Parthenocarpy, a Strategy for Fruit Development under Adverse Environmental Conditions

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Introduction

In plants fertilized ovule and ovary synthesize auxine phytohormones sustaining seeds and fruit production (Archbold and Dennis 1985). The development of fruit without pollination and fertilization is called parthenocarpy, therefore parthenocarpy fruits are seedless. Parthenocarpy offers the possibility of improving fruit set when environmental conditions are adverse for pollen production, germination and fertilization; moreover in some crops the absence of seeds can improve fruit quality (e.g. banana, eggplant, persimmon, grape, watermelon), while in other ones (e.g. Actinidia) might also improve productivity.

Parthenocarpy can have a genetic basis (genetic or natural parthenocarpy) or it can be artificially induced. Genetic parthenocarpy is called obligatory when the expression of the parthenocarpy trait is not influenced by external factors and facultative if it occurs only under conditions adverse for pollination and fertilization. Artificially induced parthenocarpy can be observed in several plant species by treating flowers with plant growth factors or by pollination with incompatible pollen or X-rays irradiated pollen.

Artificial Induced Parthenocarpy

The induction of parthenocarpy is a rather common agricultural practice for some horticultural species (Schwabe and Mills 1981). The use of irradiated pollen, natural or synthetic auxins and gibberellins has been rationalized as an exogenous (hormonal) signal able to increase in IAA content during ovary development (Tsao 1980). In eggplant, the first increase takes place during the first five days after anthesis, while a major peak of IAA appears at 20 days after anthesis in both pollinated and auxin treated flowers (Lee *et al.* 1997). These and similar data obtained with other species indicate that the exogenous signal (pollination/fertilization, phytohormones, auxin, analogues, etc.) triggers an increase in endogenous IAA content and activity in the ovary, which is responsible for promoting fruit set and development. It has been reported that parthenocarpic *pat-2* tomato fruits show a slightly higher IAA content before anthesis, but more than 10 times higher level of IAA at anthesis in comparison to control fruits (Mapelli *et al.* 1994).

Genetic Parthenocarpy

Alteration in Chromosome Number

Unbalanced development of embryo and endosperm in triploid background has been utilized to yield parthenocarpic fruit. In watermelon seedless fruits with only residual

integuments are obtained from F_1 hybrid plants derived from cross between tetraploid and diploid parents (Kihara 1951). Several banana (*Musa paradisiacal*) cultivars are poliploid (mainly triploid) and yield parthenocarpic fruits. Bunch and fruit characteristics are positively influenced by both high ploidy level and replacement of recessive alleles with dominant alleles at the parthenocarpic P_1 locus (Ortiz and Vuylsteke 1995).

Mutant Lines

In tomato (*Lycopersicum esculentum* L.) the following genes have been identified which are able to sustain the parthenocarpic traits: *pat*, *pat*-2, *pat*-3, *pat*-4 (Philouze 1983). However study lead to the conclusion that *pat*-2 gene plays the major role and *mp* gene, in the homozygous state, influences the phenotypic expression of *pat*-2 in both homozygous and heterozygous states (Vardy *et al.* 1989). In eggplant, a genetic tendency to parthenocarpy seems to be controlled by few genes with additive effect (Hennart 1996). Cucumber is one of the plant species where parthenocarpic mutants have been more intensively used to breed cultivars for greenhouse cultivation. The parthenocarpic trait appears to be controlled by a single gene (*Pa*) expressing incomplete dominance and by modifier genes (Pike and Peterson 1969).

Seedlessness in table grapes, an important trait meeting the preference of consumers, can arise from parthenocarpy or stenospermy. The latter type of seedlessness has been widely exploited by breeders to produce new seddless cultivars. In pear, both stimulative and autonomous parthenocarpy have been identified, and some commercial cultivars showed parthenocarpic fruit development (Griggs and Iwakiri 1954; Nyeki *et al.* 1998). Parthenocarpic fruit development has also been reported in other fruit trees, such as cactus pear (Weiss *et al.* 1993) and mango (Kulkarni and Rameshwar 1978).

In apple apetalous parthenocarpy has been described. The flowers lack petals and stamens, but they can produce either seedless parthenocarpic fruits or seeded fruits if hand pollinated.

Recombinant DNA Methods

The recombinant DNA methods aimed at parthenocarpic fruit production can be classified into two types depending on the approach: (1) the first type is based on unbalanced embryo development and/or to block seed production in transgenic plants, without curtailing fruit development: (2) the second type relies on the proper expression of genetic information affecting phytohormone content and/or activity in the desired organ, usually the ovary and/or its tissues (*i.e.*, ovules) to trigger parthenocarpic fruits development.

