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The role of PCD in sexual dimorphism of dioecious *Spinacia oleracea* L.

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Research Article

Abstract − The formation of non-hermaphroditic, i.e. male or female, flowers is a rare event in the plant kingdom. *Spinacia. oleracea* provides an ideal unisexual floral developmental system for studying the structural development of floral organs. These species forms non-hermaphroditic flowers; the pistil is fertile in the female flower, but the development of the stamens stops at an early phase and this organ atrophies and becomes nonfunctional, while the male flowers form four fertile stamens, but there is not any trail of the pistil, it aborts at a much early stage. We searched for the presence of programmed cell death (PCD) in abortive tissues during the ontogenetic development of these flowers. These results show curicial information on how the fertile sex organ in spinach differentiates and develops while the development of the other aborted sex organ is arrested ; The presence of PCD occur in unisexual flower development in rhe very early stage and continue short time. We also found that stamen development in the female flower and pistil development in the male flower were subject to changes that did not result in large-scale structural changes. The PCD data obtained is the first study of spinach in the previous studies. These studies are shedding additional light on the sexual specialization hypothesis. Moreover, the ability to manipulate or control the flowering of the dioecious plant by simple means holds great potential, both from an economic aspect and to increase food production for an ever-growing human population.

Keywords − *Flower, ontogeny, programmed cell death, spinach, unisexuality.*

1. Introduction

Dioecious species produce unisexual flowers : either male (staminate) or female (pistillate) flowers on separate individuals. Dioecy is existent only in~7% of flowering plants (Renner, 2014). According to Ainsworth & (1998), "dioecious species are widespread among taxa, and dioeciousness is thought to have evolved in each individual lineage independently of hermaphroditic ancestors" (Duana, Xiaofang, Shi, Zhonglai, & Zhongtao, 2018; Charlesworth, 1978).

Our working crop spinach is an economically important dioecious plant. Our working crop spinach is a unisexual flower-forming an economically important plant. It is grown up all on the world. It is the best-selling frozen vegetable in many countries. Spinach is delicious vegetables, it is including beta-carotene and folic acid, and is a source of vit C, iron, phosphorus, sodium, and potassium (Henry, Takashi, Ryutaro, & Luca, 2018). Our literature searches revealed that the mechanism of PCD in the growing of unisexual flowers of spinach species has not been studied. The study we conducted will exposed the diversity of mechanisms embracing in female or male flowers development in different plant. It can be produced in the future time and preventing yield loss in male individuals of dioecious individuals. Most unisexual flowers first contain the original androecium and gynoecium, followed by the arrest of the pistillate or staminate primordia before maturity (Diggle, Di Stilio, Gschwend, Golenberg, & Moore, 2011).

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PCD is genetically controlled process that occurs during development. It is a fundamental process that, although completely destructive at the cellular level, plays an main role in plant growth and defense. It may be detected in vary types of plant tissues (Bartoli, Forino, Durante, & Tagliasacchi, 2015). During PCD, cell shrinkage, vacuolization, degradation of cell contents, specific protease activation, chromatin condensation, DNA fragmentation (Huang, Lowe, Churchman, Schipper, & Cursons, 2016; Nawkar, Maibam, Park, Sahi, & Lee, 2013), caspase-like activities (Vardar, Aytürk, & Yanık, 2017; Michael, Robert, Randall, & Jeffrey, 2018), and mitochondrial dysfunction (Ou, Li, Wang, Li, & Quan, 2012) may occur.

A number of dioecious or monoecious plants, such as spinach species, are grown in large numbers worldwide. Most of these species act an critical role in the agricultural economy of countries. In addition to the female and male flowers produced by dioecious spinach, hermaphrodite flowers are very rarely born (Naeger and Golenberg, 2016; Rosa, 1925). Clearly, the transition from hermaphroditic to unisexual flowering is a crucial stage in the living of flowering plants.

