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Genetic modification for crop improvement

Genetic modification (GM) of crops involves the transfer of genes for specific traits between distantly related plant species using recombinant DNA technology known as transgenesis. Besides, crops can also be modified by silencing or overexpressing its endogenous gene. Transgenesis has been extensively utilized to incorporate desirable traits in plants like enhanced yield, nutritional value (high vitamin, healthy fatty acid/amino acid content), herbicide resistance, enhanced fruit shelf life, increased resistance to various biotic (bacterial, fungal, nematodes and other pathogens) and abiotic stresses(extreme temperature, salinity and drought). Currently, several varieties of GM crops such as cotton, soya, maize, potato, sugar beet, alfalfa, and canola are grown throughout the world. Tomato, soyabean and lathyrus transgenic plants expressing Oxalate decarboxylase gene (OXDC) from *Flammulina velutipes* showed significant reduction in the level of anti-nutrient oxalic acid and were resistant to fungal infection.

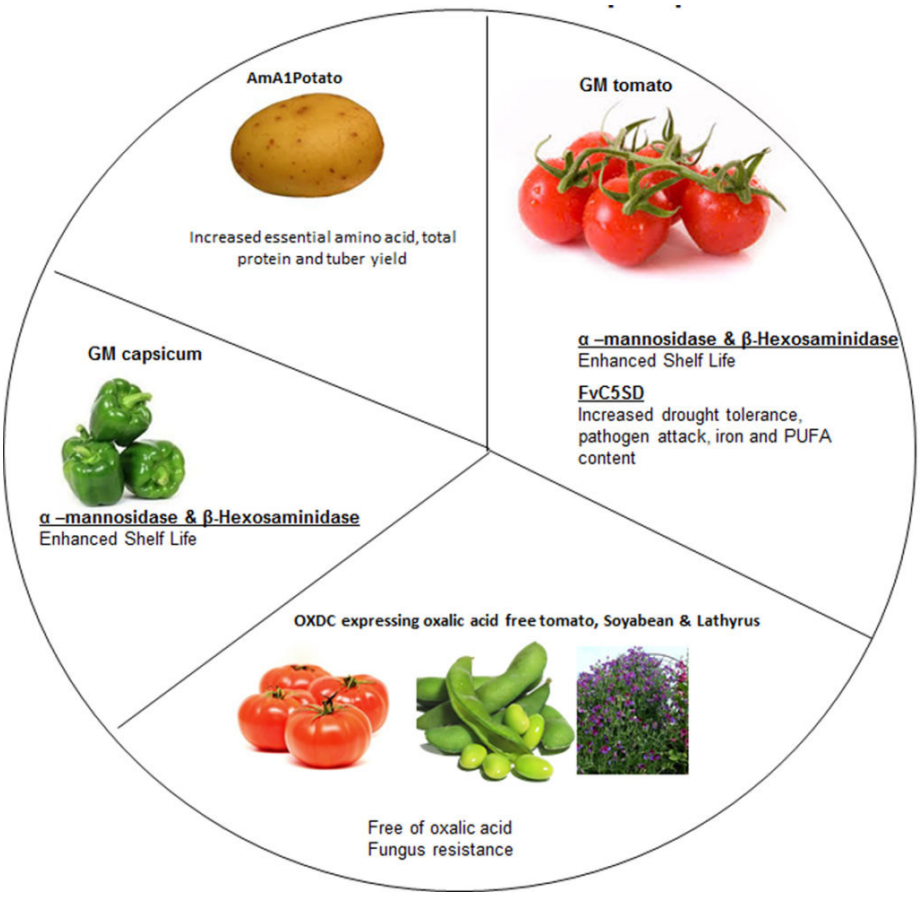

Fig. 1. Genetic modification for crop improvement.

Transgenic potato expressing AmA1, the seed albumin protein from *Amaranthus hypochondriacus* showed increased level of essential amino acids, total protein content and tuber yield. Tomato and capsicum knockdown lines of enzymes α-mannosidase (α- man) and β-hexosaminidase (β-hex) exhibited delayed

ripening and increased shelf life. Tomato transgenics expressing sterol desaturase from *F. velutipes* (FvC5SD) showed multiple beneficial traits like drought and pathogen tolerance, enhanced iron and beneficial polyunsaturated fatty acids. Being precise and rapid, transgenesis is advantageous than conventional breeding practices which are time consuming and can involve transfer of undesirable genes along with desirable genes. But, GM crops developed through transgenesis have been facing increased disapproval and lack of consumer acceptance due to the associated biosafety issues like environmental and food safety risks. GM crops developed by transgenesis have an antibiotic resistance gene for selection which can escape into the environment (non-GM crops or related wild species) and lead to health problems upon consumption. These limitations, encouraged the development of alternative concepts of cisgenesis and intragenesis that involve transformation of plants with the DNA derived from the species itself or from closely related species capable of sexual hybridization. Recombinase technology involving site specific integration of transgene can overcome limitations associated with traditional genetic engineering methods. *Agrobacterium*-mediated plant transformation and particle bombardment are based on random integration of multiple copy of transgene into plant genome leading to gene silencing and unpredictable expression pattern. Besides, site-specific recombination (SSR) technology, co-transformation is another strategy to generate marker-free transgenic plants. It involves the *Agrobacterium*- mediated co-transformation of a selectable marker gene and a gene of interest from different T-DNAs followed by segregation of the genes in the subsequent sexual generation.

The recently developed breakthrough technology of genome editing involves engineered nucleases such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspersed short palindromic repeats/CRISPR associated nuclease (CRISPR/Cas9). This is a highly advanced tool in which targeted DNA double-strand breaks (DSBs) at a locus of interest can be utilized efficiently to introduce precise modifications in the plant genomes. Genome engineering allows the modification or mutation of genes of interest without involving foreign DNA resulting in plants that might be considered equivalent to non-transgenic genetically altered plants. This would promote development and commercialization of transgenic plants with superior phenotypes even in countries where GM crops are poorly accepted. CRISPR/Cas9 is comparatively simpler, efficient, inexpensive, and user friendly tool. ZFN and TALEN methods are laborious and cumbersome and has not been utilized much by plant researchers due to several limitations associated with their design. CRISPR/Cas9 can not only be used efficiently to generate knockouts in orphan crops but can also be used to target multiple genes simultaneously.

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