Annals of Agricultural & Crop Sciences

Open Access

Austin Publishing Group

Special Article: Flower Development

Determination of the Microspore Development Concerning Floral Morphology for Improving Drought Tolerant Citron Watermelon (*Citrullus lanatus* var. *citroides*) Rootstocks Via Androgenesis.

Kurtar ES[1,*], Seymen M[1]; Alan AR[2], Toprak FC[2]; Atakul Z[3]; Metin D[3]; Lachin AR[4]; Yirmibes B[4]

[1]Department of Agriculture Faculty and Horticulture, Selcuk University, Turkey

[2]Plant Genetics and Agriculture Biotechnology Application and Research Center, Pamukkale University, Turkey

[3]Department of Horticulture, Institute of Science, Selcuk University, Turkey

[4]Institute of Science, Pamukkale University, Turkey

***Corresponding author: Kurtar ES**

Selcuk University, Horticulture Department of Agriculture Faculty, Konya, Turkey.

Email: ertansaitkurtar@selcuk.edu.tr

Received: March 20, 2023

Accepted: April 26, 2023

Published: May 03, 2023

Abstract

The negative effects of abiotic stress factors (especially drought and salinity) that arise due to global climate change are increasing in today's agricultural production. To eliminate the negative effects of these stress factors, some cultural practices have been implemented such as the breeding of tolerant cultivars and rootstock, exogenous applications of PGPR, AMF, and chemical substances, etc. Contrary to exogenous applications, providing tolerance with breeding methods has an important role in efficient and sustainable agriculture. Androgenesis is a phenomenon for the induction and development of microspore-derived pure lines, as initial material for F1 cultivar breeding, in different vegetable crops. Success in microspore embryogenesis is considerably related to the culture of microspores at an appropriate developmental stage. There is no standard protocol for the anther culture in citron watermelon (*Citrullus lanatus* var. *citroides*). Thus, a relation between floral morphology (bud length and width, bud index, sepal position, petal color), and microspore development (microspore diameter, uninucleated and binucleated phases) was investigated in citron. The male flower buds were collected at different sizes and microspore development was observed in both light and fluorescence microscopy. A strong positive relationship was detected between flower bud morphology and the specific stages of microspore development. To the author's knowledge, this is the first report to indicate that the microspore developmental stage can be estimated by flower bud morphology, and applied in the anther/microspore culture of citron watermelon.

Keywords: Drought; Rootstock breeding; Anther culture; Microspore development; Citron watermelon

Introduction

Cucurbita maxima x *Cucurbita moschata* hybrids are widely used as rootstock for watermelon production because they provide resistance and tolerance to both biotic (especially Fusarium wilt) and abiotic (drought, salinity, and low soil temperature) stress conditions. However, the drought tolerance of these rootstocks is limited and their nematode resistance is not at the desired level [4]. *C. lanatus* var. *citroides* (L.H. Bailey Mansf.), which is a wild form in the genus Citrullus, has different names such as "citron", "citron watermelon", and "forage watermelon". It is used in jam, pickles, cooking, water source in arid areas, and pectin for the food industry, in obtaining snacks and oil, and in the medical and cosmetic industry as much as for animal feed [2,16,19,37]. Due to its high drought tolerance, it is easily grown in arid and semi-arid regions [25]. In our previous studies, two different citron watermelon genotypes were compared to gourd and TZ-148 in terms of yield, phenolic compounds, and antioxidant content, and it has been revealed that they have the potential to be used as a commercial rootstock for watermelon under water stress conditions [31,40].

Citron watermelon is resistant to *Meloidogyne incognita* [33], and also strains 2 and 3 of *Fusarium oxysporum* f. sp. *niveum* have not been reported in watermelon [38]. Citron watermelon is a genetic source against biotic and abiotic stress condi-

Citation: Kurtar ES, Seymen M, Alan AR, Toprak FC, Atakul Z, et al. Determination of the Microspore Development Concerning Floral Morphology for Improving Drought Tolerant Citron Watermelon (Citrullus lanatus var. citroides) Rootstocks Via Androgenesis. Ann Agric Crop Sci. 2023; 8(2): 1130.

tions in watermelon production [5,8,10,15,18,21,26]. They can be crossed with watermelon cultivars (2n=2x=22) and used as candidates in rootstock breeding programs [6]. Its vegetation period is shorter than the winter squash (90-120 days) making it possible to get seeds twice a year [20].

