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Multigene engineering

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The current global population of 6.4 billion is expected to reach 10 billion by the year 2050. The rate of agricultural yield at present is not sufficient to meet this demand and already malnutrition and starvation are taking a toll worldwide. In the past, productivity of primary producers, namely higher plants, has been accomplished through selective breeding programs but the success in this area have reached a plateau. Bringing new acreage under cultivation is not a viable option as much of the unutilized lands in developing nations is of marginal quality and serious environmental consequences prohibit agricultural development on remaining fertile preserves. The architects of the 'green revolution' envisioned that increased global carrying capacity would result from the development of new crop cultivars, the use of irrigation systems, and the application of chemical fertilizers and pesticides. Crop species have been genetically engineered to resist viral pathogens and insect pests, tolerate drought and herbicide treatment and to enhance nutritional value through the incorporation of novel DNA into the nuclear genome.

Plant metabolic engineering has the potential to provide for the needs of an expanding population. Environmentally benign biosynthesis of novel materials and pharmaceutical proteins along with the opportunity to improve the productivity and nutritive value of crop plants has focused considerable effort towards the genetic manipulation of crop species. The most important output traits that could be conferred through biotechnology often require the coordinated expression of several foreign genes. Many of the desired agronomic traits under development will require the simultaneous engineering of multiple genes or pathways. The ability to provide the coordinated expression of multiple genes to produce valuable agronomic traits is considered the Holy Grail of plant biotechnology but this area remains a challenging one for those involved in nuclear genetic engineering.

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Nuclear genetic engineering:

Multiple genes have been skillfully engineered via the nuclear genome. A significant step in multigene engineering has been the development of a rice variety that accumulates provitamin A. It is estimated that improved Vit.A nutrition can help to prevent over one to two million deaths each year among children aged one to two years. But there are some difficulties to introduce multiple genes into the nuclear genome.

Disadvantages of nuclear genetic engineering:

- Position effect
- Gene silencing
- Stunted growth
- Sterility

One common concern arising from the use of nuclear transgenic crops expressing Bt toxins in suboptimal levels is the development of Bt-resistant pests. Plant-specific recommendations to reduce the development of Bt resistance include increasing Bt expression levels (high dose strategy), expressing the protein only in tissues highly sensitive to damage (tissue specific expression), or expressing multiple toxins (gene pyramiding).

The introduction of multiple genes via the nuclear genome requires the generation of individual transgenic plants and subsequent backcrosses to reconstitute the entire pathway or multi-subunit proteins.

Crop plants possess two genomes in addition to that of the nucleus, the organellar genomes of mitochondria and chloroplasts. Genetic engineering of higher plant chloroplasts may offer the potential to mitigate certain limitations of agricultural productivity. Technological advances, most notably the invention of the particle accelerator and the ability to express foreign genes in plastids, have provided the opportunity to explore the chloroplast genome as a new platform to address current and future demands for improved food production. The concept of chloroplast genetic engineering has been demonstrated to confer desirable plant traits including insect resistance, herbicide resistance, salt tolerance, drought tolerance, disease resistance, phytoremediation and reversible male sterility.

Plastid operons and multigene engineering :

That the plastid expression system allows for the transcription of operons from a single promoter to produce translatable polycistronic mRNAs offers great potential for metabolic engineering. The ability to transform the plastome for multiple genes in a single recombination event makes possible the expression of multi-enzyme pathways in the first transformed generation eliminating the need to cross lines recombinant for individual genes. Simultaneous integration of selectable markers along with genes of interest assures that regenerants expressing said markers will harbor the entire transformation cassette. Multi-gene engineering permitted the 'double gene single selection' system that facilitated the generation of homoplasmic cotton transformants through the ability to apply selective pressure in green as well as non-green stages of development.

Chloroplast genetic engineering:

Chloroplast genetic engineering is emerging as an alternative new technology that overcomes many of the environmental concerns of nuclear genetic engineering. One common environmental concern is the escape of foreign genes through pollen or seed dispersal, from transgenic crops to their weedy relatives, creating super weeds, or, among other crops, causing genetic pollution. Although pollen from a few plants contains metabolically active plastids, the plastid DNA itself is lost during the process of pollen maturation and hence is not transmitted to the next generation. Maternal inheritance of foreign genes through chloroplast genetic engineering is highly desirable when there is potential for outcrossing among crops or between crops and weeds.

Introducing blocks of foreign genes in a single transformation event would avoid complications inherent in putting one gene at a time into random locations in the nuclear genome, a process that results in position effects or gene silencing. This is especially true for multi-subunit biopharmaceutical proteins (such as monoclonals) in which subunits should be synthesized in stoichiometric amounts for complete assembly. Crystallization of foreign proteins should also serve as a model system for large-scale production of foreign proteins within chloroplasts in a folded configuration, which enhances their stability and facilitates single step purification via centrifugation. This is the first demonstration of the expression of a bacterial operon in transgenic plants and opens the door to engineering novel pathways in plants in a single transformation event.

Chloroplast genetic engineering has been accomplished so far only in tobacco and potato. However, genes for herbicide, insect, and pathogen resistance and drought tolerance have been expressed to very high levels, conferring the desired traits. Chloroplast genome has been shown to express biopharmaceuticals, including human proteins, at levels 300-fold higher than nuclear expression, resulting in a properly folded and fully functional configuration. Efforts are underway to extend chloroplast genetic engineering to other economically important crops both in academic and industrial laboratories. All of these findings augur well for environmentally friendly genetic engineering approaches in the next generation of transgenic crops. It is the advanced concept of inserting transgenes into functional operons and transcriptionally active spacer regions. This approach facilitated the insertion of multiple genes under the control of single promoter, enabling the coordinated expression of transgenes. Chloroplast transgenic plants showed normal growth and physiology and no pleiotropic effects. These developments should help to allay public concerns and make genetically modified food more acceptable.

Thus, plant biotechnology is at the threshold of an exciting new era in which the emphasis is on the introduction of traits that require the manipulation of metabolic pathways or coordinated expression of multisubunit proteins. The development of rice variety enriched in provitamin A is an early success story in this new era. The chloroplast transgenic approach has facilitated expression of bacterial operons and biopharmaceuticals at unprecedented levels, never before reported in the literature. These exciting achievements not only relieve concerns about gene silencing and position effects but also eliminate the need for time consuming breeding to bring multiple transgenes within a single host. In addition, these advances offer several environmentally friendly features including gene containment. The new era will rely heavily on both nuclear and chloroplast multigene engineering technologies to utilize the new knowledge acquired in the post genomic era for biotechnological applications and to understand complex metabolic pathways.

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