In this study, we aimed to investigate the role of PCD in abortive tissues of spinach that occurs during ontogenetic development of female and hermaphrodite flowers. The data to be obtained are the first study among previous studies with spinach.

2. Materials and Methods

Test material Seeds of *S. oleracea* L. Turkey were planted in potting soil and grown in growth chambers at 20 ^{0}C under long daylight conditions (18 hours light, 18 – 20 ^{0}C temperature, 6 hours dark). Flowers were randomly selected from both male and female flowers for the studies.

2.1. Stereomicroscopic Analysis

First, the mature male and female flowers were morphologically analysed using a stereomicroscope (Olympus 970931). We dissected the flowers and observed them under a digital stereomicroscope. Flowers were investigated and photographed (KAMERAM software). The sizes of the flower parts (stamens and pistil) were measured with the stereomicroscope and the samples were prepared for light analysis.

2.2. Light Microscopic Analysis

For light microscopic analysis, the material was fixed in a FAA solution (37 % formaldehyde : acetic acid : ethanol : dH_2O, 10 : 5 : 50 : 35). Later, the samples were embedded in paraffin blocks. Blocks were sectioned at 3 - 8 μm using a Leica RM2235 rotary microtome and sections were stained with Delafield's hematoxylin.

2.3. Process of Programmed Cell Death (PCD)

Cells were studied in detail using DAPI and TUNEL techniques to investigate PCD. Histological sections of male and female flowers were stained with DAPI (4-6-diamidino-2-phenylindole) (1 μg/mL) to diagnose nuclear morphology. TUNEL analysis provides the ability to detect DNA damage, especially for early stage PCD detection. Dead cells are stained with green fluorescence, and normal cells are stained with blue fluorescence (Coimbra, Torrão, & Abreu, 2004).
For the TUNEL method (TdT-mediated dUTP nick end labeling), sections were adhered to slides coated with poly-L-lysine and incubated with reagents from the ApopTag®Plus (Chemicon). (Wang, Wu, Xu, Gao, & Chen, 2010). Unlike the positive control, my TdT enzyme was not used for the negative control.

3. Results and Discussion

In this research, the development phases of unisexual two flowers of spinach and the role of PCD during formation were investigated.

We found that hermaphrodite, female (pistillate) and male (staminate) flowers are produced in spinach plants. In this study, we examined the development of unisexual flowers, so we only examined male and female flowers in monoecious plants.

Many tightly packed male and female flowers in figure 1a, on the plants are located in apex or around the branch in figure 1b, 1c.

Figure 1. Flowering of *S. oleracea* L. plant. a. Branch of monoecious plant. The pistillate (red arrow) and the staminate flowers (blue arrow) in various stages, b. Terminal branches from a monoecious plants; staminate flowers formed on plant and pistillate (red arrow) flowers, c. Mixed pistillate and hermaphrodite flowers (black arrow) are showing in the same cluster on dioecious plant. lf. Leaf.

3.1. Characteristic of Young Flowers

Young male flowers occur in the inflorescence in figure 2a. Young female flower buds are 5 of number at the shoots in figure 2b, 2c. There are four sepals in the male flowers with covered bracts in figure 2d, 2e. while the female flowers contain two sepals and the style develops on the fertile pistil in figure 2f. A male flower bears four fertile stamens, but there is no trace of a stamen of female flower buds. In mature male flowers in figure 2g, there are traces of gynoecium, while mature female flower in figure 2h, bears fertile gynoecium.

