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Ploidy estimation in pepper and eggplant via stomata characteristics

Hayati Bat[1], Fatma Nur Altındağ[1], Merve Arefe Yiğit[1], Ş. Şebnem Ellialtıoğlu[2], Nuray Çömlekçioğlu[3] *

[1]United Genetics Turkey, R&D Tissue Culture Laboratory, Mustafakemalpaşa, Bursa, Turkey
[2] Ankara University Technopolis, Ellialtioglu Tarim Teknolojileri Co. Ankara, Turkey
[3]*Osmangazi University, Faculty of Agriculture, Department of Horticulture, Eskişehir, Turkey

*Corresponding author: nrycomlekcioglu@ogu.edu.tr

Abstract

The most reliable methods to confirm the ploidy level in plants are to count the chromosomes or to measure the DNA in the cells by flow cytometry. However, these methods are laborious, time consuming, and require special equipment. In this study, the reliability of stomatal characteristics in confirming the ploidy level was investigated in haploid and spontaneous double haploid (SDH) pepper and eggplant plants. Stomatal characteristics were measured using a digital camera and related software from light microscope images in 100 samples at each ploidy level. Stomatal density, guard cell width and length were measured in random fields of view, and chloroplasts were counted. Mean stomatal lengths were determined as 28.34 µm and 40.39 µm, and mean stomatal widths were determined as 22.52 µm and 29.50 µm respectively for haploid and SDH plants in pepper. The stomatal density was 10.71 in haploid and 27.07 in SDH. Average stomatal lengths were determined as 22.32 µm and 32.00 µm, and mean stomatal widths were determined as 17.36 µm and 22.32 µm, respectively, in haploid and SDH eggplant. The stomatal density of eggplants were found to be 29.20 in haploid plants and 12.61 in SDHs. Chloroplast numbers in guard cells of SDH plants were determined to be 2 fold more than haploids. In haploid and SDH peppers 9.93 and 18.66 chloroplasts were counted, respectively, and 6.39 and 11.19 chloroplasts were counted in eggplants, respectively. There were positive relationships between stomatal size and chloroplast number and ploidy level, and negative relationships between stomatal density and ploidy level, which can be presented as an early marker to determination ploidy levels in both species.

Keywords: chloroplast, ploidy level, stomata, anther culture

Introduction

Haploid plants have very important place in plant breeding because they contain only one series of alleles at each locus allows to reveal recessive mutations and to obtain 100% homozygous pure lines in one generation by doubling the chromosome numbers. Since haploid plants cannot form gametes, they are unfertile and do not produce seeds. In order to be used in breeding programs, they must be transformed into productive double haploid (DH). Plants obtained by doubled haploid techniques can be haploid or spontaneous double haploid (SDH) (Gyulai *et al*., 2000, Alremi *et al.*, 2014, Keleş et al., 2015, Ari *et al*., 2016, Çömlekçioğlu & Ellialtıoğlu, 2018). Spontaneous genome doubling is largely dependent on plant species, and it has been reported that spontaneous genome doubling is greater than 90% in some species, while some species are reported to be resistant to spontaneous genome doubling (Mir *et al*., 2021). Grozeva *et al*. (2021), obtained 100% haploid in some genotypes and 100% SDH in some genotypes by anther culture. As the average of 17 genotypes, 59.9% of the plants were haploid and 40.1% were diploid. In previous studies on eggplant, SDH rate reported as 15.4% by Dumas de Vaulx & Chambonnet (1982), 25.6% by Rotino (1996) and 46.4% by Salas et al (2011).

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ORCID and Mail:
Bat, H.: 0000-0002-7773-4905 (hayati@uni.gen.tr); Altindag, F.N.: 0000-0001-9739-4314 (fatmanur@uni.gen.tr);
Yigit, M.A.: 0000-0002-6631-1747 (merve@uni.gen.tr); Ellialtioglu, Ş.Ş.: 0000-0002-3851-466X (ellialti@agri.ankara.edu.tr);
Comlekcioglu, N.: 0000-0001-7189-613X (*nrycomlekcioglu@ogu.edu.tr)*

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In eggplant anther culture stimulated microspores develop into callus. The most of the callus tissues were found to be mixoploid with different ploidy types ranging from haploid to tetraploid. However, 60%-70% of the regenerated plants (Corral-Martinez & Segui-Simarro, 2012; Rivas-Sendra *et al*., 2015) were confirmed to be SDH by flow cytometry. For this reason, ploidy levels of plants must be verified quickly before chromosome doubling is performed. The most reliable method is to count the chromosomes or to measure the DNA in the cells by flow cytometry. However, these methods are difficult, time consuming and require special equipment. Therefore methods to determine ploidy in a large number of plants in a short time gain importance.