1. The unbalanced and/or arrest of seed development has been proposed to be achieved by using a cytotoxic gene (Paddon and Hartley 1987; Tomes *et al.* 1996a) or regulatory genes affecting embryo and endosperm development (Grossniklaus and Vielle-Calzada 1998). Up to now no experimental data have been disclosed and, therefore, these methods are so far only of speculative interest.

2. The methods based on genetic information affecting phytohormones content have been proposed by Tomes *et al.* (1996b); Li (1997); Barg and Salts (1996). Rotino and co-

workers (Rotino *et al.* 1997) have described the use of the regulatory regions of the *DefH9* (Deficiens Homologue 9) gene from *Antirrhinum majus* to express the *iaaM* gene from *Pseudomonas syringae* pv *savastanoi* (Yamada *et al.* 1985) specifically in the placenta and ovules. A related patent (Rotino *et al.* 1996) described the use of the *DefH9* promoter to drive expression of *iaaM*, *rolB*, *iaaL* or *ILR1* genes. In this patent, *rolB* is proposed to hydrolyse indolethanol-glucoside, releasing the auxine indolethanol from indolethanol-glucoside. The product of the *iaaL* gene from *Pseudomonas syringae* pv savastanoi is the IAA-lysine synthetase that conjugates IAA to lysine and other amino acids (Glass and Kosuge 1988), while ILR1 is able to release free IAA from IAA conjugated with leucine or phenylalanine (Bartel and Fink 1995).

In all the "phytohormonal" methods so far developed, the genetic information used codes for enzymes affecting the content and/or activity of phytohormones (e.g. *iaaM, ipt, iaaL, ILR1, rolB*, gibberellin). The regulatory region(s) of the gene represents the most important genetic information to control temporal and spatial expression of the gene of interest. These two parameters are relevant both to obtain parthenocarpy and to ensure an optimal expressivity of the parthenocarpic trait without affecting the vegetative plant growth. Consequently, all the regulatory regions used to control the expression of the different chimeric genes drive gene expression in the ovary. Differences in the timing and specificity of expression have been, at least in some cases, incorporated in the methods. A third relevant aspect to consider is the strength of expression, because an excess or a defect in the expression of a phytohormone-synthetizing gene might cause the development of morphologically altered parthenocarpic fruits or an inefficient fruit set and growth, respectively.

The *DefH9* gene belongs to the MADS-box family of transcriptional regulators. The *DefH9-iaaM* chimeric gene uses the regulatory regions of the *DefH9* gene (Rotino *et al.* 1996; 1997), which are able to drive expression in the placenta, ovule and tissues derived therefrom of heterologous species (Ficcadenti *et al.* 1999). Its ovule-specific expression ensures that parthenocarpic fruit development starts before anthesis making possible a production of parthenocarpic fruits earlier in comparison to hormonal treated or pollinated flowers. Its expression in tissues derived from the ovule and ovary sustains fruit growth in later phases of fruit development. The very low level of expression of the *DefH9-iaaM* should allow to obtain parthenocarpic fruit development in most, if not all, species. Further-more, it should ensure the development of parthenocarpic fruits without malformations. For this purpose, and also considering that different species and cultivar within a species differ in the number of ovules/seeds and in their sensitivity to auxin, a novel genetic method has been recently developed and used to optimise expression of parthenocarpic gene (Pandolfini *et al.* 2002).

DefH9-iaaM and its derivatives gene *DefH9-RI-iaaM* have been utilized to transform vegetable and fruit crop species, the results so far obtained are reported below.

Eggplant (*Solanum melongena* L.)

The commercial ripeness of eggplant fruit precedes its physiological maturity because seeds considerably depreciate the value of fruits for both fresh and processed market. The negative effects associated with the presence of seeds are a faster and more intense

browning of the fruit flesh upon cutting, an increase of bitter taste and a harder flesh. These factors make seed-containing fruits unpalatable for the consumer. Out-of-season fruit yield is usually obtained from greenhouse cultivations where sub-optimal environmental conditions (low temperature and light) for fruit set and growth is counteracted by hormonal treatment of flowers (Northman *et al.* 1975; Sarma and Barman 1977). For such reasons, intense breeding activities are currently in progress to obtain parthenocarpic hybrids (Daunay 1981-1982; Hennart 1996; Restaino *et al.* 1992). However the parthenocarpic eggplant varieties so far available still need phytohormone applications to produce fruits of marketable size (Leonardi and Romano 1997).