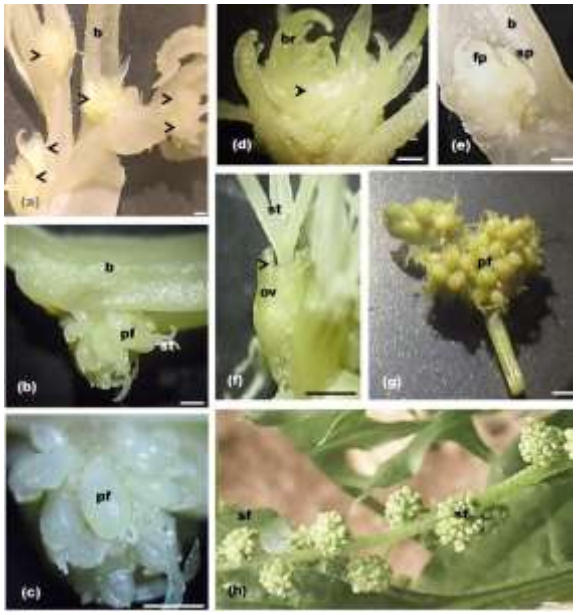

Figure 2. Flower morphology of *S. oleracea* L. viewed with a stereomicroscope. a. Male flower buds (arrows), b. Young female flower cluster, c. Details of b, d. Male flower bud (arrow) with covered bracts, e. Male flower bud in advanced stage: bracts are broken, f. A young female flower; styles reaches (arrow) into fertile pistil, g. Mature female flower cluster, h. Mature male flower cluster; the spinach plants form longer stalks of male flowers that develop and extend similar fingers from each node. an. Anther, b. Branch, br. Bracte, fp. Flower primordium, ov. Ovary, pf. Pistillate flower (female), sf. Staminate flower (male), sp. Sepal, st. Style. Scale bar, 1 mm.

3.2. Ontogenic development of flowers

In our study, we characterized the ontogenic development of flowers of *S. oleracea* L. from the beginning of the apical meristem to the mature flower stage in Figure 3.

Firstly, the apical meristem develops from the vegetative meristem in both the male and female flowers in Figures 3a, 3b. The flower meristem develops into the primordia of these floral organs, i.e., sepal, stamen, and carpel, in that order. The sepal primordia develop sequentially and circularly. Up to this stage, the development of the both flowers is similar. At this earliest stage studied, the developmental phase of unisexual flowers did not differ significantly; only the male primordium tissue faster growthed. The 3rd and 4th floral whorls (stamens and carpels) are extremely different between flowers ; nature of the organs and their number.

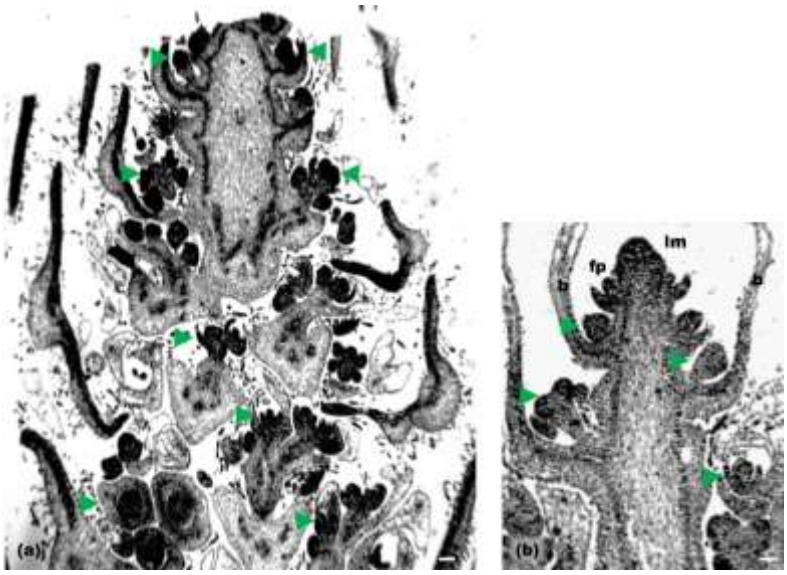

Figure 3. The two example images show the organization of inflorescences of *S. oleracea* L. in longitudinal section. Light micrographs show, a, b. Arrow indicates a flower cluster, with the oldest flowers represented by the lower symbol. b. Branch, im. Inflorescence meristem, fp. Flower primordium, scale bar, 50 μm.

