MEDDOCS Open Access Publisher

ISSN: 2637-7721

Journal of Plant Biology and Crop Research

Open Access | Research Article

Morphological Characterization of Anther and Pollen Formation in an EMS Induced Tomato Mutant with Blossom Drop Phenotype

Punyavee Dechkrong[1]; Sornsawan Srima[2]; Thanapat Nilwaranon[5]; Pumipat Tongyoo[2,3]; Hans de Jong[4]; Julapark Chunwongse[2,3,5]*

[1]Central Laboratory and Greenhouse Complex, Research and Academic Service Center, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, 73140, Thailand.

[2]Center of Excellence on Agricultural Biotechnology (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand.

[3]Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, 73140, Thailand.

[4]Laboratory of Genetics, Wageningen University & Research, Droevendaalsesteeg 1, P.O. Box 16, 6708, PB, Wageningen, The Netherlands.

[5]Department of Horticulture, Faculty of Agriculture at Kamphaeng Saen Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140.

***Corresponding Author(s): Julapark Chunwongse**

Center of Excellence on Agricultural Biotechnology (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand.

Email: julapark.c@ku.th

Received: Nov 09, 2020

Accepted: Dec 17, 2020

Published Online: Dec 23, 2020

Journal: Journal of Plant Biology and Crop Research

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Abstract

Two plants from the EMS mutagenized M2 tomato population were observed with blossom drop phenotype. One of these plants could be studied in detail by using cytological investigations, including the anther sectioning, whole tissue squashing, and a fluorescent pollen viability test. Whereas plant development displayed determinate and slower development, pollen development was disturbed, leading to aberrant or empty pollen, whereas pollination with wild type tomato pollen failed. The observed mutant demonstrated a highly variable expression suggesting that epigenetic regulation is involved in the variable abnormal phenotype. We suggest that the mutant phenotype may be caused by early flower bud hormonal regulation rather than a disturbance in the micro - and megasporogenesis pathways.

Keywords: EMS mutant; Blossom drop phenotype; Tomato; Microsporogenesis; Cytological analysis.

Cite this article: Chunwongse J, Dechkrong P, Srima S, Nilwaranon T, Tongyoo P, et al. Morphological Characterization of Anther and Pollen Formation in an EMS Induced Tomato Mutant with Blossom Drop Phenotype. Plant Biol Crop Res. 2020; 1: 1030.

Introduction

In tomato, pollination and fertilization occur inside the still-closed flowers, of which at least 98% were successful [1]. Essential processes include the pollen dropping on the stigma under biotic or abiotic conditions [2,3], followed by pollen tube formation and elongation into the style. When two sperm cells reach the ovary, fertilization occurs, and an embryo develops [2-4]. This whole series of processes depends not only on the presence of pollinators but also involves the availability of reproductive organs and the right environmental factors during the different phases of development [5,2,6,7]. In tomato, the ideal temperature for this process is about 25°C, while optimal relative humidity ranges somewhere between 40% to 70% [8-11].

However, extreme environmental conditions can affect pollen quality and viability, thus diminishing the success rate of pollination [2,12,6], and this often leads to a phenomenon called blossom drop [1,2,13,8,9,14,10,11]. In most cases, blossom drop in tomato is known to result from very high temperature and humidity, but can also be caused by water stress, nutrition deficiency, excess nitrogen fertilizer, pests and diseases [8,9,14,10,11]. Blossom drop diminishes crop yield, and so it is a severe problem of tomato culture [1,8,9,10]. Typical symptoms start with discoloration of the pedicel to greenish-yellow, finally turning the entire bloom yellow. At maturation, the blossom gets wilted, dies and dropped shortly later [15].