The first experiment with GM eggplant was carried out in 1997-98 under greenhouse at Ragusa, Sicily (Italy) for winter fruit production (Donzella *et al.* 2000). Genetic engineered parthenocarpic hybrids P1, P3, and P4 were obtained by crossing (as male parents) the primary transgenic plants (Rotino *et al.* 1997) DR2 *DefH9-iaaM* 34-1 with the lines Tal 1/1 (P1) and Tina (P3) and Tal 1/1 *DefH9-iaaM* 1-1 with the line Tina (P4). The transgenic hybrids were compared to the un-transformed control hybrids C1 (Tal 1/1 x DR2) and C2 (Tal 1/1 x Tina) and the commercial Talina F_1 hybrid (SG-Sandoz). Therefore, the hybrids P_1 and P_4 had the identical genetic constitution as C1 and C2 but for the presence of the transgene. Spraying the progenies with a kanamycin solution to select for transgenic plantlets (Sunseri *et al.* 1993) allowed also to demonstrate that the marker gene *nptII* segregated as a single locus in the three hybrid combinations.The first four harvests (winter production), occured when temperature (11 of 16°C) and light intensity (310 to 360 W/m^2) were most limiting for fruit set and plant growth. Un-transformed un-hormoned plants gave very poor yield (200-300 g/plant) while transgenic parthenocarpic plants were not affected by the hormonal sprays and gave a similar production whether or not their flowers had been sprayed (1,000-1,200 g/plant). Besides, exogenous hormone treatments did not affected the size of the fruit in the engineered parthenocarpic plants while un-transformed plants gave much bigger fruits when treated with hormones. During the subsequent ten harvests (4 March-1 June 1998) although the environmental conditions were less restrictive for flower pollination and fertilization, phytohormones still improved the yield of un-transformed plants (from 2000 to 4000 g/plant), but did not influenced that one of the transformed plants (5000 g/plant).

The second experiment with eggplant was performed in two different locations: Monsampolo del Tronto (Central Italy) and Pontecagnano (Southern Italy) for spring-summer fruit production in greenhouse. In this period hormonal spray is not requested as flowers are easily pollinated by bombus bees. Two parthenocarpic F_1 hybrids were compared with the two corresponding un-transformed hybrids and two commercial cultivars (Acciarri *et al.* 2002). Early yield (i.e. the first four harvesting) of parthenocarpic hybrids was 6 fold higher respect to their corresponding un-transformed hybrids. During the whole production cycle both transformed hybrids gave a yield 46% higher respect to corresponding controls. The average number of fruit produced per plant was 8-9 in both locations and hybrids. Therefore the higher yield was explaned only by an higher average fruit weight GM hybrids.

The third experiment with eggplant was carried out at Monsampolo del Tronto in open field during summer time (May to September). Two GM hybrids P1, P10 were compared with corresponding un-transformed control C1-C10 (Acciarri *et al.* 2002). P1 and P10

hybrids yielded respectively 36% and 76% more than their controls C1 and C10. The difference in productivity between P1 and C1 hybrid (long-shaped fruits), was caused by higher average weight of GM fruits, while difference between P10 and C10 (sub-oval fruits) was due to the increased number of fruits per plant. The total production (ten harvests) of P1 hybrids was 37% higher than control C1 eggplants. This difference was due both to the higher number of fruits/plant and to the increased weight of GM fruits. Although higher in P10 hybrid in comparison to its control C10, the total yield (the number and average weight of the fruits) was not significantly different between the two. During the whole harvesting period, fruits from both P1 and P10 parthenocarpic hybrids were seedless, whilst control fruits always had seeds. Therefore, under open field cultivation, the *DefH9-iaaM* gene had a positive influence on fruit quality, as GM *DefH9-iaaM* fruits were always seedless.

Transgenic and un-transgenic fruits from the third trial (open field) were analysed for aptitude to freezing (Maestrelli *et al.* 2003). Transgenic fruits could be maintained on the plants for a longer time without any noticeable quality modification and meeting the requirements of industry which prefers to receive the product regularly over the whole harvest period. GM fruits, both in fresh cut slices and after 15 storage months at -20°C, showed a reduced browning of the pulp, with respect to the control eggplants. The GM thawed fruits firmness was significantly less than the corresponding controls, this did not negatively influence the suitability for further use. In fact, preliminary tests have confirmed that GM fruits could be employed as slices for grilling and as ingredients in vegetable soup.