3.2.1. Development of the Flowers

In our study, we characterized the ontogenic development of flowers of *S. oleracea* L. from the beginning of the apical meristem to the mature flower stage in Figure 4.

Firstly, the apical meristem develops from the vegetative meristem in both the male and female flowers. The flower meristem develops into the primordia of these floral organs, i.e., sepal, stamen, and carpel, in that order. The sepal primordia develop sequentially and circularly. Up to this stage, the development of the both flowers is similar. At this earliest stage studied, the developmental phase of unisexual flowers did not differ significantly; only the male primordium tissue faster growthed.

The 3rd and 4th floral whorls (stamens and carpels) are extremely different between flowers ; nature of the organs and their number.

3.2.2. Development of the Male Flower

Stamens begin to develop after sepals in figure 4a. No sign of a gynoecium at the flower apex in the figure 4b, but the anther primordia formed in figure 4c. Archaespore cells form first in the anthers, and in the advanced stage the stamens have become fertile in figure 4d**.**

3.2.3. Development of the Female Flower

The sepals develop but the stamens are not visible in figure 4e, 4f. Later the gynoecium primordium develops as a small protuberance in figure 4g. In the advanced stage, the fertile gynoecium primordium occupies most of the flower bud center. Later, four dry short styles and the stigma begin to grow at the apex

of the gynoecium. The gynoecium contains a single ovule. The seed anlage is anatropous, bitegmic and basally located in figure 4h.

In some species, as we present in spinach flowers, unisexual flowers may not show remnants of the atrophic sex. For example, the female flowers of *Cannabis sativa* are able to transition directly from the beginning of perianth development to the beginning of carpel formation. These flowers bear no trace of the stamen primordium (Mohan, & Nath, 1964).

Although the developmental phases of unisexual flowers of spinach show similarities to the unisexual flower developmental phases of some species, differences are also seen in the arrest of floral meristem tissue development. In the flowers of *Zea may*s and *Ficus carica*, for example, the development of sexual organs begins. The developmental arrest take places during the loss of the gynoecium (Aytürk, & Ünal, 2016). In contrast, *Thalictrum dioicum* L. species (Di Stilio, Kramer, & Baum, 2005), rudimentary organs are not present in the mature unisexual flowers. The development of the male flower of *Laurus nobilis* L. (Aytürk & Ünal, 2012) and *S. oleracea* L. (Sherry, Eckard, & Lord, 1993) was similar, and the unisexual flowers were formed in an early phase.