One of the strategies to unravel the mechanisms underlying the blossom drop phenotype is Ethyl Methane Sulfonate (EMS) mutagenesis. In a recently obtained M2 population of EMS treated seed sample of tomato TOMAC463, we obtained 325 mutant plants, of which two exhibited the characteristic blossom drop phenotype. One of the mutants died before we finished all experiments and could not be included in the final analysis. The second mutant with aberrant plant and flower phenotype pollen morphology and development was examined in detail and compared with the wild type tomato. We also hypothesize about the possible mechanisms that are impaired in the EMS mutants leading to the blossom drop phenomenon.

Materials and methods

Plant materials

We used the tomato variety TOMAC463, a Near-Isogenic Line (NIL) obtained through repeated backcrossing of the genetically distinct Seed a tip 3, a heat stress-tolerant variety and the MomorTA 230 line harboring the *Tm-2a* tomato mosaic virus resistant gene. Seeds of the TOMAC463 were treated with 1% of Ethyl Methane-Sulfonate solution using a flask shaker machine at 150 rpm for 16 hours in a fume hood. M1 seeds were sown and grown in a nurse field at Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand, in 2017. We reared 1,662 seedlings obtained from self-pollinated M1 plants in an evaporative cooling green house. From the group of 325 M2 individuals, one mutant (M2#515) displayed a typical blossom drop phenotype.

Pollen fertility and Pollen viability

Pollen grains of mutant M2#515 and the TOMAC463 wild type were stained with 1% aceto-carmine and studied under an Olympus bright field microscope. For the pollen viability test, we followed the self-compatibility protocol of Kho and Baër [16]. Self-pollination was established by pollinating the stigma with its own pollen grains. Later, we incubated the pollinated-pistils in the vapor of a saturated solution (98% RH) of $K_2Cr_2O_7$ for 24 hours, then transferred the pistils to 1N NaOH solution for 1 hour at 25°C. Next, pistils were rinsed two times with distilled water and then transferred to a 0.1% (w/v) Methyl Blue solution in water for 24 hours at 6°C. Pollen tube development was studied under an Olympus fluorescence microscope with epifluorescence illumination and appropriate UV/blue excitation/emission filters.

Cytological preparation of the blossom specimen

The blossoms of the mutant and wild type were harvested for microscopic analysis. The blossoms of both mutant and wild type at different lengths were pre-fixed overnight in a 2.5% glutaraldehyde solution at 4°C, followed by rinsing three times with 0.1M sodium-potassium phosphate buffer (Na_2HPO_4 + KH_2PO_4 buffer, pH 7.0) for 10 minutes. The material was then post-fixed in an aqueous 1% (w/v) osmium tetroxide solution at 4°C for 1 hour and rinsed three times with distilled water. Before embedding in the resin, specimens were dehydrated through a graded ethanol series (30%, 50%, 70%, 80% and 90% of EtOH) for 15 minutes each, and absolute EtOH two times for 15 minutes, later replaced with *n*-butyl glycidyl ether for 1 hour. Then the material was incubated in Spurr's resin for 1 hour and refreshed for another 1 hour. In the final step, specimens were embedded into the resin and polymerized in an oven at 65 °C for 24 hours. We sectioned the blocks into 200 µm slices using a Leica Ultra cut UCT-GA-D/E-1/100 microtome for the histological analysis. The sectioned specimens were stained with 3% Toluidine Blue O in distilled water and observed using a bright field light microscope.

Results

Plant Phenotype

The M2#515 mutant plant phenotype clearly differed from wild type tomato (TOMAC463). Its growth displayed a determinate and shorter development (Figure 1B), whereas that of the wild type was a semi-determinate type (Figure 1A). The wild type generally generated flowers and fruits (data not shown), including set seeds while the mutant formed flowers but fail to set seed and produce fruits as the result of blossom drop before or after pollination. Pollination with wild type pollen on the stigma of the young mutant flowers did not result in successful fertilization suggesting the failure of megasporogenesis. Other organs, including leaf shape and size of the mutant plants were not different from the wild type tomato.