It has been shown by many researchers that haploid plants have smaller stomata and lower stomatal density than diploids (Omidbaigi *et al*., 2010; Głowacka *et al*., 2010; Hannweg et al., 2013; Xie, 2015; Widoretno, 2016; Comlekcioğlu & Ozden, 2019). Chloroplast count, DNA content, stomatal size and morphological observation were compared for haploid and diploid pepper (Abak *et al*., 1998), haploid and diploid watermelon (Sarı *et al* 1999), diploid and tetraploid watermelon (Şimşek *et al*., 2013) plants. It has been reported that the ploidy level in plants can be selected quite practically as a result of using the obtained morphological and cytological data together. The chloroplast numbers in stomatal guard cells show significant differences according to species (Lawson, 2009). While most researchers reported that different ploidies have similar chloroplast numbers, Jacobs & Yoder (1989) reported no similarity between genetically similar diploid and tetraploid tomato chloroplast numbers. On the other hand, stomata size is not controlled only by genome size. Stomata size shows significant variation according to leaves, plants and ecological factors (McGoey *et al*., 2014, Tekin & Yılmaz, 2018). It has been reported that neither stomatal density nor stomatal size can be used to determine the ploidy level of diploid and tetraploid watermelon (Jaskani *et al*., 2005), and mixoploid and tetraploid ginger (Soonthornkalump et al., 2017).

The rapid increasing of the use of doubled haploid methods in plant breeding has also increased the need for rapid ploidy screening of the obtained plants. In this study, the relationship between stomatal densities, stomatal size, number of chloroplasts in guard cells and ploidy level in plants obtained by anther culture method in pepper and eggplant were re-examined.

Material and Method

This study was carried out in the greenhouse and tissue culture laboratories of United Genetics Vegetable Seeds Company (Mustafakemalpaşa, Bursa, Turkey). The pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena*) genotypes tested in the study are the breeding lines of the company.

Morphological traits such as leaves size, plant height, vigor, internodes height, and fertility, presence of pollen and seeded fruit of regenerated androgenic plants were compared with the donor plants. Regenerates were grouped as haploid or SDH based on morphological observation. In both species, 100 plants were studied in each ploidy group. Stomata were visualized using a Leica DMLB light microscope. Images were captured and measured using a digital camera and related software.

Stomata Count

Young leaves that have completed their development at the shoot tip (usually 4-5 leaves from top to bottom) were used. The leaf membranes taken from the lower epidermis (middle part of the leaf) of the leaves were placed on the slide and a drop of $AgNO_3$ (silver nitrate) solution was dropped on it (AgNO3 makes stomata and chloroplasts appear brighter). Stomata in 1 random microscopic field of view were counted at 10X40 magnification.

Stoma sizes

The width and length of 100 stomata, from each ploidy level, were measured as µm.

Number of chloroplasts

Chloroplasts in guard cells of 100 stomata counted at each ploidy level were counted.

The experiment was carried out in a randomized block design with 100 replications (plants). The data were subjected to analysis of variance (ANOVA) using Tarist (Açıkgöz *et al.*, 2004) and biplot (principal component method) by Minitab 17 Statistical Software (Anderson, 1998).. Mean separation was performed by Fisher's Least Significance Difference (LSD) ($p < 0.01$).

Results and Discussions

It was determined that there were significant differences according to ploidy levels in terms of all traits examined in both plant species ($P < 0.001$). The summary of analysis of variance (ANOVA) of pepper and eggplant for stomatal characteristics were presented in Table 1 and Table 2, respectively.

The highest F value was calculated in chloroplast number for pepper (Table 1). It is understood that there is a very high difference in the number of chloroplasts according to ploidy levels. Likewise, it was determined that there was a more significant difference between the haploid and SDH plants in terms of both the number of stomata and the stomatal length compared to the stomatal width, and they were more reliable traits to confirm of ploidy level than the stomatal width.

In eggplant, the highest F value was determined in the number of stomata according to ploidy levels. This is followed by stomatal length and chloroplast number. In eggplant, the lowest F value was calculated in stomatal width as in pepper.

Higher stomata density was found in haploid plants compared to diploids. Haploid plants had 2.5 times more stomata in pepper than diploid ones and 2.3 times more in eggplant. Similar stomatal numbers were determined in pepper and eggplant at the same ploidy levels. The average number of stomata per unit area was 27.07 in haploid pepper, 29.20 in eggplant, 10.71 in diploid pepper and 12.61 in eggplant.

Haploid and SDH plants also showed significant differences in stomatal sizes. It was determined that the mean stomatal width was 22.52 µm in haploid pepper, 17.36 µm in eggplant, and 29.50 µm and 22.32 µm in diploid pepper and eggplant, respectively, and the difference between ploidy levels was significant. The width of stomata was 1.3 times wider than haploids in DH plants in both species.

