

Review Article

<https://doi.org/10.20546/ijcmas.2020.904.092>

Intragenesis as a Sustainable Crop Improvement Method: A Review

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ABSTRACT

Keywords

Genetically modified organisms, Intragenic modification, Sexually compatible gene pool

Article Info

Accepted:
07 March 2020
Available Online:
10 April 2020

Our society has a great concern about transgenic crops regarding mixing of genetic materials between species whose hybridization is not possible naturally. Different public policies on the development and use of genetically modified organisms (GMOs) are of high concern with defining proper risk management strategies. Researchers are in agreement that this technology “tampers with nature” in an unacceptable manner. This has been recognised as an objection to the crossing of species borders in generation of transgenic organisms. There are recent projects of genetic modification which is aimed at accommodation of the above mentioned concerns by alteration of expression of endogenous genes rather than introduction of genes from other species. Several surveys show higher public acceptance of intragenic/cisgenic crops compared to transgenic crops. Therefore, although the intragenic and cisgenic concepts were internationally introduced only in last decade, several different traits in different variety of crops have recently been modified according to these concepts. The main moral reason for intragenic modification, is the requirement to respect the “otherness” of nature.

Introduction

The area of agricultural land utilized for the production purpose of transgenic (genetically modified or GM) crops has augmented at an

unprecedented swiftness as the journey of these crops were commenced about 22 years ago. Even though the introduced Genetically Modified crops have been exceedingly triumphant, it is evident that only a part of the

potential of genetic modification of crop plants is being grasped (Holme *et al.*, 2013).

The technological as well as economical inconveniences are, however, only a fraction of the dilemma. Worldwide, the GM-technology has been met considerable scepticism among the common people and in effect thereof by the producers also, the industry along with the retailers.

Various studies evidently demonstrate that one of the chief anxieties of the public about transgenic crops is the combination of genetic elements artificially derived from diverse organisms that are not crossable by the natural means (Gaskell and Bauer, 2001; Bauer and Gaskell, 2002; Lassen *et al.*, 2002).

This reluctance is linked time and again to a kind of respect for mother nature and also comes into view to be inter-linked with worries for impending health jeopardies and for the scattering of novel gene-combinations into the environment.

Bearing public worries about safety issues in mind concerning the GM crops in addition to the aspire of meeting these doubts and at the same time certifying an environmentally sound and resourceful plant production, the concept of intragenesis was developed as an efficient substitute to the transgenic crop development (Jacobsen and Schouten, 2007).

The concept is based on the elite utilization of genetic material from the same species or genetic material from the species that are closely-related and able to perform sexual hybridization.

This is in distinguishing to transgenesis where the genes as well as DNA sequences can be shifted between any species (Rommens *et al.*, Jacobsen and Schouten, 2007).

Issues associated with traditional plant breeding

Unintentional transfer of undesirable genes

In their hard works to have room for the evolving requirements of growers as well as consumers, plant breeders take up any accessible tool to recognize the strongest feasible traits. In one application, a quantity of the genetic diversity that is obtainable within a sexually compatible group is captured by making crosses between the cultivated varieties and the wild relatives and screening the consequential F₁ hybrids for a novel trait. Through performing extensive backcrossing, a part of the wild DNA, representing at least ~1% of the whole genome and encompassing the selected trait, is introgressed into the existing varieties.

Significantly, the DNA segment that is introgressed may include one or numerous genes that are linked with detrimental characteristics. Over and above frequently observed linkage yield drag (Kopisch-Obuch *et al.*, 2005), introgression can consequence in ambiguous alterations linked to lower quality of food. For example, transfer of “crisp chip” and “high starch” traits from *Solanum chacoense* to cultivated potato (*Solanum tuberosum*) augmented the levels of glycoalkaloid in the resultant variety Lenape to approximately twice concentration of the highest allowed limit (354 µg kg⁻¹) (Akeley *et al.*, 1968; Zitnack *et al.*, 1970).