Blossom drop phenotype in M2 mutant generation

Flower buds of the M2 # 515 mutant were on average at 0.76 cm which smaller than those of 1 – 1.1 cm wild type (Figure 2). The flowers of this mutant remained closed and wilted and dropped before opening. This blossom drop was most noticeable when pedicels and sepals turned from green to yellow, and ultimately to brown (Figure 2E – 2G), without producing fruits and seeds.

Pollen fertility

We next compared the pollen grain fertility of this mutant with the wild type tomato. To this end, we stained anthers and pollens in a 1% aceto-carmine (Figure 3). Anthers in the wild type tomato contained darkly stained and spherical pollens (Figure 3A & 3D), while pollens in the anthers of the mutant were transparent and shrunken (Figure 3B & 3E). In a few cases, we even observed anthers with empty locules (Figure 3C). Some of

the anthers contained dysfunctional, empty pollen grains, while anthers in other flowers did not produce pollen at all.

Pollen viability

To establish pollen viability in the mutant and wild type tomatoes, we performed the self-compatibility test with pollen grains with germinated pollen tubes on a stigma using the pollen viability test with methyl blue staining according to the protocol of Kho and Baër [16]. In the fluorescence photomicrographs, we observed that the wild type pollen grew and developed well inside its style only in six hours after pollination (Figure 4A & 4C). In contrast, pollen grains of the M2#515 mutant could not germinate on the pollen tube and did not grow within the style of pistil, although it was pollinated 24 hours before (Figure 4B & 4D).

Histological investigation of anther development in the

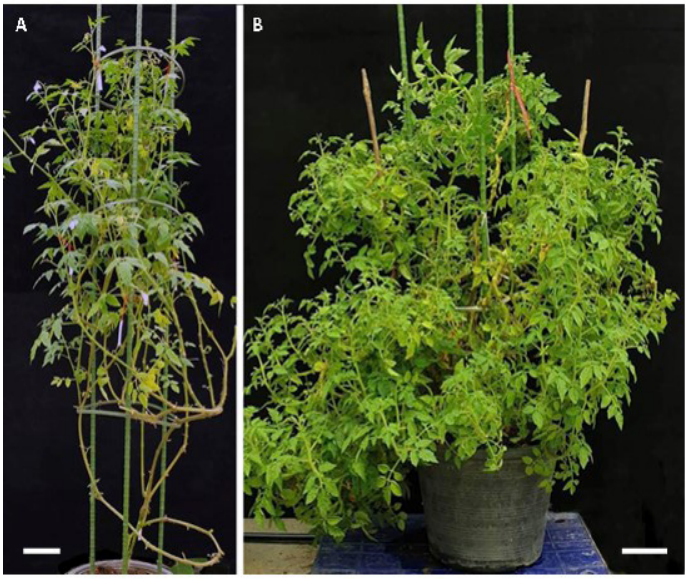

Figure 1: The growth habit of wild type tomato TOMAC463 (A) and the M2#515 mutant (B). Scale bar = 10 cm.