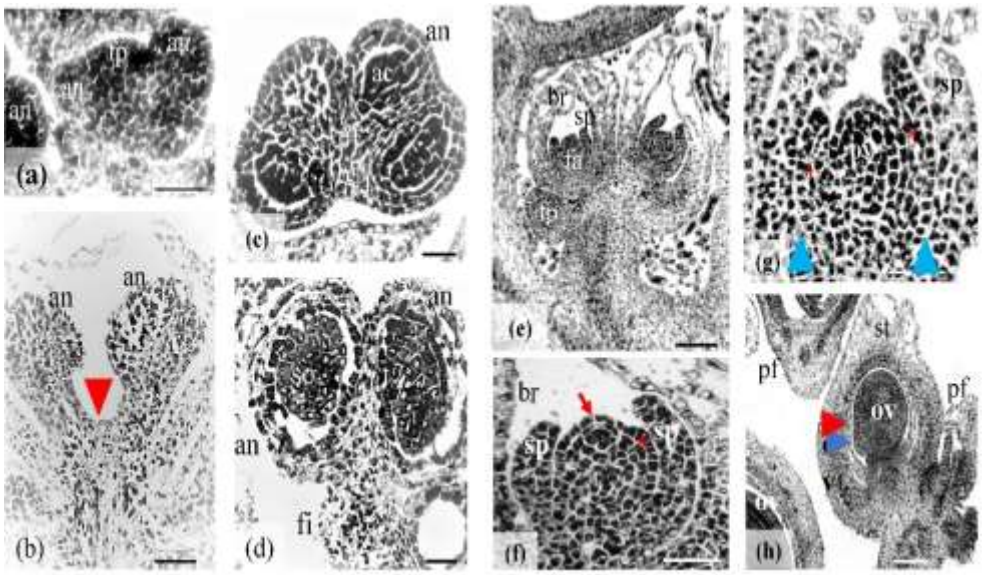

Figure 4. Developmental stages of flowers of *S. oleracea* L. in longitudinal section. Floral organogenesis in the flower viewed with a light microscope: a-d. male flower, e-h. female flower. a. First anther primordia, b. No evidence of gynoecium is seen at the floral apex (arrow), c. Young anthers have archesporial cells, d. Young anthers have parietal and sporogenic tissue. e. Two ascending floral primordia, f. Pistil initiation (asterisk); regular cell layers of floral apex (rarrow), g. Flower gynoecium (asterisk); no evidence of a stamen can be seen on floral primordium (arrows), h. Extension of gynoecium and outer (red arrow) and inner (blue arrow) integument around single ovule. ac. archesporial cells, an. Anter, br. Bract, fa. Floral apex, fp. Flower primordium, ov. Ovary, pf. Pistillate flower, so. sporogenous tissue, sp. Sepal, st. Style. Scale bar, 100 μm.

3.3. Programmed Cell Death

3.3.1. DAPI staining

We performed DAPI staining as a precursor to TUNEL analysis to determine whether atrophy was present in the stamens of the pistillate flower and the pistil of the staminate flower.

We identified three stages for female and male flowers based on our observations above, that PCD may occur,

I. the initial stage of the sepal primordium,

II. the initial stage of the pistil primordium tissue in the male flower,

III. the stamen primordium tissue in the female flower.

We identified the stages based on our researches : During the morphological observations with the stereomicroscope, we noticed that the male organs of the female flower and the female organ of the male flower stop their development at young stage, and remain small later.

In the stage I, in the both flower, the elliptical nuclei of the sepal primordium cells glow light blue and are evenly distributed in the chromatin nucleus in figure 5a.

In the stage II, in the male flower in figure 5b, chromatin condensed in the nuclear periphery of the primordium cells of the pistil is clearly visible in figure 5c, while pistil tissue in the female flower, the nuclei in all cells of this tissue fluoresce light blue and chromatin is evenly distributed in figure 5a.

In the stage III, in the female flower in figure 5d, the chromatin condensed in the nuclear periphery of the stamen primordium cells are clearly visible, whereas stamens tissue in the male flower fluoresce light blue in the all cells nuclei and the chromatin is equally spreated in the nucleus.

Our observation, we got an idea of which tissues we need for TUNEL analysis.

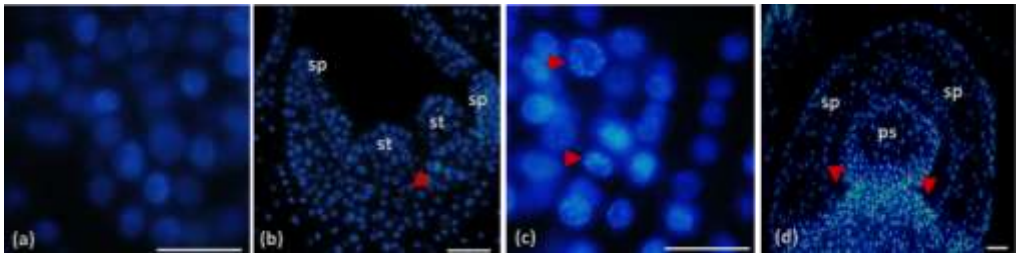

Figure 5. Images of DAPI staining. a. In stage I, cells with spherical nuclei, chromatin evenly distributed in the nucleus. b. Image of pistil primordium (arrow) of male flower at stages II, clear nuclear condensation (arrows) in nuclei (c). d. Image of stamen primordium (arrow) of female flower at stage III. Scale bars, 10µm (a,c)*, 50µm (b,d). * *Similar cell morphology was showed in stamens and pistil tissues, so only photos of male floral cells are shown.*