Table 1. Summary of ANOVA for pepper stomatal characteristics

Source of Variation	Degrees of Freedom	Number of Stomata		Stomata Width		Stomata Length		Number of Chloroplasts	
		Mean Square	*F* –Value	Mean Square	*F* –Value	Mean Square	*F* –Value	Mean Square	*F* –Value
Replication	99	23.16	0.85 ns	7.51	0.85 ns	15.07	0.84 ns	6.35	1.29 ns
Ploidy Level	1	13382.48	488.43**	2436.30	275.66**	7258.80	404.45**	3810.65	773.29 **
Error	99	27.40		8.84		17.95		4.93	
Total	199	92.40		20.37		52.90		24.76	

** Significant at alfa level 1% ns; non-significant

Tablo 2. Summary of ANOVA for pepper stomatal characteristics

Source of Variation	Degrees of Freedom	Number of Stomata Stoma Sayısı		Stomata Width Stoma Eni		Stomata Length Stoma Uzunluğu		Number of Chloroplasts	
		Mean Square	*F* –Value	Mean Square	*F* –Value	Mean Square	*F* –Value	Mean Square	*F* –Value
Replication	99	17.88	0.91 ns	6.98	1.30 ns	12.58	1.13 ns	3.08	1.10 ns
Ploidy Level	1	13761.41	702.22**	1234.30	229.73**	4681.06	419.10**	1152.00	410.25**
Error	99	19.60		5.37		11.17		2.81	
Total	199	87.80		12.36		35.34		8.72	

** Significant at alfa level 1% ns; non-significant

The mean stomatal length was 28.34 µm in haploid pepper plants and 40.39 µm in diploids, and 22.32 µm and 32.00 µm in eggplant haploid and diploid plants, respectively. DH plants were found to be 1.4 times longer than haploids in both pepper and eggplant in stomatal length as well as in stomatal width. However, 252% more stomata were found in pepper and 231% more in eggplant compared to DH plants in haploid plants (Table 3).

The stomatal dimensions and chloroplast images of haploid and SDH plants of pepper and eggplant are presented in Figure 1 and Figure 2.

Tablo 3. Certain stomatal characteristics of haploid and SDH plants of pepper and eggplant

Plant Species	Ploidy Level	Number of Stomata	Stomata Width µm	Stomata Length µm	Number of Chloroplasts
Capsicum annuum	Haploid	27.07 a	22.52 b	28.34 b	9.93 b
	Double Haploid	10.71 b	29.50 a	40.39 a	18.66 a
	LSD %1	1.94	1.10	1.57	0.83
Solanum melongena	Haploid	29.20 a	17.36 b	22.32 b	6.39 b
	Double Haploid	12.61 b	22.32 a	32.00 a	11.19 a
	LSD %1	1.64	0.86	1.24	0.62

Means followed by the same letter in column do not differ according to least significance difference (LSD) test (P≤ 0.01). Similarly, Abak *et al* (1998) reported that stomatal density and especially the number of chloroplasts in guard cells, in androgenic pepper plants determined to be haploid and SDH by root tip chromosome count, are reliable traits for the estimation of ploidy level from a particular genotype. Significant differences have been detected in stomatal cell density, size and number of chloroplasts of diploid and tetraploid plants of *Solanum aethiopicum* (PI 636107), known as Ethiopian eggplant. In plants whose ploidy level was confirmed by flow cytometry, stomatal length and diameter were positively correlated with ploidy level, and the number of stomata negatively correlated (Sakhanokho & Islam-Faridi 2014). Soonthornkalump *et al*. (2017) reported that the stomatal length and the number of chloroplasts per stomata were higher in tetraploid ginger plants than diploid plants, whereas the highest stomatal density was found in diploid plants. It has been reported that the stomatal diameter of diploid plants is significantly lower than that of tetraploid plants. The stomatal length increased and stomatal density decreased with the increase of ploidy level in also *Arabidopsis thaliana* plants (Robinson *et al*. 2018). Comlekcioglu & Ozden (2019) determined that the stomatal density of gooseberry plants, which were determined to be diploid and tetraploid by flow cytometry, showed significant differences according to the ploidy level, and that diploid plants had more than twice stomata density (175.53 and 77.73, respectively) compared to tetraploids. Diploid and tetraploid plants showed clear differences in stomatal sizes.

It should be kept in mind that the stomatal width may differ significantly depending on whether the stoma is open or closed

during the taking of the leaf epidermis layer for measurement. While measuring, care should be taken to measure in closed stomata or to take samples from leaves incubated for a certain period of time in a controlled environment for CO_2, humidity, and light (Monda *et al*., 2011). If the stoma is open or closed, the stoma length remains the same, making it a reliable (stable) traits (Beaulieu *et al*., 2008).

While the average number of chloroplasts in guard cells was very similar between the same ploidy plants in both species, increase of the number of chloroplasts in SDH plants was significant. It was determined that the chloroplast numbers in the haploid and DH plant guard cells were significantly different according to the ploidy levels in both pepper and eggplant. On average, 9.93 chloroplasts were counted in guard cells in haploid peppers, while 18.66 chloroplasts were counted with an increase of 87.91% in diploid plants. The number of chloroplasts in eggplant was determined as 6.39 in haploid plants, and 11.19 (75.11% more) chloroplasts were counted in DH plants (Table 3). This shows that chloroplast number is the most reliable marker among the investigated traits in determining the ploidy level.