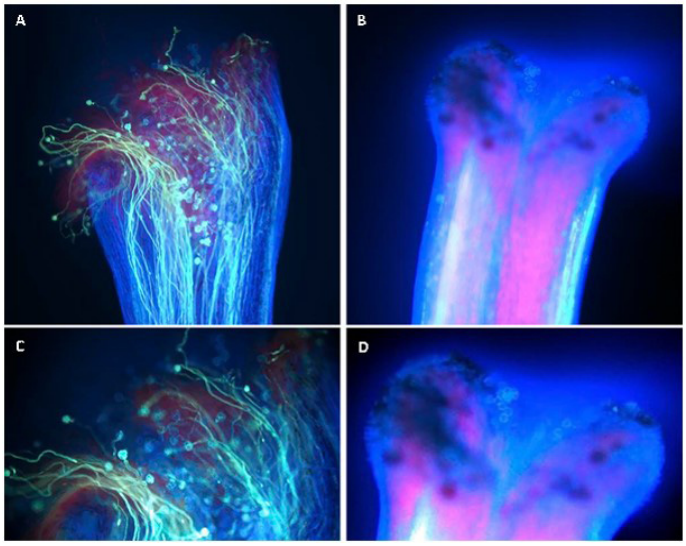

Figure 4: Fluorescence photomicrograph of pollen grains with germinated pollen tubes on a stigma using the pollen viability test with methyl blue staining. In the wild type, tomato pollen germinated well, and their tubes grow through the style (left images A, C). In the M2#515 mutant, there is no pollen germination and growth (right images B, D). Scale bars in A, C = 25 µm and in B, D = 50 µm.

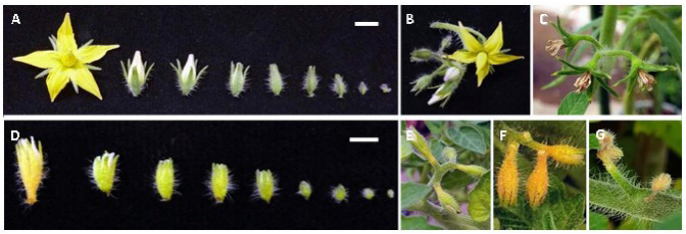

Figure 2: Phenotype of wild-type tomato flowers of TOMAC463 (top row, A, B, C) and the M2#515 mutant (bottom row, D, E, F, G).Scale bars = 5 mm.

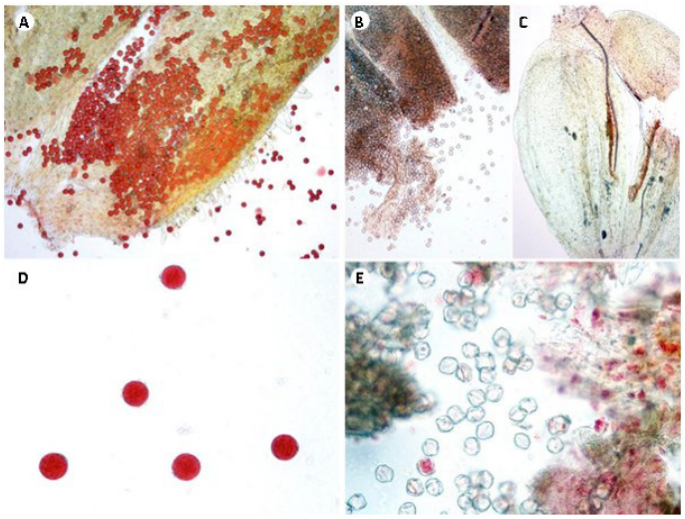

Figure 3: Squashed anther and pollen grains stained with 1% acetocarmine. Left images of anther and well-stained fertile pollen of the wild-type tomato mutant (A,D). The right images of the M2#515 mutant display empty anthers (B,C) and dead pollen (E). Scalebars in A, B, C = 200 µm, in D, E = 50 µm.

M2#515 mutant

To follow up on the results of the pollen fertility staining of the mutant showing empty pollen or no pollen at all, we performed a histological study of the anthers to establish the mutation effect in the developmental processes. Transverse sections of some anthers demonstrated the expected pollen mother cells in each of the four locules, which underwent meiosis and generated tetrads of haploid microspores (Figure 5B), similar toin wild type tomato (Figure 5A). Callose walls surrounding the tetrads degenerated, and free microspores were released (Figures 5D & 5E). Then, microspores differentiated to form pollen grains (Figure 5G, 5H). Prior to tetrad stage, mutant anthers were indistinguishable from those of the wild type until the late development of anther in which microspores of the mutant formed empty pollen grains (Figure 5K), while those of the wild type generated complete pollen grains (Figure 5J). M2#515 mutant frequently produced these dysfunctional pollens; however, some flowers of this mutant also produced abnormal anthers which failed to produce pollen in the microsporangia. Meiotic cells of abnormal anther displayed seemingly regular meiotic division and formed tetrads. After callose degradation, malformed haploid microspores were released within the locule of abnormal anther and degenerated (Figure 5C, 5F, 5I). Noticeably, malformed microspores in the upper locules of the anthers degraded faster than in other parts of the anthers and became empty locules that contained tapetal debris. At the same time, unlike upper locules, lower locules of the abnormal anthers still appeared as malformed microspores. However, abnormal mi-