In conclusion the *DefH9-iaaM* chimeric gene inserted in eggplant genotypes allowed the following practical advantages:

- normal production of fruit in greenhouse during winter time, without hormonal spray which is absolutely necessary for un-transformed eggplant cultivars;
- around 50% and 37% yield increasing respectively in greenhouse during spring time and in open field due to bigger size of fruit;
- better quality of fruit obtained in open field cultivations due to absence of seeds;
- reduced browning of the pulp and consequence more convenient utilization as slices for grilling and as ingredient of vegetable soups.

Tomato

Tomato is one of the most important vegetable crop of the world. Tomato fruits are consumed either fresh or processed. During winter cultivation in greenhouse the flowers are treated with auxinic phytohormones which cause parthenocarpic fruit development and production under environmental conditions (10-15°C shor day and low light intensity) adverse for fruit set and growth (Schwabe and Mills 1981). However, either higher sensitivity to auxins or an excess of exogenous phytohormones causes malformations of the tomato fruit (Santangelo and Soressi 1990). Consequently, breeding programs for fresh market tomatoes have usually screened tomato lines for an optimal response of the flowers to phytohormonal sprays.

In order to evaluate the effect of the *DefH9-iaaM* gene on table tomato fruit set and growth, the transgene was introduced into the two genotypes CM and L276 differing in the characteristics of their fruit (Ficcadenti *et al.* 1999). Eight independent transgenic tomato

plants (4 of the genotype CM and 4 of the genotype L276) were grown in summer under favourable environmental conditions with average temperatures ranging from 12°C to 30°C. In these conditions, it was possible to evalutate whether the parthenocarpic trait had negative effect on fruit set and/or fruit growth. Emasculated flowers from all transgenic tomato plants developed seedless parthenocarpic fruits whilst control plants did not set fruit at all. When hand pollinated, fruits with seeds were set both in transgenic plants and in the controls. Fruit setting of un-pollinated flowers ranged from 62% to 100% in all transgenic tomato plants, values similar to those observed in pollinated flowers. Obviously, un-transformed controls set fruit only from pollinated flowers. Seven out of eight independent transgenic tomato plants tested, gave the same fruit weight in transformed and un-trasformed control, only one genotype (i.e. CM 11) increased fruit weight in trasformed background. The soluble solids concentration (°Brix) was significantly elevated in all the transgenic parthenocarpic fruits of the genotype L276. The pH of parthenocarpic fruits was not significantly different, with the exception of line L276 4-1. With regard to skin colour and flesh consistency, the parthenocarpic fruits were indistinguishable from the fruits obtained by pollination. This results demonstrate that the chimeric *DefH9-iaaM* gene is able to sustain parthenoxarpic fruit development in table tomato.

In the second experiment carried out in 1999 four parthenocarpic F_1 hybrids for the presence of *DefH9-iaaM* gene were compared with the correspondent un-transformed hybrids under greenhouse conditions at Monsampolo del Tronto (Acciarri *et al.* 2000). All transgenic hybrids yielded an higher number of fruit per plant (21 against 14) specially during the early period of production when the average temperature of about 13°C limited fruit set. An additional positive effect of *DefH9-iaaM* gene was represented by higher fruit weight which was nearly double for all transformed hybrids respect to their control (150-160 g against 80-90 g).

Industrial tomatoes are cultivated in open field, and consequently their cultivars have not been selected for an adequate response to exogenous auxin treatment, which is a common practice only in greenhouse cultivation. So, flowers from different tomato lines and/or cultivars might differ in their response both to an exogenous phytohormonal treatment and to the action of the *DefH9-iaaM* gene.

The *DefH9-iaaM* gene when introduced in the typical industrial tomato cultivar UC82, caused parthenocarpic development of the fruit, but the fruits were misshapen, umbonated and empty as those obtained with an excess of exogenous auxin treatment. Thus, the working hypothesis to solve the "Pickelhauben" problem has been to reduce the expression and consequently the action of the *DefH9-iaaM* gene. The best solution consisted in downregulate the level expression of this gene at post-transcriptional level (Pandolfini *et al.* 2002). For this purpose 87 bp long DNA sequence derived from intron of the *rolA* gene was used to modify the 5'ULR of the *DefH9-iaaM* gene and the derivative gene *DefH9-RI-iaaM* was obtained.