3.3.2. TUNEL assay

In the stage I, cells subjected to TUNEL analysis did not indicate bright green, i.e., they showed a negative result in figure 6a. At stage II, the pistil primordium tissue cells of the male flower in figure 6b. bright green was visible in figure 6c. At stage III in contrast to stage II, bright green was observed in the stamen primordium cells in figure 6d, confirming PCD. The bright colours were observed at a very short phase in flowers. After these stages, only autofluorescence was detected in these tissues.

We have achieved our main goal in this research on unisexual spinach flowers : we have proved the presence of PCD in unisexual flower development by studying flower ontogeny. We also found that stamen development in the female flower and pistil development in the male flower were exposed changes that did not result in large-scale structural changes. *Asparagus officinalis* (Bracale et al., 1997), *Zea maize* (DeLong Calderon-Urrea, & Dellaporta, 1993), and *Silena latifolia* (Grant, Hunkirchen, & Saedler, 1994) showed that PCD is responsible for the interruption of sexual organs development.

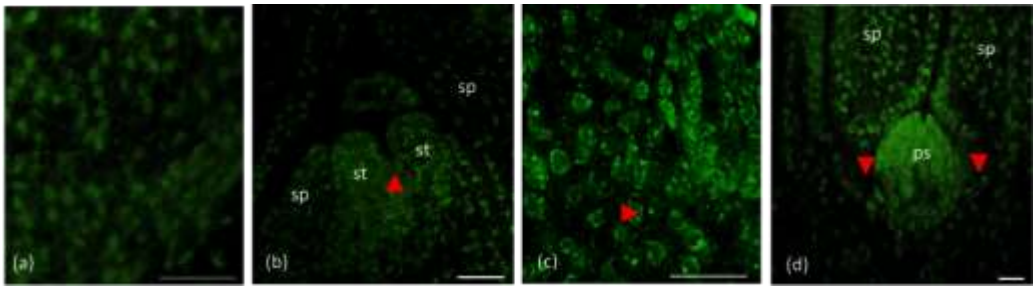

Figure 6. Images of TUNEL analysis. **a.** Cells without bright green indicating no PCD in stage I. **b.** Images of the beginning of the pistil primordium at the male flower (arrow), **c**. Enlarged image of cells with PCD, note the bright green cells (arrows). **d.** The stamen primordium (arrow) of the female flower. Scale bars, 10 μm (**a,c**)*, 50 μm (**b,d**). ***Similar cell morphology was showed in stamen and pistil tissues, so only photos of cells from male flower cells are shown.*

4. Conclusion

This analysis is designed to an response to the following question was explored: do PCDs play a role in the abortive of dioecious spinach flowers ? In this work on spinach, we have examined two basic parts of development. Firstly, we investigated how ontogeny occurs in the sex organs of unisexual flowers by comparing two unisexual flowers. These results suggest that sexual differentiation of spinach flowers begins at a early stage and is accompanied by early dimorphism. Base on our findings, we showed the analysis that plant sex type in spinach is determined by PCD, but there is no obvious PCD in the primordial tissue. These are preliminary results. Our project result needs to be supported by molecular analysis, a more detailed project on same topic needs to be studied. Our study of sex determination of the spinach floral organ development will be particularly important in determining how the novel morphologies in spinach arose.

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Author Contributions

Özlem Aytürk : Conceptualization of research, designing of experiments, contribution of experimental materials, application of all tests and info collection, analysis of data and interpretation, write of the manuscript.

Özal Mutlu : Designing of experiments, prepared of the test materials, analysis and interpretation of data, write of the manuscript.