crospores from each locule of abnormal anthers were found degraded (Figure 5L). As a result of the histological investigation, two expressions of the male-sterile phenotype were purposed for the mutant, i.e., anthers that contain dysfunctional pollen or anthers that completely lack pollen.

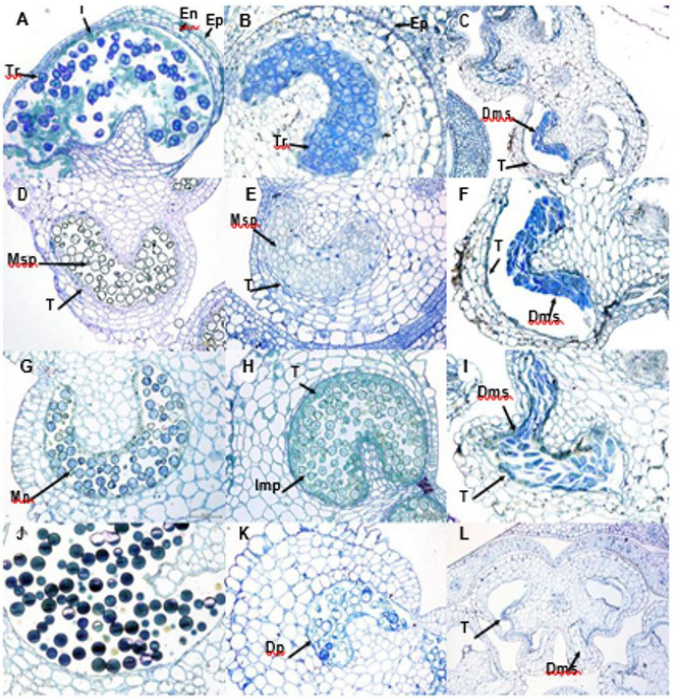

Figure 5: Transverse section of anthers of the wild type (left column, images A, D, G, J), of the M2#515 mutant with anthers containing empty pollen (middle column, images B, E, H, K), and anthers which do not contain pollen at all (right column, images C, F, I, L). Tetrad stage: A, B; young microspore stage: C, D, E, F, I; pollen mitosis: G,H; mature pollen stage: J, K, L. Ep: Epidermis; En: Endothecium; Dms: Degraded-Microspore; Dp: Dysfunctional Pollens; Imp: Immature Pollen; Mp: Mature Pollen; Msp: Microspore; T: Tapetum; Tr: Tetrads.

