



## Scrutiny of Strategies in developing Parthenocarpic Tomato (*Solanum lycopersicum*)

### KEYWORDS

Nancy D

Plant and Microbial Biotechnology Lab, Department of Biotechnology, Periyar University, Salem, Tamil Nadu

**ABSTRACT** Fruit development occurs after victorious completion of early development following pollination, fertilization and maturation. At the end of early development; a green fruit is obtained, which has the size of a mature fruit and the maturation phase commences. Parthenocarpy is the growth of the ovary into a seedless fruit in the dearth of pollination and/ or fertilization. Nevertheless, the current agricultural practices to achieve seedlessness have in-built disadvantages. A considerable difference in dry matter content, sugar and soluble solids were observed in parthenocarpic fruits. The successfully available seedless tomatoes are "Montfavet 191" (pat mutant) developed by mutation in two recessive genes in US market, *sha* and *pat1* "Sweet". World's first seedless tomato, "CV MicroTom" by regulation of genes for auxin synthesis (*iaaM*) or responsiveness (*rolB*) driven by *DefH9* or the Inner No Outer (*INO*) promoter from *A. thaliana* and "Monalbo / porphyre" (single *pat-2* gene mutant). Beyond the seedless nature, these fruits are sweet with firm gel and juiciness. This review has been focused on different strategies available to develop parthenocarpic tomatoes that includes; environmental factors controlling fruit set, gene modifications and terminator technology, which provides an in-depth view towards magnitude of seedlessness.

### INTRODUCTION

A fruit is the consequence of the ovary development. It provides a suitable environment for seed development and supports efficient dispersal of seeds (Gorguet, 2005). Fertilization of the ovule generally triggers the ovary development into a fruit. Fruit development normally involves; fruit setting, cell division and cell expansion. Parthenocarpy may occur naturally or can be induced artificially with the application of various hormones. Evidenced, that seedless forms of grapes (*Vitis vinifera*) have been prized for many centuries. Seedless tomato fruits exceed seeded fruits in dry-matter content by upto 1%, amid more sugars and more soluble solids. The shelf life of seedless fruits is longer since the seeds generate hormones that trigger senescence (Gorguet, 2005). An unfavorable environmental condition for tomato fruit set includes; low and high humidity, low light intensity, low or high temperatures (affect endothelium formation that prevents pollen release) and soaring wind. Pollen tube growth and development of functional gametes are also influenced by environmental factors that play a critical role in parthenocarpy (Testa, 2002).

The pollen produced gibberellins and the exogenous gibberellins can augment auxin content in the ovary of an unpollinated flower, that consequently trigger fruit setting in absence of fertilization. As well, the developing embryo reins the rate of cell division in the contiguous fruit tissue. It is generally considered that developing seeds support cell expansion within the fruit by the production of auxin and other mysterious molecules. Gibberellin that triggers fruit setting in the course of ovary auxin increase is shore up by its increased transcription at the time of fruit set and by its expression in the mitotic cell layers of the placenta (Testa, 2002). Hence this review has been strenuous on different strategies available to develop seedless tomatoes like; temperature controlled growth, hormone regulated, *pat* gene mutants, terminator technology and *Agrobacterium tumefaciens* genes (*iaaM* and *iaaH*).

### AGRONOMIC MODIFICATIONS REGULATED PARTHENO-CARPY

Parthenocarpic tomato plants (cultivar Severianin) produce a high yield and fruit set in colder temperatures than seeded cultivars. Thus, parthenocarpy is potentially useful for producing vegetables in winter months or to ensure yield stability in case of unfavorable pollination conditions. Many plants (tomato, eggplant and pepper) will produce sufficient fertile pollen grains only in specific climatic condition. In tomato, pollination occurs in a very narrow range of temperatures 15.8°C–21.8°C (night) and 30.8°C–35.8°C (day). Major limiting factor for fruit set is the farthest sensitivity of microsporogenesis and pollination to moderately high or low temperatures and inadequate humidity.