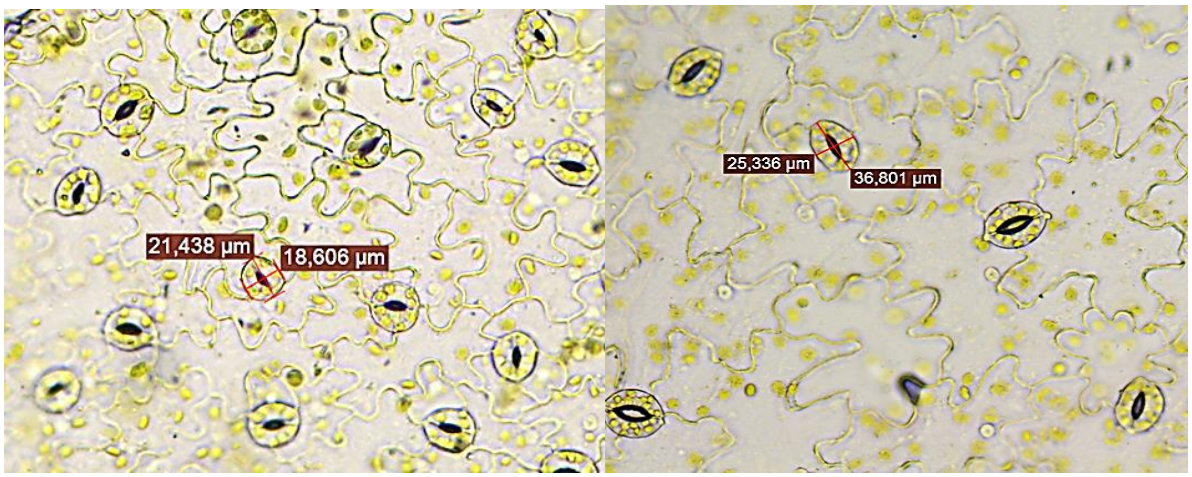

Figure 1. Stomata sizes and chloroplasts in haploid (left) and SDH (right) peppers.

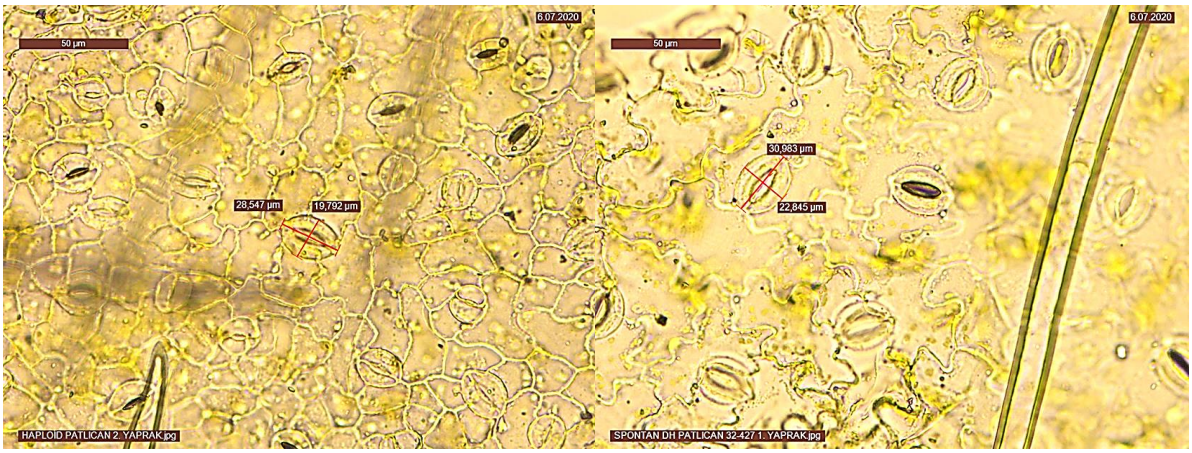

Figure 2. Stomata sizes and chloroplasts in haploid (left) and SDH (right) eggplant.

Our results were fully consistent with the results of Qin & Rotino (1995) for pepper with an average of 9.93 chloroplasts per stoma in haploid and with an average of 18.66 chloroplasts in diploid. The researchers measured the number of chloroplasts per stoma and the length of the stomata in 17 androgenic plants (whose ploidy level have been confirmed by chromosome count) obtained by anther culture from three pepper genotypes. It was found that the plants grouped according to the chloroplast number were the same as the ploidy level defined by the root tip chromosome count. The number of chloroplasts in haploid plants varied between 8.3-11.0 and the average was 9.3 chloroplasts. Garcia-Arias *et al*. (2018) reported that there is a significant correlation between chromosome number and chloroplast number, which can be an indirect and effective indicator of ploidy in gooseberry plants. While there were 5.2 chloroplasts on average in haploid (2x=24) plants, it ranged from 7 to 10 (mean 9.3) in tetraploid (4x=48) donor plants and 4-32 in mixoploid plants. Mixoploid plants with 48 chromosomes presented more than 7 chloroplasts as did tetraploid plants. It has been reported that if a flow cytometer is not available, stomatal size and density will allow a quick evaluation, especially when working with large numbers of plants. Matteij et al., (1992) reported that genetically similar 2x, 4x, 6x and 8x potato plants were able to distinguish accurately by their chloroplast numbers in guard cells. Tepe et al., (2002) reported that there is a high correlation between ploidy level and the number of stomata in the leaf unit area in mint plants. When stomata counts and chromosome counts were evaluated together, it was concluded that there was a high correlation between these two traits. They found that as the number of chromosomes increased, the cell size increased and the number of stomatal cells per leaf unit area decreased.