The first step in producing an F1 hybrid variety is the development of pure parental lines and it takes 6-8 generations of inbreeding to reach the desired purity in open-pollinated species such as watermelon. This is a time-consuming, laborious, and high-budget process. Completely 100% homozygous pure lines can be obtained in one generation by anther/microspore culture. Utilization of these techniques allows the production of Doubled Haploid (DH) lines, shortens the process of recombinant pure line development, and allows the creation of high-quality F1 hybrid varieties with DH plants in the early stages of a breeding program. In this way, breeding efficiency is enhanced and the production of new varieties can be accelerated.

As aforementioned, success in anther/microspore culture depends on multiple factors such as microspore development stage, genotype, growing conditions, pretreatments, media composition, incubation conditions, and media refreshment. The microspore development stage is critical for inducing embryogenesis and haploidy frequency. Immature microspores can change direction from gametophytic to sporophytic development (embryogenic re-programming) [32]. It is well known that microspores at the uninucleate stage are generally favorable for the anther culture for many species and genotypes. But it differs among species and genotypes from tetrad, early to late uninucleate to the binucleate stage [3,9,23,27,30,39]. Because of many limitations, the anther culture-based double haploidization process is not generated in citron watermelon as a recalcitrant species.

So, the goals of this study were to compare the shapes of flower buds and the stages of microspore development, as well as to figure out the right size of bud for improving drought-tolerant citron watermelon (*Citrullus lanatus* var. *citroides*) rootstocks through androgenesis.

Materials and Methods

Experimental Area, Plant Material, Cultural Practices

The research was carried out in controlled greenhouse conditions. The soil of the research area has a clay-loam structure and contains 2.43% organic substance. It was determined that the EC was 1.05dS/m, the pH was 7.82 and the lime content was 7.3%, and the soil condition was relatively suitable for cultivation. Citron watermelon line SÜ-3 at the S3 level was selected as a donor from the gene pool at the Vegetable Growing and Breeding Department, Faculty of Agriculture, Selcuk University. SÜ-3 has vigorous plant growth habits, oval fruits of medium size, and hard fruit flesh (Figure 1). 2–3 days before planting the seedlings, 175kg/ha of MAP (mono ammonium phosphate) was applied with drip irrigation according to soil analyses. The black plastic mulch was used as a soil cover material to minimize the weed, disease, and pest hazards. The seedlings were planted with 100x60cm row spacing. Conventional cultural practices (disease and pest management, and fertilization) were performed during the entire growing period. Plants were pruned, trained, and developed a single stem morphology.

Figure 1: Plants and fruit of line SÜ-3.

Collection of Flower Buds

A series of flower buds were collected from healthy and vigorous plants at full flowering time in the morning (7:30–9:00 am). Then, the buds were immediately transported to the laboratory for microscopic observations. The buds were classified based on their sizes ranging from 4.5mm to 18.0mm in width and from 7.50mm to 20.0mm in length and numbered separately (Figure 2 & Table 1).

Table 1: Bud classification and size.

No	Length (mm)	Width (mm)
1	7.5 – 9.0	4.5 – 5.5
2	9.0 – 10.0	5.5 – 6.5
3	10.0 – 11.0	6.5 – 7.5
4	11.0 – 12.5	7.5 – 9.0
5	12.0 – 14.5	9.0 – 11.5
6	14.5 – 18.0	11.5 – 16.0
7	18.5 – 20.0	16.5 – 18.0

Figure 2: The classification of bud size in *Citron watermelon* line SÜ-3.