Persistent incidence of plant-produced toxins or allergens

More than 99% of the total dietary intake of toxins has been estimated to be generated by the food crops themselves (Ames *et al.*, 1990). Products, for example, acrylamide, exhibit properties of being toxic as well as carcinogenic and gather in, such as, bread

crusts along with the surface of potato chips in addition to French fries (Tareke *et al.*, 2002). The normal acrylamide dietary intake level is 28 $\mu\text{g day}^{-1}$ (www.cfsan.fda.gov/~dms/acryexpo.html.) and it could be lessened to slight quantities by the use of wheat and potato varieties accumulating stumpy levels of asparagine.

Unfortunately, breeders have not yet selected for low asparagine, and there are no acceptable varieties available at present that unexpectedly demonstrate this trait. Thus, labours to produce low-asparagine potato or wheat would necessitate both identification of source and introgression of trait, a procedure that may take a long time of 20 years (Rommens, 2007).

The availability of genes in crops of these days that are allergen-encoding is an even more vital concern. A solitary peanut can be menacing to people's life liable to budding allergy reactions, and the consumption of bread often injures the intestinal lining of 0.8% of the Americans unintentionally that suffer from gluten sensitivities (Harrison *et al.*, 2007).

Although a number of allergen-encoding genes have been inactivated through the use of mutagenesis, it would be complicated to get rid of all 20-80 genes from the crops such as rice, wheat, soybean, peanut, and apple that encode allergens or suspected allergens (Gendel and Jenkins, 2006).

The regulatory agencies resist the deliberate utilization of genes that are identified to generate toxins, allergens, or anti-nutritionals (Kaeppler, 2000) but can accomplish petite to stop the unchecked transfer of such genes through conventional breeding (Bradford *et al.*, 2005). There is another major issue of dormant traits activation.

Intragenesis

Rommens introduced the definition of the concept of intragenic transformation in the year 2004. In intragenesis, diverse plant genetic components are recombined *in-vitro* to raise an expression gene construct that is then introduced into a plant falling within the same sexually compatible gene pool (Rommens 2004; Rommens *et al.*, 2007).

Thus, the source of the genes for the genetic modification in case of intragenesis is from the same or a species that falls within crossable limit. Intragenes are hybrid genes. Selected genetic elements from various genes can be merged together in intragenesis (Rommens *et al.*, 2007). Intragenesis permits for the design of cassettes uniting definite genetic elements from plants belonging to the identical sexually compatible gene pool. So, coding regions of one gene (with or without the introns) can be combined with the promoters and the terminators from various genes from the same sexually compatible gene pool.

Moreover, silencing constructs can be designed by merging a number of different genetic elements from the group having the same sexual compatibility. When employing *Agrobacterium*-mediated transformation, the T-DNA border sequences should derive from the sexually compatible DNA pool (P-DNA borders). The phenotype of a plant achieved through the intragenesis is not attainable through the approaches of traditional breeding as the expression profile of the freshly accumulated gene may vary from that detected naturally (Schaart and Visser 2009; Devi *et al.*, 2013). Antisense or RNA interference (RNAi) can be utilized for achieving the purpose of silencing the gene(s) in case of intragenesis (Schaart and Visser 2009) (Table 1 and 2).

Table.1 Examples of traits that can be incorporated into a plant by either transferring or modifying the expression of native genes