In Brassica species, the number of chloroplasts in guard cells have been reported to be 4.2-7.8 and 7.9-13.6 for *B. rapa* haploids and diploids, respectively, 7.5-12.4 and 14.1-20.3 for *B. napus* amphihaploids and amphidiploids, respectively and 7.7-9.9, 11.7- 7.9 and 18.0-26.5 for *B. oleracea* haploids, diploids and tetraploids, respectively. No significant effect of plant vegetative or generative developmental stage or growth temperature on chloroplast number was determined (Monakhos *et al*., 2014). The size of the stomata and the number of chloroplasts were significantly increased in the polyploid plants when compared to the diploids of ginger for which ploidy confirm by flow cytometry. With the help of stomata size and chloroplast numbers, tetraploid and diploid plants were determined, but tetraploid and mixoploid plants could not be confirmed (Soonthornkalump *et al*., 2017).

Alsahlany *et al*. (2019) compared the chloroplast count, genome-wide Single Nucleotide Polymorphism genotyping and flow cytometry methods to determine ploidy in diploid and tetraploid potato genotypes. It has been reported that three ploidy determination methods give the same results for all evaluated plants and that chloroplast count can be used as a reliable and inexpensive method for determining the ploidy level.

While Kramer & Bamberg (2019) suggested chloroplast counting using iodine-based staining for potato, they found that different traits could be fast and reliable for estimating ploidy with different methods and stomatal length was as accurate as chloroplast counts, and scoring faster. They observed that the number of stomata per unit leaf area indicates the ploidy level, with tetraploids having more than diploids, but suggested that the length of guard cells is easier to measure.

When the correlation of ploidy level and stomatal characteristics was evaluated in both species, a significant positive correlation was determined between stomatal size and chloroplast number, and a significant negative correlation was determined between ploidy level and stomatal density (Table 4 and Table 5).

Table 4. Correlation between ploidy level and stomatal characteristics in pepper

	Ploidy level	**Stomata number**	**Stomata width**	**Stomata length**
Ploidi level				
Stomata number	-0.853**			
Stomata width	0.775**	-0.667**		
Stomata length	0.830**	-0.728**	0.792**	
Choloroplast number	0.879**	-0.741**	0.788**	0.817**

** Significant at alfa level 1%

Table 5. Correlation between ploidy level and stomatal characteristics in eggplant

	Ploidy level	**Stomata number**	**Stomata width**	**Stomata length**
Ploidi level				
Stomata number	-0.888**			
Stomata width	0.708**	-0.670**		
Stomata length	0.816**	-0.787**	0.790**	
Choloroplast number	0.815**	-0.742**	0.710**	0.807**

** Significant at alfa level 1%

In both species, a negative relationship was found between the number of stomata and the size of stomata and the number of chloroplasts. It was determined that there was a positive relationship between stomatal sizes and the number of chloroplasts in guard cells, and these properties were stable and reliable parameters. It has been confirmed once again that ploidy is an important factor in stoma characteristics. PC1 (principal component) 82.9%, and PC2 8.5% constituted 91.4% of the total variation between stomatal characteristics and ploidy level in pepper and PC1 81.3%, and PC2 8.3% constituted 89.6% of the total variation in eggplant (Figure 3 and Figure 4).