Plants of industrial tomato cultivar UC82 were transformed with *DefH9-RI-iaaM* gene. In the first experiment, carried out in greenhouse, fruit set of emasculated flowers was an average 85% against 95% obtained with *DefH9-iaaM* gene. Parthenocarpic fruit of *DefH9-RI-iaaM*, *DefH9-iaaM* and untransformed (selfed) plants weighted respectively 46 g, 77 g, 58 g. In conclusion, the *DefH9-RI-iaaM* gene caused parthenocarpic development without any fruit malformations.

In 2003 two UC82 transgenic lines for the presence of *DefH9-RI-iaaM* gene, one un-transformed line of UC82 cultivar and the All-flesh un-transformed cultivar were evaluated in open field. All-flesh line gave the highest fruit yield (1.9 Kg/plant) while both transgenic lines performed as the control cv UC82 (1.3-1.5 Kg/plant). The number of fruit per plant was significantly higher for both transgenic lines respect to their control (43 and 34 against 22) while the average fruit weight was significantly lower (37 and 38 against 65 g). The percentage of fruit with seed was 27 and 20 for the two GM lines, 85 and 87 respectively for UC82 and all-flesh cultivars. It is to point out that the very high temperature during the summer 2003 in Italy (average 35°c and maximum above 40°C). Thus ones again it was demonstrated the effect of *DefH9-RI-iaaM* gene in partenocarpic fruit development of industrial tomato cultivar UC82 under very high temperature. Chemical and technological analysis evidenced the same results for UC82 cultivars and the two GM lines which different from All-flesh cultivar (Rotino *et al.* in press).

In conclusion for table tomato cvs the introgression of *DefH9-iaaM* gene allowed the following advantages:

- fruit yield under greenhouse cultivation at low temperature (10-15°C) and light intensity, without exogenous growth regulators spray;
- yield improvement under greenhouse cultivation at normal or high temperature and probably also in the open field.

For industrial tomato the introgression of *DefH9-RI-iaaM* gene has been demon-strated to allow a normal development of fruits and probably yield even under environmental conditions limiting pollination and fertilization of flowers (low temperature, raining).

Strawberry and Raspberry

In the research carried out by Universities of Ancona (Central Italy) and Verona (Northern Italy) the *DefH9-iaaM* gene has been inserted into three species belongings to *Rosaceae* species: diploid strawberry (*Fragaria vesca*); exaploid strawberry (*Frageria x ananassa*) and raspberry (*Robus idaeus*).

In emasculated flowers, the presence of the *DefH9-iaaM* gene in the genome of both *F. vesca* and *F. x ananassa* plants caused parthenocarpic development of the achenes and some swelling of the receptacle. All emasculated flowers of *DefH9-iaaM F. vesca* plants were able to sustain achene development whilst 80% (24 out of 30) of emasculated flowers in control plants did not develop achenes. A similar result was found in *F. x ananassa* where 28 out of 30 *DefH9-iaaM* emasculated flowers developed achenes, whereas only 4 out of 30 emasculated control flowers developed achenes. Raspberry plants transgenic for the *DefH9-iaaM* gene showed parthenocarpic fruit development from emasculated flowers, Twenty-seven out of 30 emasculated flowers from *DefH9-iaaM* raspberry plants developed fruits, whereas only 3 out of 30 emasculated flowers of control plants showed fruit develop-ment. Thus, in three species belonging to *Rosaceae*, the *DefH9-iaaM* gene conferred parthenocarpic fruit development to emasculated flowers. However, the commercial fruits obtained from emasculated flowers of both strawberry (i.e. receptacle) and raspberry did not develop fully. In all three species, the percentage of fruit set in self-pollinated flowers, was 100% both transgenic and control (Mezzetti *et al.* 2004).

In raspberry, the *DefH9-iaaM* increased fruits weight by 14%, inflorescence number per plant by 22% and fruit number per inflorescence by 47%. The overall effect of the *DefH9-iaaM* gene doubled (+108%) the yield. The sugar content of the fruit was not altered by the presence of the *DefH9-iaaM* gene in any of the three species tested in the present study.

Conclusions

Among the recombinant DNA techniques so far proposed to induce parthenocapic development of fruit, that based on the use of *DefH9-iaaM* and *DefH9-RI-iaaM* genes appears to be the most promising for a practical utilization because several field trials under normal cultivation confirmed agronomical and commercial advantages. The positive results obtained in *Solanaceae* (tomato, eggplant), *Rosaceae* (strawberry, raspberry), *Vitaceae* (grapes) families lead to think that both genes could be used to improve yield and quality of other crops, including tropical species.

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