Discussion

In this study, we have shown that the tomato mutant with blossom drop phenotype found in an M2 EMS population displays abnormal microsporogenesis leading to degenerated pollen or no pollen formation at all. A causal relation between male meiotic dysfunction would lead to the failure of pollination and blossom shed seemed a first plausible explanation. However, our attempts to pollinate young flower buds of the mutant with wild type pollen remain unsuccessful, suggesting that both micro- and megasporogenesis were disrupted. Hence, plant developmental processes are blocked in the earlier stage of flower development. It is generally known that various biotic and abiotic stress factors can cause blossom drop. These factors, including extreme temperature and relative humidity, unbalanced nitrogen nutrition, but also by lack of water, reduced or extended light exposure, excessive wind, insect damage, foliar disease, excessive pruning or heavy fruit set [17]. One of the purposed methods used to reduce blossom drop damage is spraying plant hormones directly on the flowers [18,9]. Several studies have further confirmed the role of auxin and gibberellin in the initiation of tomato fruit growth and fruit set. Notable that their effects are particularly sensitive to environmental conditions [19,20]. Its developmental regulation begins in the floral meristem, where the architecture and organization of this tissue are controlled and endures shortly before fruit ripening is achieved. During the initial phase of tomato fruit development, the CLAVATA - WUSCHEL (CLV-WUS) feedback loop is known for controlling meristem activity and regulating the floral meristem size. A consequence of the regulation of carpel number in flowers which lead to the seed locules in fruits [20]. Silva et al. [21] observed that the interaction of gibberellin and two unrelated microRNA-controlled modules determine floral induction and development. The age-regulated microRNA156 demonstrated a comparable effect on flower control in diverse species, whereas gibberellin and the microRNA319 - regulated TCP transcription factors promote flowering in the facultative long-day Arabidopsis but suppress it in the day-neutral tomato.

Reichardt et al. [17] revealed that drought-induced blossom drop in tomato plants is controlled by the peptide hormone Phytosulfokine (PSK), which in reaction to drought stress depends on phytaspase 2, a subtilisin-like protease of the phytaspase subtype. This enzyme synthesizes the peptide hormone by aspartate-specific processing of the PSK precursor in the tomato flower pedicel. The premature blossom drop in response to environmental stress is triggered by this PSK in tomato. Nevertheless, the mechanism of peptide hormone that interferes with auxin and ethylene-mediated regulation of abscission zone activity is still not elucidated. Epigenetic instability was purposed as a vital role in the occurrence of blossom drop in the gardening of commercial tomato cultivars [8,9]. Also, it is also known that EMS mutagenesis may affect the epigenome. Mittelsten Scheid et al. [22] showed that such mutations were shown to reactivate a silent transgenic test locus in trans. The genetic and epigenetic effects of environmental mutagens and carcinogens have been reviewed in detail by Pulliero et al [23].

Blossom drop is a highly complex phenomenon that most likely originates from aberrant processes in complex hormonal pathways. Unfortunately, the plant materials in this study both male and female are infertile, so it was not possible to elucidate their mechanism by genetic analyses. Not only, the implementation of genes in the phytaspase 2 pathway [17] sequencing but also elucidating the eventual epigenetic regulation are promising strategies to explore the development mechanism.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author contribution statement

PD, HdJ, and JC conceived the explanation of the observed phenomena; PD, SS, and TN carried out the experiments; PD and HdJ wrote the manuscript; PT and JC contributed to the final version of the manuscript and JC supervised the project. All authors have read the manuscript and agreed with its content.

Acknowledgments

The authors gratefully acknowledge colleagues from the Center for Agricultural Biotechnology, Kasetsart University, Thailand, for financially supporting this research. Mrs Yuphin-Srihirun and Mrs ApinonSonongare acknowledged for the specimen preparationfor the semi-thin section. Laboratory of Applied Microbiology supported an optical microscope of the 60x objective lens for higher magnification.

References

1. Levy A, Rabinowithch HD and Kedar N. Morphological and physiological characters affecting flower drop and fruit set or toma-

toes at high temperatures. Euphytica. 1978; 27: 211-218.

2. Picken AJF. A review of pollination and fruit set in the tomato (Lycopersicon esculentum Mill.). Journal of Horticultural Science. 1984; 59: 1-13.

3. Mascarenhas JP. Gene activity during pollen development. Annu. Rev. Plant. Physiol. Mol. Biol. 1990; 41: 317-338.

4. Li W, Ma H. Gametophyte development. Current Biology. 2002; 12: R718-R721.

5. Kuo CG, Chen BW, Chou MH, Tsai CL, Tsay TS. Tomato fruit-set at high temperatures. Proceedings of the First International Symposium on the Tropical Tomato, October 1978, Asian Vegetable Research and Development Center, Shanhua, Taiwan. 1979; 94-108.