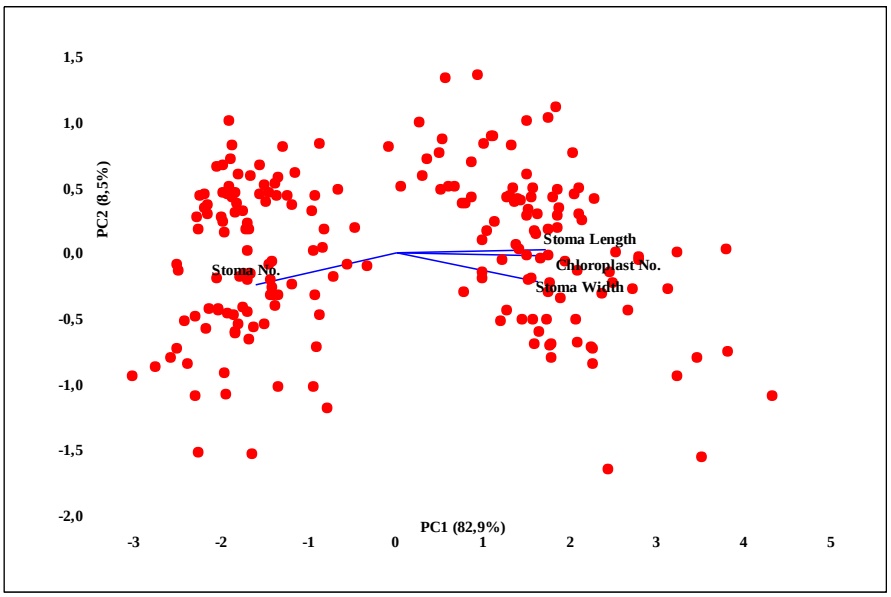

Figure 3. The relationships between ploidy level and stomatal characteristics according to biplot analysis of principal components in pepper

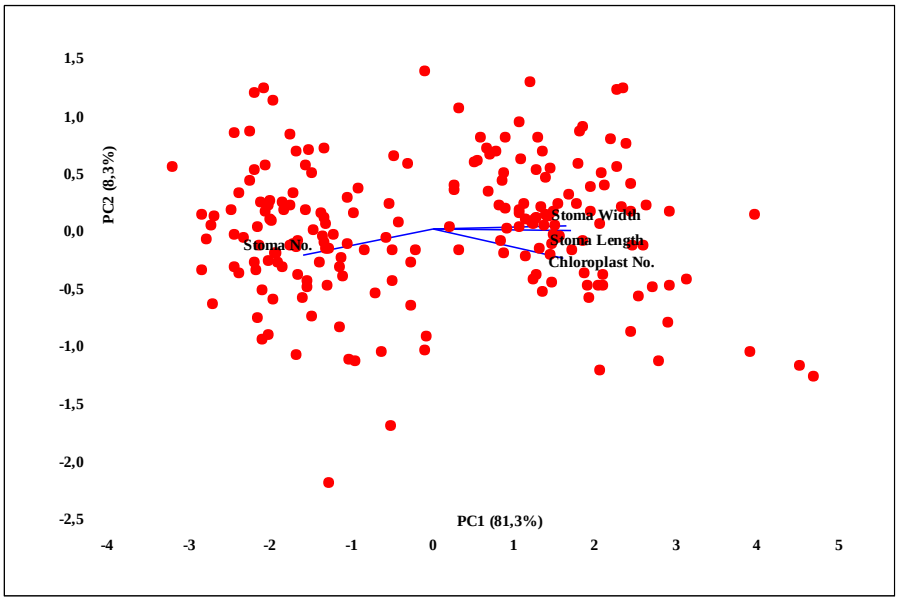

Figure 4. The relationships between ploidy level and stomatal characteristics according to biplot analysis of principal components in eggplant

Conclusions

Haploid plant needs to undergo chromosome duplication to obtain a fertile plant. For successful use of DH plants in plant breeding, it is important to confirm the status of the ploidy level. The chromosome doubling can be spontaneous or induced. There are advantages and disadvantages to using any method to determine ploidy. Chromosome counting is undoubtedly the most reliable method for determining the ploidy level in plants.

However, it was concluded that stomatal characteristics, especially chloroplast numbers, can be used safely in making the first groupings in case of a large number of plants. Pre-ploidy screening based on stomatal characteristics of androgenic plants is useful for reducing population size and can accelerate breeding programs. In the absence of a flow cytometer, stomatal features other than mixoploids can be used to successfully confirm haploid and DH plants. Allows a quick and reliable evaluation when working with a large number of plants. Identifying haploid and diploid plants with epidermal stoma characteristics is faster and cheaper than chromosome counting or flow cytometry, and is an important alternative that does not require expensive equipment.

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Author Contributions: HB; planned of overall research; NC, SSE: statistical analyses, writing-review and editing: FNA, MAY; collected and prepared of samples and laboratory analysis.

References

Abak. K., Comlekcioglu N., Buyukalaca S. & Sari, N. (1998). Use of stomatal characteristics to estimate ploidy level of haploid and dihaploid pepper plants. Xth EUCARPIA Meeting on Genetics and Breeding of *Capsicum annuum* & Eggplant. Avignon, France. p 179-182.

Açıkgöz, N., İlker, E., & Gökçöl, A. (2004). *Biyolojik araştırmaların bilgisayarda değerlendirilmeleri*, Ege Üniversitesi Tohum Teknolojisi Uygulama ve Araştırma Merkezi Yayınları No: 2, Ege Üniversitesi Ziraat Fakültesi ofset atölyesi (in Turkish).

Al Remi, F., Taskın, H., Sönmez, K., Büyükalaca, S., Ellialtıoglu. Ş. (2014). Effect of Genotype and Nutrient Medium on Anther Culture of Pepper (*Capsicum annuum* L). Turkish J. Agr. Natural Sci. 1:108–116.