Determination of Bud Size, Bud Index Value, Sepal Position, Petal Color, and Microspore Developmental Phase

The length and width of 40 male buds were measured by a binocular microscope, and the average bud size was determined for each bud class. Microspore development was observed in both light and fluorescence-based microscopy (DAPI). For light microscopy analysis, about 50 pollen mother cells from 10 male buds were processed at each specific bud size. Anthers were extracted from buds, stained with 1% acetocarmine solutions for 5 min, and squashed over a microscope slide. Then excessive tissues were discarded, a cover slip was placed over them, and the diameter of the microspores was measured in three different observation fields at 400 (40x10) magnifications. The bud index was calculated separately for each bud examined and expressed as a width/length ratio. The position of the sepal (calyx) relative to the petal (corolla) and petal color were determined by visual observations.

For the fluorescence microscopy, five male buds were processed for each size. Buds were fixed in Carnoy solution (3:1 absolute ethanol and glacial acetic acid) for 24h and then immersed in 1NHCl for 30 min to digest the exine. Anthers were extracted from buds, stained with 1-2 drops of DAPI (4'-6-di-

amidino-2-phenylindole), and observed under the fluorescent microscope at 400 (40x10) magnifications.

Data Evaluation

The study was carried out in a randomized plot design with 3 replications. The data obtained were subjected to variance analysis in the JMP-13 package program. Significant differences between the applications were determined according to the 5% significance level and interpreted.

Results and Discussion

A limited number of researchers were reported to have produced double haploid lines in watermelon and citron via anther culture. It is well known that immature anthers including the late-uninucleated or early-binucleated phase at the beginning of the first mitotic division are amenable material for the initiation of anther culture for many species. Thus, microspore development (microspore diameter, uninucleated and binucleated phases) and floral morphology (bud length and width, bud index, sepal position) were investigated to identify the appropriate stage for the anther culture in citron watermelon.

We observed 7 distinct bud sizes associated with microspore developmental phases. The intact and autofluorescent-bearing exine was an important factor in preventing the identification of appropriate microspore development phases for anther culture of citron watermelon. Therefore, examinations with the acetocarmine staining technique failed to determine the microspore development stages. A similar bottleneck was reported in cassava [35]. However, the application of HCl, optimized for citron watermelon, provided successful digestion of the exine and facilitated the detection of microspore development stages.

Statistical analyses revealed that there is a strong relationship between microspore diameter, bud size, bud index, sepal position, and petal colour and their corresponding microspore development phase and depicted that bud morphology is an indicator and a convenient parameter to predict the appropriate microspore development stage, and could be used reliably in anther culture of citron watermelon. Similar results were reported for anther/microspore culture in many crops [3,7,17,22,23,34,41].

In our study different bud sizes produced different stages of microspores development. The average microspore diameter increased as the microspore development phase progressed, and reached its maximum size at the mature pollen (late two-celled pollen) phase. Each microspore developmental phase is characterized by a certain bud shape and size (Table 2 & Figure 3).

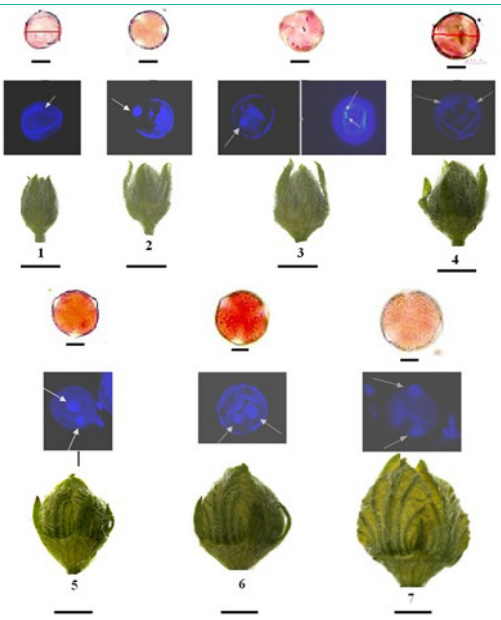

Figure 3: Flower bud and pollen development in '*Citron watermelon* line SÜ-3'. (1) Early uninucleated (2) Late Uninucleated (3) Late Uninucleated -Early Binucleated (4) Binucleated (5) Binucleated-Immature (6) Binucleated-Premature (7) Binucleated-Mature (Bar of microspores = 20µm; Bar of buds = 5mm).

