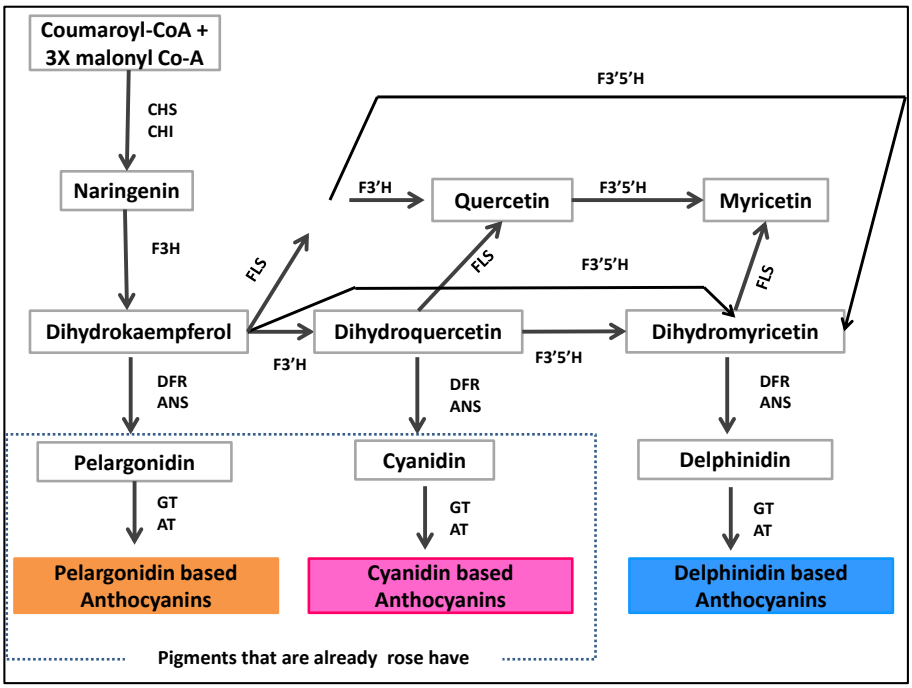

Fig 4: Generalised flavonoid biosynthesis pathway relevant to flower colour pigmentation. Native rose petals only accumulate pelargonodin and cyanidin-based anthocyanins, mainly pelargonidin and cyanidin 3, 5-diglucoside. Lack of delphinidin-based anthocynanins, which is attributed to deficiency of F3'5'H, has hampered the generation of rose flowers having blue and violet hues. The expression of a hetelorogous F3'5'H gene in rose is expected to generate delphinidin and, thus, a novel flower colour with a blue hue. CHS: chalcone synthase; CHI: chalcone isomerase; F3H: flavanone 3-hydroxylase; F3'H: flavonoid 3'-hydroxylase; F3'5'H: flavonoid 3', 5'-hydroxylase; FLS: flavonol synthase; FNS: flavone synthase; DFR: dihydroflavonol 4-reductase; ANS: anthocyanidin synthase; GT: anthocyanidin glucosyltransferase; AT: anthocyanin acyltransferase

There are some limitations also like Re- transformation can induce transgene silencing; Need for a range of selectable marker gene so that a different one can be used with each sequential transformation.

Example: Cotton-Bollgard™ II (examples are taken from the GM approval database 2017).

Conclusion

The first stacked get regulatory approval in 1995 is dual hybrid cotton stack producing by crossing Bollgard™ cotton that express Bt toxin Cry IA(b) and Round up Ready™ cotton that express epsps gene conferring resistance to herbicide glyphosate. However there are some technological concerns in molecular stacking w including 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. The site-specific gene recombination systems in conjunction with the use of engineered DNA cutting enzymes and the artificial gene assembly known as mini-chromosome are some promising technologies.

References

1. Bizily S, Rugh CL, Meagher RB. Phytodetoxification of hazardous organomercurials by genetically engineered plants. Nat Biotechnol. 2000; 18:213-217.
2. Golden rice project, Golden rice humanitarian board http://www.goldenrice.org/Content2-How/how1_sci.php
3. Halpin C. Gene stacking in transgenic plants – the cha llenge for 21st century plant biotechnology. Plant Biotechnology Journal. 2005; 3:141-155
4. International Survey of Herbicide Resistant Weeds. Herbicide-Resistant Weeds by Site of Action, 2017. http://www.weedscience.org/Summary/SOASummary.aspx.
5. ISAAA. ISAAA. GM Approval Database, 2017. http://www.isaaa.org/gmapprovaldatabase/event/default.asp.
6. Ma JK, Hikmat BY, Wycoff K, Vine ND, Chargelegue D, Yu L, Hein MB, Lehner T. Characterization of a recombinant plant monoclonal secretory antibody and preventive immunotherapy in humans. Nat Med. 1998; 4:601-6. PMID:9585235;
7. Ma JK, Lehner T, Stabila P, Fux CI, Hiatt A. Assembly of monoclonal antibodies with IgG1 and IgA heavy chain domains in transgenic tobacco plants. Eur J Immunol. 1994; 24:131-8. PMID:8020548.
8. Naqvi S, Farre G, Sanahuja G, Capell T, Zhu C, Christou P. When more is better: multigene engineering in plants. Trends in Plant Science. 2009; 15:48-56.
9. Que Q, Chilton M, de Fontes C, He C, Nuccio M, hu Z, *et al*. Trait stacking in transgenic plants – challenges and opportunities. GM Crops. 2010; 1(4):220-229.
10. Storer NP, Thompson GD, Head GP. Application of pyramided traits against Lepidoptera in insect resistance management for Bt crops. GM Crops and Food. 2012; 3(3):154-162.
11. Tanaka Y, Brugliera F, Chandler S. Recent Progress of flower colour modification by biotechnology. Int. J Mol. Sci. 2009; 10:5350-5369
12. Ye XD, Al Babili S, Kloti A, Zhang J, Lucca P, Beyer P, Po trykus I. Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science. 2000; 287:303-305.
13. Yukihisa Katsumoto, Masako Fukuchi-Mizutani, Yuko Fukui, Filippa Brugliera, Timothy A Holton, Mirko Karan, *et al*. Engineering of the Rose Flavonoid Biosynthetic Pathway Successfully Generated Blue-Hued Flowers Accumulating Delphinidin. Plant Cell Physiol. 2007; 48(11):1589-1600.