| Trait | target plant | target gene | approach | Year | Reference |
|-------------------------------|-------------------------|----------------|---|------|------------------------------------|
| Extended shelf life | tomato | Pg | fruit-specific silencing | 1988 | Sheehy <i>et al.</i> , 1988 |
| Extended shelf life | tomato | Acc oxidase | fruit-specific silencing | 1991 | Oeller <i>et al.</i> , 1991 |
| Extended shelf life | tomato | Acc synthase | fruit-specific silencing | 1998 | Liu <i>et al.</i> , 1998 |
| Increased vitamin-E content | Arabidopsis | gmt | seed-specific overexpression | 1998 | Shintani <i>et al.</i> , 1998 |
| Reduced glycemic index | potato | Sbe I + Sbe II | tuber-specific silencing | 2000 | Schwall <i>et al.</i> , 2000 |
| Increased xanthophyll content | tomato | Lcy + Chy | fruit-specific overexpression | 2002 | Dharmapuri <i>et al.</i> , 2002 |
| Heat-stable vegetable oil | cottonseed | Fad2 | seed-specific silencing | 2002 | Liu <i>et al.</i> , 2002 |
| Increased zeaxanthin content | potato | Zep | tuber-specific silencing | 2002 | Romer <i>et al.</i> , 2002 |
| Increased anthocyanin content | tomato | Ant1 | fruit-specific overexpression | 2003 | Mathews <i>et al.</i> , 2003 |
| Increased vitamin-C content | strawberry ^a | GalUR | constitutive overexpression ^b | 2003 | Agius <i>et al.</i> , 2003 |
| Increased vitamin-E content | soybean | Vte3 + Vte4 | seed-specific overexpression ^c | 2003 | Van-Eenennaam <i>et al.</i> , 2003 |
| Late blight resistance | potato | RB | use of original promoter | 2003 | Song <i>et al.</i> , 2003 |
| Reduced allergen content | soybean | Gly m Bd 30 K | constitutive silencing ^d | 2003 | Herman <i>et al.</i> , 2003 |
| Enhanced aroma | potato | Cgsb | constitutive overexpression ^b | 2003 | Di <i>et al.</i> , 2003 |
| Increased flavonol content | potato | Chi | tuber-specific overexpression | 2004 | Lukaszewicz <i>et al.</i> , 2004 |
| Heat-stable vegetable oil | soybean | Fad3 | seed-specific silencing | 2004 | Fillatti <i>et al.</i> , 2004 |

| | | | | | |
|---|--------------|----------------|--|------|---------------------------------------|
| Bruise tolerance | potato | Ppo | tuber-specific silencing | 2004 | Rommens <i>et al.</i> , 2004 |
| Increased carotenoid and flavonoid content | tomato | Det1 | fruit-specific silencing | 2005 | Davuluri <i>et al.</i> , 2005 |
| Reduced allergen content | apple | Mal d 1 | constitutive silencing ^d | 2005 | Gilissen <i>et al.</i> , 2005 |
| Reduced allergen content | peanut | Ara h 2 | constitutive silencing ^d | 2005 | Dodo <i>et al.</i> , 2005 |
| Extended shelf life | tomato | Dhs | constitutive silencing ^b | 2005 | Wang <i>et al.</i> , 2005 |
| Increased α-carotene content | potato | Lcy-e | tuber-specific silencing | 2006 | Diretto <i>et al.</i> , 2006 |
| Enhanced aroma | tomato | Aadc1A | constitutive overexpression ^b | 2006 | Tieman <i>et al.</i> , 2006 |
| Enhanced flavor | potato | R1 + PhL | tuber-specific silencing | 2006 | Rommens <i>et al.</i> , 2006 |
| Reduced heat-induced acrylamide content | potato | R1 + PhL | tuber-specific silencing | 2006 | Rommens <i>et al.</i> , 2006 |
| Reduced lignin content | alfalfa/feed | C3H | silencing in vascular tissues | 2006 | Ralph <i>et al.</i> , 2006 |
| Reduced allergen content | tomato | Ltpg1 or Ltpg2 | constitutive silencing ^d | 2006 | Le <i>et al.</i> , 2006 |
| Increased folate content | tomato | Acds | fruit-specific overexpression | 2007 | Diaz de la Garza <i>et al.</i> , 2007 |
| Reduced heat-induced acrylamide content | potato | Apg1 | tuber-specific overexpression | 2007 | Rommens, 2007 |
| Reduced heat-induced acrylamide content | potato | Asn1 + Asn2 | tuber-specific silencing | 2007 | Rommens, 2007 |

^aConcept demonstrated in Arabidopsis. ^bMolecular strategies may be improved upon by employing tissue-specific promoters. ^cGene isolated from Arabidopsis. ^dMultigene silencing constructs may be used to simultaneously inactivate various allergen-encoding genes.