### HORMONAL REGULATIONS FOR PARTHENO-CARPY

Parthenocarpic condition led to different ovary growth patterns. Compared to the wild type, *pat* anthers were often shorter and had distorted lobes. Some anthers were abnormally curved with complex structures developing on the adaxial surface (Andrea, 1998). In *pat* mutants, the complete machinery for fruit set and development is switched on earlier. On highly abnormal locules of *pat* stamens, meiosis was disturbed and pollen production was hampered by complete degeneration of the sporogenous tissue, which correlates with hypertrophy of the tapetum (Table 1). A new potential use of seedlessness arose with the development of plant genetic engineering. In case of transgene association with seedless character, the transgene would be hard to disseminate by seed dispersal (e.g. dispersal on the ground after consumption or by birds). The only possible dissemination of the transgene would be by pollen. That creates a problem, depending on the ability of the species concerned to cross-hybridize with the surrounding plants. The resulting hybrids would be seedless only if the transgene is genetically linked to the seedless character.

The mutant phenotype was first ascribed to the action of two tightly linked genes, *sha* for 'short anthers' and *pat* for 'parthenocarpic fruit'. The discovery of a spontaneous *sha-pat* mutation in the line 'Montfavet 191' proved the single

recessive mutation with pleiotropic effects and the gene was finally named *pat*. The expression of parthenocarpy in *pat* plants is based on an enhanced ovary growth rate during the first 10 days after anthesis. It has higher auxin, gibberellin and DNA contents in the ovaries. The gibberellic acid (GA) biosynthesis seems to be the key in parthenocarpic fruit development initiation both in *pat-2* and *pat-3 / pat-4* lines (Fos, 2001). Polyamines are also involved in early fruit development, which is necessary for parthenocarpic growth of *pat-2* ovaries. Fos (2003) elucidated partial parthenocarpic fruit set induction by adding polyamines. But it is not convinced, whether gibberellins and polyamines act together or independently on fruit set in tomato as the primary signal. To conclude, *pat-2* and *pat-3 / pat-4* genes stimulate one or more steps in the GA biosynthesis pathway that enhances GAs expression and induce parthenocarpic fruit development (Gorget, 2005).

In the *pat* mutant forms, aberrations in ovule production play a role in seed set of *pat* fruits. However, it gives a very low seed set (Andrea, 1998). Reduction in fruit size and seed collection made *pat* gene less attractive for breeding. *Pat* gene has been mapped on long arm of chromosome 3, that unfasten a gate for cloning of the gene (Beraldi, 2004). The *pat-2* gene (single recessive gene) or double with *mp* gene has minor effect. The plants are smaller and less vigorous in yield, fruit set and fruit's firmness reduced with determinate plant growth (Tomato lines; "*Monalbo*" or "*Porphyre*"). Deleterious pleiotropic effects can be overcome by suitable genetic background. The *pat-3 / pat-4* genes involved in high level of parthenocarpy without obvious effects and two or five genes control this trait. To bring to a close seedless fruits, the plant should be grown in conditions adverse to pollination and/or fertilization, or the flowers have to be emasculated. Parthenocarpic mutant genes *pat-2* and *pat-3 / pat-4* are not allelic. Polygenic inheritance and different fruit sizes makes the use *pat-3 / pat-4* genes less attractive for breeding (Gorget, 2005).

### Terminator Seed Technology

Spena (2001) reviewed to distinguish between the approaches to develop parthenocarpic plants; as based on unbalanced embryo development, modulating the phytohormone content and/ or activity in specific organ; in which the second approach is widely proficient. Among all the strategies available to produce seedless fruits, terminator technology is the only one to have unleashed the wrath of the media. One potential use is to prevent genetically modified plants being used without payment to the seed companies. One query, which has not yet been fully answered, is how the auxin induction is able to substitute for seeds in the developing fruit. Terminator technology could be a strategy to limit the occurrence of legal action. Genetic engineering/ stenospermocarpy/ parthenocarpy in wider range of species can bring the same advantages as terminator technology for the plant biotechnology industry. However, seedless fruits are far more attractive for farmers and consumers than sterile seeds.