The average diameter of the microspores was measured at 41.24µm, and their green buds were 8.24mm in length and 5.23 mm in width at the Early Uninucleated (EU) phase. The Bud Index (BI) was calculated at 0.633 and the sepal was above the petal. The Late Uninucleated (LU) microspores were 46.01µm in diameter, and their green buds measured 9.47mm in length, 6.18mm in width, and 0.651 BI. The sepal position of buds contained LU microspores above the petal.

The microspores between the LU and Early Binucleate (EB) phases contained both uninucleated and binucleated microspores, mostly uninucleated. It has been determined that the nuclei in the binucleated microspores (with a big vacuole) were quite small and difficult to observe. The microspores between LU-EB were found in buds of 51.13µm in diameter and their sizes were 10.51mm in length and 6.94mm in width. The BI value was determined at 0.661, and the sepal position of green buds was above the petal.

The First Binucleated (FB) microspores were found in relatively light-green and oval-shaped buds with a BI of 0.706. Their length and width were measured at more than 11mm and 8mm, respectively. The diameter of microspores was 54.96µm, and the sepal was mostly equal to the petal. The buds had more than 0.661 BI and contained only two nuclei of pollen.

Table 2: Bud Class (BC), Microspore Diameter (MD), Bud Size (BS), Bud Index (BI), Sepal Position (SP), Petal Color (PC), and Microspore phase (MS).

BC	MD (µm)	BS (mm)		BI	SP	PC	MS
		Length	Width				
1	41.24 e	8.24 e	5.23 e	0.633 e	Above	Green	EU
2	46.01 d	9.47 d	6.18 e	0.651 d	Above	Green	LU
3	51.13 c	10.51 d	6.94 de	0.661 d	Above	Green	LU - EB
4	54.96 bc	11.81 cd	8.32 d	0.706 c	Mostly Equal	Light Green	FB
5	57.04 b	13.56 c	10.61 cd	0.782 bc	Below	Green-Yellow	BI
6	60.57 ab	16.39 ab	14.09 b	0.860 a	Below	Yellow-Green	BP
7	63.14 a	18.81 a	16.47 a	0.876 a	Below	Mostly Yellow	BM (OBA)
	LSD: 2.61	LSD: 2.39	LSD: 1.97	LSD: 0.32			

EU: Early Uninucleated; LU: Late Uninucleated; EB: Early Binucleated; FB: Binucleated; BI: Binucleated-Immature; BP: Binucleated-Premature; BM: Binucleated-Mature; OBA: One day Before Anthesis

The diameter of microspores at Binucleated Immature (BI) was 57.04mm. They were clearly and easily observed and had larger (vegetative) and relatively smaller (generative) nuclei. The buds were green-yellow and their petal was prominently longer than their sepal. They were 13.56µm in length and 10.61µm in width. The microspores and the buds continued to grow and develop. The Binucleated-Premature (BP) pollens were detected in yellow-green and relatively globe-shaped buds. They had 16.39mm length and 14.09mm width, with a shorter sepal (nearly 50% of the petal) and 0.860 BI. The average diameter of the microspores was measured at 60.57µm.

Binucleated-Mature (BM) pollen grains had 63.14µm diameter and visible quite large two nuclei with a big vacuole. Its bud shapes were nearly globe-shaped with a BI of 0.876, and buds were 18.81mm and 16.47mm in length and width, respectively. The color of the petals was predominantly yellow, with an easily visible green vessel. The sepals were quite small, hardly visible, and sometimes deformed. The average size of citron microspores and pollen grains is considerably narrower than squash, pumpkin, and winter squash [13] but similar to melon [23].

A positive correlation was found between flower-bud size (length and diameter) and microspore development stages in the carob tree [7], cassava [35], alfalfa [41], and melon [24]. On the other hand, the correlation (R^2=0.59) between bud size, anther length, and microspore development stages showed that anther length was a reliable indicator in the tomato [3]. Contrary to the findings of Adhikari and Kang (2017), a considerable amount of publications suggested that anther sizes should not be used as a parameter in determining the appropriate microspore development stage in tomato [30], eggplant [28] and melon [24].