Alsahlany, M. Zarka D., Coombs J. & Douches, D.S. (2019). Comparison of Methods to Distinguish Diploid and Tetraploid Potato in Applied Diploid Breeding. American Journal of Potato Research (2019) 96:244–254 https://doi.org/10.1007/s12230-018-09710-7

Anderson T.W. (1984). An introduction to multivariate statistical analysis, Second Edition. John Wiley & Sons.

Ari, E., Bedir, H., Yildirim, S., Yıldırım, T. (2016), Androgenic Responses of 64 Ornamental Pepper (*Capsicum annuum* L.) Genotypes to Shed-Microspore Culture in Autumn Season. Turkish Journal of Biology, 40 (3): 706-717. https://doi.org/10.3906/biy-1505-41

Beaulieu, J.M., Leitch, I.J., Patel, S., Pendharkar, A. and Knight, C.A. (2008). Genome size is a strong predictor of cell size and stomatal density in angiosperms. New Phytologist, 179: 975-986. https://doi.org/10.1111/j.1469-8137.2008.02528.x

Çömlekçioğlu N., & Ellialtıoğlu, Ş.Ş. (2018). Review on the research carried out on in vitro androgenesis of peppers (*Capsicum annuum* L.) in Turkey. Review Paper: Research Journal of Biotechnology. Vol. 13 (6).

Comlekcioglu, N., & Ozden M. (2019): Polyploid induction by colchicine treatment in goldenberry (*Physalis peruviana*), and effects of polyploidy on certain traits. The Journal of Animal & Plant Sciences, 29(5): 1336-1343. ISSN: 1018-7081.

Corral-Martinez, P. & Segui-Simarro, J.M. (2012). Efficient production of callus-derived doubled haploids through isolated microspore culture in eggplant *(Solanum melongena* L.) Euphytica (2012) 187:47–61. https://doi.org/10.1007/s10681-012-0715-z

Dumas de Vaulx, R., & Chambonnet, D. (1982). Culture in vitro d'anthe`res d'aubergine (*Solanum melongena* L.): stimulation de la production de plantes au moyen de traitements a` 35°C associe´s a` de faibles teneurs en substances de croissance. Agronomie 2:983–988.

Garcia-Arias, F., Sanchez-Betancourt, E., & Nunez, V. (2018). Fertility recovery of anther-derived haploid plants in Cape gooseberry (*Physalis peruviana* L.). Agron Colomb 36(3):201–209

Głowacka, K., Jezowski, S. & Kaczmarek, Z. (2010). In vitro induction of polyploidy by colchicine treatment of shoots and preliminary characterisation of induced polyploids in two *Miscanthus species*. Ind Crops Prod 32: 88-96.

Grozeva, S.,Todorova, V. & Nankar, A.N. (2021). Creation of pepper doubled haploids and morphological characterization of androgenic plants. Euphytica 217;113. https://doi.org/10.1007/s10681-021-02840-w

Gyulai, G., Gemesne, Juhasz A., Sagi, Z., Zatyko, L., Heszky, L.,& Venczel, G. (2000). Doubled haploid development and PCRanalysis of F1 hybrid derived DH-R2 paprika (*Capsicum annuum* L.) lines. J Plant Physiol 156:168–174. https://doi.org/10.1016/S0176-1617(00)80302-8

Hannweg, K., Sippel, A. & Bertling, I. (2013). A simple and effective method for the micropropagation and in vitro induction of polyploidy and the effect on floral characteristics of the South African iris, Crocosmia aurea. S Afr J Bot. 88: 367-372.

Jacobs, J.P., & Yoder, J.I. (1989). Ploidy levels in transgenic tomato plants determined by chloroplast number. Plant Cell Reports 7: 662–664.

Jaskani, M.J., Kwon S.W., & Kin, D.H. (2005). Flow cytometry of dna contents of colchıcıne treated watermelon as a ploıdy screenıng method at M1 stage. Pak. J. Bot., 37(3): 685-696,

Keleş, D., Pınar, H., Ata, A., Taşkın, H., Yıldız, S., & Büyükalaca, S., (2015). Effect of pepper types on obtaining spontaneous doubled haploid plants via anther culture. HortScience, 50(11), 1671-1676.

Kramer, L.J. & Bamberg, J. (2019). Comparing Methods of Ploidy Estimation in Potato (*Solanum*) Species. *Am. J. Potato Res.* **96,** 419–426. https://doi.org/10.1007/s12230-019-09729-4

Lawson, T. (2009). Guard cell photosynthesis and stomatal function. New Phytologist (2009) 181: 13–34. https://doi.org/10.1111/j.1469-8137.2008.02685.x

Mattheij, W.M., Eijlander, R., de Koning, J.R.A. & Louwes. K.M. (1992). Interspecific hybridization between the cultivated potato *Solanum tuberosum* subsp. *tuberosum* L. and the wild species *S. circaeifolium* subsp. *circaeifolium* bitter exhibiting resistance to *Phytophthora infestans* (Mont.) de Bary and Globodera pallida (stone) Behrens. 1. Somatic hybrids. Theoretical and Applied Genetics 83: 459–466.