### Auxin biosynthesis induction by expression of *iaaM* and *iaaH* genes

Exploitation of facultative parthenocarpy is complicated. The possible alternative might be expression of the *A. tumefaciens* genes *iaaM* and *iaaH* under an ovary-specific promoter, which in concert encode for the biosynthesis of the auxin (IAA) from tryptophan. Subsequently, induction of parthenocarpy by expression of *iaaH* gene specifically in the ovary (Szechtman, 1997), hydrolyzed the auxin precursor

naphthaleneacetamide (NAM) to naphthalene-1-acetic acid (NAA) by the *iaaH*- encoded enzyme indoleacetamide hydrolase (Klee, 1987). Similar reports were seen with expression of *iaaM* under the ovule-specific promoter DefH-9 induced parthenocarpy in eggplant, tobacco and tomato (Donzella, 2000).

### Induction via specific expression of *roB* gene in ovary

Ovary-specific expression of the *A. rhizogenes* derived gene *roB* was chosen, because it manifests several syndromes characteristic of auxin treatment. Tomato plants transformed with a chimeric construct containing the *roB* gene fused to the ovary and young fruit specific promoter TPRP-F1 developed parthenocarpic fruits. Fruit size and morphology, including jelly fill in the locules of the seedless fruits, were comparable to those of seeded fruits of the parental line. Although it is not known whether ROLB signals for the same cassette of genes involved in fertilization-dependent fruit development, it yields seedless fruits (Barg, 2000).

Based on the steady-state level of *roB* mRNA in ovaries (7 days of post-anthesis) there appears to be an association between the abundance of transcripts of the transgene that creates to be the strength of parthenocarpy. The transcript peaks in the very early stages of ovary development, declines shortly thereafter. This mode of expression is expected of a gene driven by the TPRP-F1 promoter, which is highly ovary specific (Salts, 1991). Further insights into the distribution of the *roB* transcripts within the pericarp/embryos require RNA *in situ* analysis. Thus, the ROLB protein synthesized at these stages is sufficient to fully substitute for the contribution of the seeds to fruit shape and final size. Bangerth (1989) suggested that dominance among fruits at the same truss results from the polar IAA export from earlier developed sinks, which inhibits IAA export from the later developed ones. Reduction in the percentage of fruit set has also been reported for auxin-induced fruit development (Abad, 1989). However, ROLB does not affect IAA metabolism, therefore this hypothesis seem unlikely to apply to TPRP-F1: *roB*- based parthenocarpy (Nilsson, 1997).

### Resveratrol Biosynthesis Induction

A novel strategy to induce parthenocarpy is by the induction of Resveratrol biosynthesis in flower tissues. The expression of the stilbene synthase gene affected flavonoid metabolism in different manner in the transgenic lines. In tomato, pollination does not occur when either the night temperature is <13°C or when daytime temperature is >38°C for 5 hours (Ilaria, 2011). This was explained as due to competition between the introduced enzyme and the endogenous chalcone synthase for the substrates 4-coumaroyl CoA and malonyl CoA. Transgenic line expressing stilbene synthase cDNA under the control of the tomato lipoxygenase B (*TomLoxB*) gene promoter drives the gene expression preferentially in mature fruits (Introno, 2009).

### GENES INVOLVED IN PARTHENO-CARPY

The deviations in flower development suggest that, parthenocarpy is a secondary effect of the gene activity that controls earlier floral development. Genes for parthenocarpy affect the pattern of hormone production, transport and/or metabolism. This overcomes the growth substances threshold concentration during the critical period of anthesis, to promote ovary growth lacking pollination and fertilization. The link between genes involved in parthenocarpy and gibberellin metabolism is found in the parthenocarpic mutant of *A. thaliana* called SPINDLY (*SPY*) (Smith,

1999). In addition to parthenocarpy, the *pat* gene causes aberrations that affect androecium of *pat* flowers to be short, irregular and apparently unfused anthers that leave the stigma exerted. Its dehiscence is external with fertile pollen, but with reduced number of grains.

The link between flower aberrations and parthenocarpy in the *pat* mutant could be a defect in the regulation of GA levels or sensitivity in the flower, which is different in contiguous floral whorls. The report of a longer sequence for the *GAD<sub>3</sub>* gene (EMBL sequence: AJ277945) obtained from a cDNA library of young parthenocarpic fruits proved a hypothesis that this gene plays an important role in parthenocarpic development. Its transcription is tissue specific undergoing rapid cell division after fertilization (Andrea, 1998).