6. Storme DN, Geelen D. The impact of environmental stress on male reproductive development in plants: biological processes and molecular mechanisms. Plant, Cell and Environment. 2014; 37: 1-18.

7. Chaban IA, Kononenko NV, Gulevich AA, Bogoutdinova LR, Khaliluev MR, and Baranova EN. Morphological Features of the Anther Development in Tomato Plants with Non-Specific Male Sterility. Biology. 2020; 9: 1-17.

8. Ozores-Hampton M, and McAvoy G. What causes blossom drop in tomatoes? The Tomato Magazine. 2010; 14: 4–5.

9. Ozores-Hampton M, Kiran F and Mcavoy G. Blossom Drop, Reduced Fruit Set, and Post-Pollination Disorders in Tomato. University of Florida, Institute of Food and Agricultural Sciences, Electronic Data Information Source. 2012; 1-6.

10. Trinklein D. Understanding tomato fruit set. Integrated Pest Management University of Missouri. 2013.

11. Harel D, Fadida H, Slepoy A, Gantz S, Shilo K. The Effect of Mean Daily Temperature and Relative Humidity on Pollen, Fruit Set and Yield of Tomato Grown in Commercial Protected Cultivation. Agronomy. 2014; 4: 167-177.

12. Pressman E, Peet MM, Pharr DM. The effect of heat stress on tomato pollen characteristics is associated with changes in carbohydrate concentration in the developing anthers. Annals of Botany. 2002; 90: 631–636.

13. Sato S, Peet MM, and Thomas JF. Physiological factors limit fruit set of tomato (Lycopersicon esculentum Mill.) under chronic, mild heat stress. Plant Cell and Environment. 2000; 23: 719–726.

14. Giorno F, Wolters-Arts M, Mariani C and Rieu I. Ensuring Reproduction at High Temperatures: The Heat Stress Response during Anther and Pollen Development. Plants. 2013; 2: 489-506.

15. Mohan A, and Senthilkumar D. A survey of tomato blossom and flower drop to the influence of environmental phenomena (Solanum lycopersicumL.). Int. J. Agric. Environ. Food Sci. 2019; 3: 15-20.

16. Kho YO and Baër J. Observing pollen tubes by means of fluorescence. Euphytica. 1968; 17: 298–302.

17. Reichardt S, Piepho H-P, Stintzi A, Schaller A (2020). Peptide signaling for drought-induced tomato flower drop. Science. 367: 1482 – 1485.

18. Minges PA, Mann LK. Improving tomato fruit set. Use of hormone spray treatment for improvement of fruit set for spring and early summer harvest. California Agriculture. 1949.

19. de Jong M, Mariani C, and Vriezen, WH. The Role of Auxin and Gibberellin in Tomato Fruit Set. Journal of Experimental Botany. 2009; 60: 1523–32.

20. Quinet M, Angosto T, Yuste-Lisbona FJ, Blanchard-Gros R, Bigot S, et al. Tomato Fruit Development and Metabolism, Frontiers in Plant, Frontiersin.org Frontiers in Plant Science. 2019; 10: 1554.

21. Silva GFF, Silva EM, Correa JPO, Vicente MH, Jiang N, et al. Tomato Floral Induction and Flower Development Are Orchestrated by the Interplay Between Gibberellin and Two Unrelated Micro RNA-Controlled Modules."New Phytologist. 2018; 221: 1328–1344.

22. Mittelsten Scheid O, Afsar K, Paszkowski J. Release of Epigenetic Gene Silencing by Trans-Acting Mutations in Arabidopsis. Proceedings of the National Academy of Sciences. 1998; 95: 632–637.

23. Pulliero A, Cao J, dos Reis Vasques L, Pacchierotti F. Genetic and Epigenetic Effects of Environmental Mutagens and Carcinogens. BioMed Research International. 2015; 1–3.