McGoey, B.V., Chau, K. & Dickinson, T.A. (2014). Stomata Size in Relation to Ploidy Level in North American Hawthorns (*Crataegus*, Rosaceae). Madrono 61(2), 177-193, https://doi.org/10.3120/0024-9637-61.2.177

Mir, R., Calabuig-Serna, A., & Seguí-Simarro, J.M. (2021). Doubled Haploids in Eggplant. Biology, 10, 685. https://doi.org/10.3390/biology10070685

Monakhos, S.G., Nguen, M.L., Bezbozhnaya, A.V., Monakhos, G.F. (2014). A relationship between ploidy level and the number of chloroplasts in stomatal guard cells in diploid and amphidiploid Brassica species. Agric Biol 5:44–54. https://doi.org/10.15389/agrobiology.2014.5.44eng

Monda, K., Negi, J., Iio, A., Kusumi, K., Kojima, M., Hashimoto, M., Sakakibara, H.,& Iba, K. (2011). Environmental regulation of stomatal response in the Arabidopsis Cvi-0 ecotype. Planta 234: 555–563

Omidbaigi, R., M. Mirzaee, M.E. Hassani, & S.M. Moghadam (2010). Induction and identification of polyploidy in basil (*Ocimum basilicum* L.) medicinal plant by colchicine treatment. Int J of Plant Prod. 4(2): 87-98.

Qin X., & Rotino, L. (1995). Chloroplast number in guard cells as ploidy indicator of in vitro-grown androgenic pepper plantlets. Plant Cell Tissue Org Cult 41(2):145–149

Rivas-Sendra, A.,Corral-Martinez, P., Camacho-Fernandez, C., & Segui-Simarro, J.M. (2015). Improved regeneration of eggplant doubled haploids from microspore-derived calli through organogenesis Plant Cell Tiss Organ Cult. 122:759–765. https://doi.org/10.1007/s11240-015-0791-6

Robinson, D.O., Coate, J. E., Singh, A., Hong, L., Bush, M., Doyle, J.J., & Roeder, A.H.K. (2018). Ploidy and Size at Multiple Scales in the Arabidopsis Sepal [OPEN] The Plant Cell, 30: 2308–2329.

Rotino, G.L. (1996). Haploidy in eggplant. In: Jain SM, Sopory SK, Veilleux RE (eds) In vitro haploid production in higher plants, vol 3. Kluwer, Dordrecht, pp 115–141.

Sakhanokho, H. F. & Islam-Faridi, M. N. (2014). Spontaneous Autotetraploidy and Its Impact on Morphological Traits and Pollen Viability in *Solanum aethiopicum.* HortScience 49(8). 997-1002. https://doi.org/10.21273/HORTSCI.49.8.997

Salas, P., Prohens, J., & Segui-Simarro, J.M. (2011). Evaluation of androgenic competence through anther culture in common eggplant and related species. Euphytica 182:261–274.

Sari, N. Abak, K.& Pitrat M. (1999). Comparison of ploidy level screening methods in watermelon: *Citrullus lanatus* (Thunb.) Matsum and Nakai. Scientia Horticulturae, 82; 265-277.

Şimşek, İ. , Göçmen, M. & Sarı, N. (2013). Diploid ve tetraploid karpuz bitkilerinde morfolojik ve sitolojik farklılıkların belirlenmesi. Derim, 30 (1), 1-14. http://www.derim.com.tr/tr/pub/issue/4574/62652

Soonthornkalump, S., Chuenboonngarm, N. Jenjıttıkul, T., Thammasırı, K. & Soontornchaınaksaeng, P. (2017). Morphological and Stomatal Guard Cell Characteristics of in vitro *Kaempferia rotunda* L. (Zingiberaceae) through Colchicine Induced Polyploidy. Walailak Journal of Science and Technology 2017; 14(3): 235-242.

Tekin M. &, Yılmaz, G. (2018). Anatomıcal and palynological studies on endemic *Verbascum weidemannianum* Fisch. & Mey. (Scrophulariaceae) in Turkey. International Journal of Agriculture, Forestry and Life Science. 2 (2) 6-15.

Tepe, Ş., Ellialtıoğlu, Ş., Yenice, N., & Tıpırdamaz, R. 2002. In vitro kolhisin uygulaması ile poliploid nane (*Mentha longifolia* L.) bitkilerinin elde edilmesi. Akdeniz Üniv. Ziraat Fakültesi Dergisi, 15(2),63-6

Widoretno, W. (2016). In vitro induction and characterization of tetraploid Patchouli (*Pogostemon cablin* Benth.) plant. Plant Cell Tiss Organ Cult. 125:261-267.

Xie, X., Aguero, C.B., Wang, Y. & Walker, M.A. (2015). In vitro induction of tetraploids in Vitis x Muscadinia hybrids. Plant Cell Tiss Organ Cult. 122:675-683