## DISCUSSION

Seedless fruits provide secure commercial protection for investment in plant breeding. But the farmers need to purchase seeds annually. Seedless mutants become seeded varieties due to pleiotropic effect associated with unfavorable characteristics; such sterility, undersized misshapen fruits and cross Pollination. Parthenocarpic mutants, offers suitable experimental systems to identify genes involved in fruit set and early ovary development (Testa, 2002). Nevertheless, even though the production of seedless fruit is expensive, ample proof of seedlessness is reflected by the increase in the number of patents and articles concerning seedlessness. An apparent problem concerns the sexual propagation of the parental lines in all cases, were obligate parthenocarpy is the preferred phenotype. Maintenance

of such transgenic lines can be accomplished following several alternative molecular approaches: for example, the transgene can be transiently silenced in the parental line, via RNAi using a viral vector bearing antisense sequence of *rolB* (Liu, 2002). Alternatively, transcription of the *rolB* gene can be blocked in the parental line and in the hybrid cultivar *rolB* transcription will be activated via removal of the blocking fragment by site-specific recombination.

## CONCLUSION

Economic production of fruit crops relies on the delicate phases of pollen formation, migration and interaction with female organs, as a prerequisite for high fruit set and yield. In *pat* mutants, the flowers usually senesces and fails fruit set. Parthenocarpic genes stimulate active GA synthesis, which trigger fruit development in absence of pollination and fertilization. In the near future, seedlessness could be an improvement introduced into a wider range of fruits and vegetables. New insights into stilbene synthase expression restricted to flower tissues could open up a novel hybrid seed system in tomato. In particular, in the consequences of unintended spread of genetic material through pollen and the risk of cross-pollination with non-transformed plants, would be avoided by using genetically modified sterile male plants and by comparative analyses.

## ACKNOWLEDGEMENT

I gratefully acknowledge for the support through the University Research Fellowship by the Periyar University, Tamil Nadu. I express my gratitude on the institutional incharges and the lab mates, for their immense help.

**Table 1: Developmental Stages of Ovary in Tomato (Andrea, 1998 and Testa, 2002)**

Stages	Wild Type	Pat Mutant
-1(flower bud)	Ovule initiation and Carpel Fusion.	First aberrations on anthers.
0(flower bud)	Androecium differentiation and Microsporogenesis. Evident hair initiation restricted to lateral & adaxial surface of anther lobes.	Androecia had abnormal anther number and irregularly orientated locules. Dehiscence line pushed out towards corolla & anthers distorted.
1(flower bud)	Uninucleate pollen grains release from tetrads.	Meiosis proceeds normally and uninucleate pollen was present, meiosis started and callose deposited.
2(flower bud)	Megagametogenesis.	Pericarp cell layers started to increase.
3(opening flower)	Pollination and fertilization. Nucellus degenerated.	Consistent ovary enlargement and stamina cone crushed.
4(full anthesis)	Mature 7- celled Polygonum type embryo sac formation	Pericarp thickness increases.
5(2 dpa*)	Synchronous beginning of pericarp division, placenta growth, ovule development and mitotic activity triggered.	Few ovules developed will increase in diameter.
6(6 dpa)	Pericarp thickness increases & pollen tubes grow on placenta surface. Unfused, glabrous, tetrasporangiate anthers with 2 lobes- regular spaced along lateral surface.	Abnormally curved anthers on the adaxial surface, which are carpeloid. Exhibits stigma-like surface. Placenta- like region bears external ovules and devoid of hairs.
Characteristic features	Ovary doesn't increase in size till stage 5. 8 to 12 cell layers in ovaries. Ovules grow to 180µm, till stage 5. Polygonum type embryo sac.	Smaller ovules than wild type. Regularly shorter & distorted lobes. Major ovules are undeveloped and no increase in size after anthesis. Callose accumulated on hypostatic region. Smaller seeds than wild type.