Asuman Karadeniz : Execution of field / lab experiments.

Conflicts of Interest

The authors have declared no conflict of interest

References

Ainsworth, C., Parker, J., & Buchanan-Wollaston, V. (1998). *Sex determination in plants*. Currient Topics in Development Biology, 38: 167-223. https://doi.org/10.1016/S0070-2153(08)60247-1

Aytürk, Ö., & Ünal, M. (2012). *Structural Analysis of Reproductive Development in Staminate Flowers of Laurus nobilis* L. Notulae Scientia Biologicae, 4(1), 31-43 https://doi.org/10.15835/nsb416383

Aytürk, Ö., Unal, M. (2016). *Comparison of Female, Gall and Male Flower Development with Microscopic and Molecular Tecniques in Dioecious Ficus carica* L. PhD, Marmara University, Istanbul, Turkey. https://tez.yok.gov.tr/UlusalTezMerkezi/tezSorguSonucYeni.jsp

Bartoli, G., Forino, L.M.C., Durante, M., & Tagliasacchi, A.M. (2015). *A lysigenic programmed cell death-dependent process shapes schizogenously formed aerenchyma in the stems of the waterweed Egeria densa*. Annals of Botany, 116: 91-99 https://doi.org/10.1093/aob/mcv067

Bracale, M., Caporali, E., Galli, M.G., Longo, C., Marziani-Longo, G., Rossi, G., Spada , A., Coave, C., Falavigna, A., Raffaldi, F., Maestri, E., Restivo, F.M., & Tassi, F. (1991) Sex determination and differentiation in *Asparagus officinalis* L. Plant Sci. 80: 67 ± 77 https://doi.org/10.1016/0168-9452(91)90273-B

Charlesworth, D., & Charlesworth, B. (1978). *A model for the evolution of dioecy and gynodioecy*. The American Naturalist, 112: 975-997 https://doi.org/10.1086/283342

Coimbra, S., Torrão, L., & Abreu, I. (2004) Programmed cell death induces male sterility in *Actinidia deliciosa* female flowers. Plant Physiol. Biochem., 42, 537–541. https://doi.org/10.1016/j.plaphy.2004.05.004

DeLong, A., Calderon-Urrea, A., & Dellaporta, S.L. (1993). Sex determination gene TASSELSEED2 of maize encodes a short-chain alcohol dehydrogenase required for stage-specific floral organ abortion. Cell ,74: 757 ± 7. https://doi.org/10.1016/0092-8674(93)90522-R

Di Stilio, V.S., Kramer, E.M., & Baum, D.A. (2005). *Floral mads box genes and homeotic gender dimorphism in Thalictrum dioicum (Ranunculaceae)-a new model for the study of dioecy*. The Plant Journal, 41: 755-766 https://doi.org/10.1111/j.1365-313X.2005.02336.x

Diggle, P.K., Di Stilio, V.S., Gschwend, A.R., Golenberg, E.M., Moore, R.C., Russell, J.R.W., & Sinclair, J.P. (2011). Multiple developmental processes underlie sex differentiation in angiosperms. Trends Genet. 27, 368–376. https://doi.org/10.1016/j.tig.2011.05.003

Duana, T., Xiaofang, D., Shi, C., Zhonglai, L., & Zhongtao, Z., (2018). *Evolution of sexual systems and growth habit in Mussaenda (Rubiaceae): Insights into the evolutionary pathways of dioecy*. Molecular Phylogenetics and Evolution, 123: 113-122. https://doi.org/10.1016/j.ympev.2018.02.015

Durand, D., & Durand, A. (1991). Sex determination and reproductive organ differentiation in mercurialis. Plant science, 80, 49-65. https://doi.org/10.1016/0168-9452(91)90272-A

Grant, S., Hunkirchen, B., & Saedler, H. (1994). Developmental differences betweenmale and female flowers in the dioecious plant *Silene Latifolia*. Plant J. 6: 471 ± 480 https://doi.org/10.1046/j.1365-313X.1994.6040471.x