Our findings indicated that the buds were 5.0-6.0mm in width and 8.0-9.5mm in length and contained only uninucleated microspores. The microspores between uninucleated and binucleated were obtained from buds that measured 7.0mm in width, and 10.5mm in length. These stages have been identified as a characteristic and critical point for success in the anther-based double haploidization process of citron watermelon. Hence, the anther that contained pollen at the uninucleated stage shows a better response and uninucleated pollens were observed in flower buds that had 4.5-5.0mm length in melon [11]. The middle to late uninucleated microspore stages was the best in anther culture of winter squash (*Cucurbita maxima* Duch.) and pumpkin (*Cucurbita moschata* Duch.) [13]. In *Lagenaria siceraria* (Molina) Standl, pollen at the uninucleated stage collected from 5-7mm buds, is the most appropriate for androgenesis [12]. Male flower buds (10-12mm in length) containing microspores at the late-uninucleated stage are the best material for haploidization in watermelon [1,29].

The sepal (calyx) position of green buds contained microspores from uninucleated to uni-binucleated was longer than petal (corolla). The buds with mostly equal petals and sepals contained binucleated microspores in citron watermelon. Besides, Wang et al. (2015) argued that the microspore development stage was closely related to bud size and anther color, the petals were calyx slightly and the anthers were yellow and contained productive microspores at late-uninucleated in melon. On the other hand, the visual parts of the sepal and petal were a less reliable morphological feature in tomatoes [23].

Conclusion

Breeding of new cultivars and rootstocks tolerant to abiotic (drought and salinity) stress factors could be more profitable than exogenous applications in sustainable agriculture. Double Haploidization (DH) is a unique process to obtain pure lines that form the basis of F1 hybrid cultivar breeding programs. Anther culture is a way for the induction and development of microspore-derived plants in many species. It's well known that, this technique is complex and its efficiency is affected by many factors. Success in anther culture-based DH is considerably associated with the culture of microspores at an appropriate developmental stage. A close relationship was observed between bud morphology and the specific stages of microspore development. It could be safely used for the selection of an appropriate bud size containing the best development stage of microspore and applied in the anther/microspore culture of citron watermelon as a recalcitrant species. However, our findings established a citron watermelon line, to generalize these findings; it would be beneficial to study with a large number of genotypes.

Acknowledgments

This study was funded by the Scientific and Technological Research Council of Turkey (TÜBİTAK-1001) (Project No: 221O338).

References

1. Abdollahi MR, Darbandi M, Hamidvand Y, Majdi M. The influence of phytohormones, Wheat ovary co-culture, and temperature stress on another culture response of watermelon (Citrullus lanatus L.). Brazilian Journal of Botany. 2015; 38: 447–456.

2. Acar R, Behiç C, Mustafa A, Abdullah Ö. Determination of the change in feed value in different-sized fruits of forage watermelon (Citrullus lanatus var. citroides). Selcuk Journal of Agricultural Sciences. 2015; 2: 27–32.

3. Adhikari PB, Kang WH. Association of floral bud and anther size with microspore developmental stage in Campari tomato. Horticultural Science and Technology. 2017; 35: 608-617.

4. Aydınlı G, Kurtar ES, Mennan S. Screening of Cucurbita maxima and Cucurbita moschata genotypes for resistance against Meloidogyne arenaria. M. incognita. M. javanica. and M. luci. Journal of Nematology. 2019; 51: 1-10.

5. Balkaya A, Gungor B, Saribas Ş, Yildiz S. Determination of the effects of pumpkin rootstock on yield and fruit quality in mini watermelon cultivation. YYU J Agr Sci. 2018; 28: 237–246.

6. Bullitta S, Cifarelli S, Gladis T, Hammer K, Laghetti G. Collecting crop genetic resources in the Mediterranean agricultural island: Corsica (Part 1- north Corsica). 2007; www.corsica-isula.com.

7. Custódio L, Carneiro MF, Romano A. Microsporogenesis and anther culture in carob tree (Ceratonia siliqua L.). Scientia Horticulturae. 2005; 104: 65-77.