**Note:** \*dpa- Days Post Anthesis

## REFERENCE

1. Gorguet, B., Van Heusden, A.W. and Lindhout, P. (2005). A Review on- Parthenocarpic Fruit Development in Tomato. *Plant Biology*, 7: 131-139. | 2. Testa, G., Riccardo, C., Francesca, T., Gian, P.S. and Andrea, M. (2002). Sequencing and characterization of tomato genes putatively involved in fruit set and early development. *Sex Plant Reprod*, 14: 269-277. | 3. Andrea, M., Anna, R.T. and Gian, P.S. (1998). The parthenocarpic fruit (pat) mutant of tomato (*Lycopersicon esculentum* Mill.) sets seedless fruits and has aberrant anther and ovule development. *Development*, 125: 107-114. | 4. Fos, M., Proano, K., Nuez, F. and Garcia Martinez, J.L. (2001). Role of gibberellins in parthenocarpic fruit development induced by the genetic system pat-3/ pat-4 in tomato. *Physiologia Plantarum*, 111: 545- 550. | 5. Fos, M., Proano, K., Alabadi, D., Nuez, F. and Carbonell, J. (2003). Polyamine metabolism is altered in unpollinated parthenocarpic pat- 2 tomato ovaries. *Plant Physiology*, 131: 359- 366. | 6. Beraldi, D., Picarella, M., Soressi, G.P. and Mazzucato, A. (2004). Fine mapping of the parthenocarpic fruit (pat) mutation in tomato. *Theoretical and Applied Genetics*, 108: 209- 216. | 7. Spena, A. and Rotino, G.L. (2001). Parthenocarpy: state of the art, Current trends in the Embryology of Angiosperms (Bhojwani SS and Soh WY eds). Dordrecht: Kluwer Academic Publishers, 435- 450. | 8. Szechtman, A.D., Salts, Y., Carmi, N., Shabtai, S. and Pilowsky, M. (1997). Seedless fruit setting in response to NAM treatment of transgenic tomato expressing the *iaaH* gene specifically in the ovary. *Acta Hort*, 447: 597-698. | 9. Klee, H., Horsch, R., Hinchee, M.A., Hein, M.B. and Hoffman, N.L. (1987). The effects of overproduction of two *Agrobacterium tumefaciens* T-DNA auxin biosynthetic gene products in transgenic petunia plants. *Genes Dev*, 1: 86-89. | 10. Donzella, G., Spena, A. and Rotino, G.L. (2000). Transgenic parthenocarpic eggplants: superior germplasm for increased winter production. *Mol Breed*, 6: 79-86. | 11. Barg, R. and Salts, Y. (2000). Method for the induction of genetic parthenocarpy in plants. US Patent: 6,114,602. | 12. Salts, Y., Wachs, R., Gruissem, W. and Barg, R. (1991) Sequence coding for a novel proline-rich protein preferentially expressed in young tomato fruit. *Plant Mol Biol*, 17: 149-150. | 13. Bangerth, F. (1989). Dominance among fruit/sinks and the search for correlative signal. *Physiol Plant*, 76: 608-614. | 14. Abad, M. and Monteiro, A.A. (1989). The use of auxins for the production of greenhouse tomatoes in mild-winter conditions: a review. *Sci Hort*, 38: 167-192. | 15. Nilsson, O. and Olsson, O. (1997). Getting to the root: the role of the *Agrobacterium rhizogenes*- *rol* genes in the formation of hairy roots. *Physiol Plant*, 100: 463-473. | 16. Ilaria, I., Stefania, B., Stefania De, D., Barbara, L. and Giovanna, G. (2011). Over expression of a grape stilbene synthase gene in tomato induces parthenocarpy and causes abnormal pollen development. *Plant Physiology and Biochemistry*, 49: 1092- 1099. | 17. Introno, A.D., Paradiso, A., Scoditti, E., D'Amico, L. and De Paolis, A. (2009). Antioxidant and anti-inflammatory properties of tomato fruit synthesizing different amount of stilbenes. *Plant Biotechnol J*, 7: 422-429. | 18. Smith, V.A. and Koltunow, A.M. (1999). Genetic analysis of growth regulator induced parthenocarpy in Arabidopsis. *Plant Physiol*, 121: 437-451. | 19. Liu, Y., Schiff, M. and Dinesh-Kumar, S.P. (2002). Virus-induced gene silencing in tomato. *Plant J*, 31: 777-786. |