Henry, I.M., Takashi, A., Ryutaro, T., & Luca, C.1. (2018). *One Hundred Ways to Invent the Sexes: Theoretical and Observed Paths to Dioecy in Plants*. Annual Review of Plant Biology, 69: 553-575. https://doi.org/10.1146/annurev-arplant-042817-040615

Huang, Y-T., Lowe, D.J., Churchman, G.J., Schipper, L.A., & Cursons, R. (2016). *DNA adsorption by nanocrystalline allophane spherules and nanoaggregates, and implications for carbon sequestration in Andisols*. Applied Clay Science,120: 40-50. https://doi.org/10.1016/j.clay.2015.11.009

Michael, R., Robert ,L., Randall, J. M., & Jeffrey, D.K. (2018). *Plant Mating Systems Often Vary Widely Among Populations. Frontiers in Ecology and Evolution*, 6: 38 https://doi.org/10.3389/fevo.2018.0

Mohan Ram, H.Y., & Nath, R. (1964). *The morphology and embryology of Cannabis sativa Linn. Phytomorphology*, 14, 414-429.

Naeger, J., & Golenberg, E. (2016). *Mode and tempo of sequence and floral evolution within the Anserineae*. Plant Systematic Evolution, 302: 385-398. https://doi.org/10.1007/s00606-015-1269-z

Nawkar, G.M., Maibam, P., Park, J.H., Sahi, V.P., & Lee, S.Y. (2013). *UV-Induced cell death in plants.* International Journal of Molecular Sciences, 14: 1608-1628 https://doi.org/10.3390/ijms14011608

Ou, X.H., Li, S., Wang, Z.B., Li, M., & Quan, S. (2012). *Maternal insulin resistance causes oxidative stress and mitochondrial dysfunction in mouse oocytes.* Human Reproduction, 27: 2130–2145. https://doi.org/10.1093/humrep/des137

Renner, S.S. (2014). *The relative and absolute frequencies of angiosperm sexual systems: dioecy, monoecy, gynodioecy, and an updated online database*. American Journal Botany, 101: 1588-1596. https://doi.org/10.3732/ajb.1400196

Rosa, J. (1925). Sex expression in spinach. Hilgardia, 1: 259-274 https://doi.10.3733/hilg.v01n12p259

Sather, D.N., York, A., Pobursky, K.J., & Golenberg, E.M. (2005). *Sequence evolution and sex-specific expression patterns of the C class floral identity gene, SpAGAMOUS, in dioecious Spinacia oleracea* L. Planta, 222 (2): 284-92 https://doi.org/10.1007/s00425-005-1544-2

Sherry, R.A., Eckard, K.J., & Lord, E.M. (1993). *Flower development in dioecious Spinacia oleracea (Chenopodiaceae).* American Journal Botany, 80: 283-291 https://doi.org/10.1002/j.1537-2197.1993.tb13801.x

Vardar, F., Aytürk, Ö., & Yanık, F. (2017). *Programmed cell death evidence in wheat (Triticum aestivum* L.*) roots induced by aluminum oxide (Al2O3) nanoparticles*. Caryologia, 70: (2), 112-119 https://doi.org/10.1080/00087114.2017.1286126

Wang, C. L., Wu, J., Xu, G. H., Gao, Y. B., & Chen, G. (2010). *S-RNase disrupts tip-localized reactive oxygen species and induces nuclear DNA degradation in incompatible pollen tubes of Pyrus pyrifolia*. Journal Cell Science, 123 (24): 4301–4309. https://doi.org/10.1242/jcs.075077

Zaitchik, B.F., Le Roux, L.G., & Kellogg, E.A. (2000) Development of male flowers in *Zizania aquatica* (North American wild-rice: Gramineae). International Journal of Plant Sciences 161 : 345 – 351. https://doi.org/10.1086/314268