8. García-Mendívil HA, Sorribas FJ. Effect of Citrullus amarus accessions on the population dynamics of Meloidogyne incognita and M. javanica and watermelon yield. Scientia Horticulturae. 2021; 275: 109680.

9. Gorecka K, Kowalska U, Krzyżanowska D, Kiszczak W. Obtaining carrot (Daucus carota L.) plants in isolated microspore cultures. J Appl Genet. 2010; 51: 141-147.

10. Hwang JH, Ahn SG, Oh JY, Choi YW, Kang JS, et al. Functional characterization of watermelon (Citrullus lanatus L.) EST–SSR by gel electrophoresis and high-resolution melting analysis. Scientia Horticulturae. 2011; 130: 715–724.

11. Islam A, Misoo S, Ishii T. Selection of flower buds and carbon source for anther culture in melon (Cucumis melo). The IUP Journal of 2 Genetics & Evolution. 2011; 4: 3.

12. Kouakou KL, Doubi TS, Koffi KK, Kouassi KI, Kouakou TH, et al. Androgenic potential and anther in vitro culture of Lagenaria siceraria (Molina) Standl an edible-seed cucurbit. Int J Biol Chem Sci. 2015; 9: 1779-1789.

13. Kurtar ES, Balkaya A, Ozbakır M, Ofluoglu T. Induction of haploid embryo and plant regeneration via irradiated pollen technique in pumpkin (Cucurbita moschata Duchesne ex. Poir). Afr J Bio. 2009; 8: 5944–5951.

14. Kurtar ES, Balkaya A, Kandemir D. Evaluation of haploidization efficiency in winter squash (Cucurbita maxima Duch.) and pumpkin (Cucurbita moschata Duch.) through anther culture. Plant Cell Tissue and Org Cul. 2016; 127: 497–511.

15. Kurum R, Celik I, Eren A. Effects of rootstocks on fruit yield and some quality traits of watermelon (Citrullus lanatus). Derim. 2017; 34: 91–98.

16. Lauoku AL, Gnakri D, Dje Y, Kippre AV, Malice M, et al. Macronutrient composition of three cucurbit species cultivated for seed consumption in cote d'Ivoire". African Journal of Biotechnology. 2007; 6: 529-533.

17. Lauxen MS, Kaltchuk-Santos E, Hu C, Callegari-Jacquesi SM, Bodanese-Zanettini MH. Association between floral bud size and developmental stage in soybean microspores. Braz. Arch. Biol. Technol. 2003; 46: 515-520.

18. Levi A, Jarret R, Kousik S, Patrick Wechter W, et al. Genetic resources of watermelon. In: Grumet R. Katzir N. Garcia-Mas J. (eds) Genetics and Genomics of Cucurbitaceae. Plant Genetics and Genomics: Crops and Models. 2017; 20.

19. Minsart LA, Bertin P. Relationship between genetic diversity and reproduction strategy in a sexually-propagated crop in a traditional farming system. Citrullus lanatus var. citroides. 2008.

20. Mustafa AB, Alamin AAM. Chemical composition and protein degradability of watermelon (Citrullus lanatus) seeds cake grown in Western Sudan. Asian J Anim Sci. 2012; 6: 33–37.

21. Nawaz MA, Han X, Chen C, Zheng Z, Shireen F, et al. Nitrogen use efficiency of watermelon grafted onto 10 wild watermelon rootstocks under low nitrogen conditions. Agronomy. 2018; 8: 259.

22. Niazian M, Shariatpanahi ME, Abdipour M, Oroojloo M. Modeling callus induction and regeneration in an anther culture of tomato (Lycopersicon esculentum L.) using image processing and artificial neural network method. Protoplasma. 2019; 256: 1317–1332.

23. Nguyen ML, Ta THT, Huyen TNBT, Voronina AV. Anther-de¬rived callus formation in bitter melon (Momordica charantia L.) as influenced by microspore development stage and medium compo¬sition. Selskokhozyaystvennaya Biologiya = Agricultural Biology. 2019; 54: 140-148.

