

Review Article

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## Intragenesis as a Sustainable Crop Improvement Method: A Review

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### ABSTRACT

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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 F<sub>1</sub> 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<sup>-1</sup>) (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](http://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 glyceic 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 <sup>a</sup>	GalUR	constitutive overexpression <sup>b</sup>	2003	Agius <i>et al.</i> , 2003
Increased vitamin-E content	soybean	Vte3 + Vte4	seed-specific overexpression <sup>c</sup>	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 <sup>d</sup>	2003	Herman <i>et al.</i> , 2003
Enhanced aroma	potato	Cgsb	constitutive overexpression <sup>b</sup>	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

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

<sup>a</sup>Concept demonstrated in Arabidopsis. <sup>b</sup>Molecular strategies may be improved upon by employing tissue-specific promoters. <sup>c</sup>Gene isolated from Arabidopsis. <sup>d</sup>Multigene 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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