Table.2 Intragenic crops developed or currently under development

| Crop | Type | Prom./term./spacer from | Gene | Trait | Reference |
|--------------------|----------------|---|--------------------|--|--------------------------------|
| Potato | Silencing | GBSS/nos Spacer: GBSS-fragment GBSS/GBSS Spacer: GBSS-fragment | GBSS | High amylopectin | de Vetten <i>et al.</i> , 2003 |
| Potato | Silencing | GBSS/Ubi3 Spacer: Ubi7-fragment | Ppo | Preventing black spot bruise | Rommens <i>et al.</i> , 2004 |
| Potato | Silencing | GBSS/Ubi3 Spacer: Ubi7-fragment | Ppo, R1, PhL | Preventing black spot bruise. Limiting cold-induced degradation | Rommens <i>et al.</i> , 2006 |
| Potato | Silencing | Prom.Agp/Prom.GBS* Spacer: Ubi7-fragment | StAs1,S tAS2 | Limit acrylamide in French Fries | Rommens <i>et al.</i> , 2008 |
| Potato | Silencing | Prom.Agp/Prom.GBS* Spacer: Ubi7-fragment | StAs1 | Limit acrylamide in French Fries | Chawla <i>et al.</i> , 2012 |
| Apple | Expression | RbcS/RbcS | HcrVf2 | Scab resistance | Joshi <i>et al.</i> , 2011 |
| Strawberry | Overexpression | FaExp2/FaExp2 | PGIP | Gray mould resistance | Schaart, 2004 |
| Alfalfa | Silencing | PromPetE/PromPetE* Spacer: Comt-fragment | Comt | Reduced levels of lignin | Weeks <i>et al.</i> , 2008 |
| Perennial ryegrass | Overexpression | n.m. but from species itself | Lpvp1 | Drought tolerance | Bajaj <i>et al.</i> , 2008 |

*This type of ‘convergent transcription’ silencing construct with two promoters was shown to be very efficient (Yan *et al.*, 2006). n.m.: not mentioned

Present status of intragenic crops’ regulation

The simplicity, timeframe and cost of endorsement of the intragenic crops are under development will be dependent upon the future regulations of these crops. In the majority of the countries, the release of intragenic crops falls currently under the

regulatory guidelines as similar as the crops that are transgenic.

Limitations of intragenics

Although the intragenic technology is enlightening considerable advantages more than the transgenic counterpart, conversely, still there are a petite number of limitations

present related to this novel tool. One of the chief disadvantages contributed by the intragenic approach compared to transgenic, is that characters that are outside to the sexually well-matched gene pool cannot be introduced. In addition, extraordinary proficiency and time are compulsory for successful development of intragenic crops as compared to the transgenic crops.

The application of intragenic practice boosts the opportunity to introgress the ideal favoured genes into the fresh cultivars, without troubling their constructive characteristics. Conventional methods in plant breeding rely on random modifications of genome and are not easy to apply to either eradicate undesirable features or activate the dormant traits. Moreover, improvements of definite characteristics could be coupled with inadvertent as well as unnoticed changes in genetic level that compromise quality of food. These issues are addressed efficiently by accurately recombining native elements *in vitro* and introducing the resulting expression cassettes into plants using marker-free and all-native DNA transformation. An evaluation of these low-risk crops should be focused on any potential safety issues to the consumer. We propose to lessen the regulatory yoke for intragenic crops as compared to transgenic crops.

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How to cite this article:

Mainak Barman, Saipayan Ghosh, Kingsuk Das and Sabir Ahmed Mondol. 2020. Intrageneration as a Sustainable Crop Improvement Method: A Review. *Int.J.Curr.Microbiol.App.Sci*. 9(04): 773-782. doi: <https://doi.org/10.20546/ijcmas.2020.904.092>