24. Nguyen ML, Huyen TNBT, Trinh DM, Voronina AV. Association of bud and anther morphology with developmental stages of the male gametophyte of melon (Cucumis melo L.). Plant Genetics and Biotechnology. 2022; 26: 146-152.

25. Nkoanaa DK, Mashiloc J, Shimelis H, Ngwepeb RM. Nutritional phytochemical compositions and natural therapeutic values of citron watermelon (Citrullus lanatus var. citroides): A Review. South African Journal of Botany. 2021; 1-13.

26. Pal S, Rao ES, Hebbar SS, Sriram S, Pitchaimuthu M, Rao VK. Assessment of Fusarium wilt resistant Citrullus sp. rootstocks for yield and quality traits of grafted watermelon. Scientia Horticulturae. 2020; 272: 109497.

27. Perera PI, Vidhanaarachchi R. Anther culture in coconut (Cocos nucifera L.). Jose M. Seguı´-Simarro (ed.). Doubled Haploid Technology: Volume 3: Emerging Tools. Cucurbits. Trees. Other Species. Methods in Molecular Biology. 2021; 2289.

28. Salas P, Rivas-Sendra A, Prohens J, Segui-Simarro JM. Influence of the stage for anther excision and heterostyly in embryogenesis induction from eggplant anther cultures. Euphytica. 2012; 184: 235- 250.

29. Sari N, Solmaz I. Doubled Haploid Production in Watermelon. Methods in Molecular Biology. 2021; 2289: 97-110.

30. Seguí-Simarro JM, Nuez F. Meiotic metaphase I to telophase II as the most responsive stage during microspore development for callus induction in tomato (Solanum lycopersicum) anther cultures. Acta Physiol Plant. 2005; 27: 675–685.

31. Seymen M, Yavuz D, Ercan M, Akbulut M, Çoklar H, et al. Effect of wild watermelon rootstocks and water stress on chemical properties of watermelon fruit". Hortic Environ Biotechnol. 2021.

32. Soriano M, Li H, Boutilier K. Microspore embryogenesis: establishment of embryo identity and pattern in culture. Plant Reprod. 2013; 26: 181–196.

33. Thies JA, Buckner S, Horry M. Influence of Citrullus lanatus var. citroides rootstocks and their F1 hybrids on yield and response to root-knot nematode. Meloidogyne incognita. in grafted watermelon. Hortscience. 2015; 50: 9–12.

34. Tomasi P, Dierig DA, Backhaus RA, Pigg KB. Floral bud and mean petal length as morphological predictors of microspore cytological stage in Lesquerella. Hort Science. 1999; 34: 1269-1270.

35. Wang C, Lentini Z, Tabares E, Quintero M, Ceballo H, et al. Microsporogenesis and pollen formation in cassava. Biologia Plantarum. 2010; 55: 469-478.

36. Wang WW, Yang XP, Fan SY, Liu G, Zhang M, et al. Correlation between microspore development period and floral organ morphology of Cucumis melo L. Journal of Northwest A & F University - Natural Science Edition. 2015; 43: 108-112.

37. Wehner TC. Watermelon. http://cuke.hort.ncsu. edu/cucurbit/wehner/articles/ book16.pdf. 2011.

38. Wechter WP, Kousik C, McMillan M, Levi A. Identification of resistance to Fusarium oxysporum f. sp. niveum race 2 in Citrullus lanatus var. citroides plant introductions. Hort Science. 2012; 47: 334–338.

39. Winarto B, Teixeira da Silva JA. Microspore culture protocol for Indo¬nesian Brassica oleracea. Plant Cell Tissue Organ Cult. 2011; 107: 305-315.

40. Yavuz D, Seymen M, Süher S, Yavuz N, Türkmen Ö, et al. How do rootstocks of citron watermelon (Citrullus lanatus var. citroides) affect the yield and quality of watermelon under deficit irrigation?. Agricultural Water Management. 2020; 241: 106351.

41. Yi D, Sun J, Su Y, Tong Z, Zhang T, Wang Z. Doubled haploid production in alfalfa (Medicago sativa L.) through isolated micro¬spore culture. Sci Rep. 2019; 9: 9458.