Yüzüncü Yıl Üniversitesi
Fen Bilimleri Enstitüsü Dergisi

http://dergipark.gov.tr/yyufbed

Araştırma Makalesi

Ruanda'dan Temin Edilen Pili-Pili Çeşidinin Androgenesis Kapasitesinin Türkiye'de Araştırılması

Flavien SHIMIRA[1], Serhat YILDIZ[2], Gökhan BAKTEMUR[1], Davut KELEŞ[2], Mehmet Zahit AYDIN[1], Saadet BÜYÜKALACA[1], Hatıra TAŞKIN[1*]

[1]Department of Horticulture, Faculty of Agriculture, Cukurova University, 01330 Adana, Turkey
[2]Alata Horticultural Research Institute, 33740 Mersin, Turkey
*hatirataskin1@gmail.com

Makale Bilgileri

Geliş: 27.09.2019
Kabul: 15.11.2019
Online Yayınlanma Aralık.2019

Anahtar Kelimeler
Capsicum chinense
Biber
Anter kültürü
Ruanda

Öz: Bu çalışmada, Ruanda'dan temin edilen Pili-Pili biber çeşidinin (*Capsicum chinense*) anter kültürü performansı, düşük (A111 genotipi-Kahramanmaraş Türk tipi) ve yüksek (Inan 3363 çeşidi-Urfa Türk tipi) androgenesis kapasitesine sahip iki Türk *Capsicum annuum* biber materyali kontrolü ile karşılaştırılmalı olarak Türkiye'nin Akdeniz Bölgesi'nde değerlendirilmiştir. Anterler ilk aşamada, 30 g L^{-1} sakkaroz, 2.5 g L^{-1} aktif kömür, 15 mg L^{-1} gümüş nitrat ($AgNO_3$), 4 mg L^{-1} 1-Naftalen asetik asit (NAA), 0.5 mg L^{-1} 6-Benzilaminopürin (BAP) ve 6.5 g L^{-1} agar içeren Murashige ve Skoog (MS) besin ortamında kültüre alınmışlardır. Anterler, daha sonra 30 g L^{-1} sakkaroz ve 6.5 g L^{-1} agar içeren MS besin ortamına aktarılmışlardır. Elde edilen embriyolar, aynı ortamda kültür edilmişlerdir (ikinci ortam). Çalışma sonunda, Inan 3363 çeşidinden 19.4 embriyo/100 anter, A111 genotipinden ise 4.46 embriyo/100 anter kaydedilmiştir. Beklenildiği gibi Inan 3363 çeşidi, A111 genotipinden daha iyi performans göstermiştir. Ruanda Pili-Pili çeşidi için özellikle uygun anter kültürü safhasının tespiti gibi tüm çabalarımıza rağmen, bu çeşitten embriyo elde edilememiştir. Sitolojik araştırmalar, klasik biber anter kültürü morfolojik markırlarının, Ruanda Pili-Pili çeşidinde çalışmadığını göstermiştir. Bu çeşidin anter kültürüne tepkisinin zayıf olduğu sonucuna varabilmek için Ruanda'da denemelerin tekrar edilmesi gerekmektedir. Ruanda'da cevap alınamazsa, bu çeşidin anter kültürüne cevap vermediği sonucuna varılabilir.

Investigation of Androgenesesis Capacity of Rwandan Pili-Pili Variety (*Capsicum chinense* L.) in Turkey

Article Info

Recieved: 27.09.2019
Accepted: 15.11.2019
Online Published December 2019
DOI:
Keywords
Capsicum chinense
Pepper
Anther culture
Rwanda

Abstract: In this study, anther culture performance of Rwandan Pili-Pili pepper variety (*Capsicum chinense*) was assessed in the Mediterranean Region of Turkey and compared to two Turkish *Capsicum annuum* pepper controls having low (A111 genotype-Kahramanmaraş Turkish type) and high (Inan 3363 variety-Urfa Turkish type) androgenesis capacity, respectively. The anthers sampled were cultured in Murashige and Skoog (MS) medium including 30 g L^{-1} sucrose, 2.5 g L^{-1} activated charcoal, 15 mg L^{-1} silver nitrate ($AgNO_3$), 4 mg L^{-1} 1-Naphthaleneacetic acid (NAA), 0.5 mg L^{-1} 6-Benzylaminopurine (BAP), 6.5 g L^{-1} agar in the first stage. The anthers were then transferred to MS medium containing 30 g L^{-1} sucrose and 6.5 g L^{-1} agar. The embryos obtained were cultured in the same nutrient medium (second one). At the end of the study, 19.4 embryos per 100 anthers were obtained from the control Inan 3363 and 4.46 from A111. As expected, Inan 3363

showed better performance than A111 genotype. In spite of all efforts such as especially investigating proper anther culture stage for this Rwandan variety, no embryo could be obtained in the Pili-Pili variety. Cytological investigations showed that classical pepper anther culture morphological markers did not work in Rwandan Pili-Pili variety. In order to ascertain that the anther culture response of this variety is weak, other trials should be repeated in Rwanda. If there is no response in Rwanda, it could be concluded that this variety does not respond to anther culture.

1. Introduction

Pepper is one of the most produced and consumed vegetables in the world. There are many different species and types of this vegetable originated from South America. The consumption of cultivated species and types of these species may vary by habits and cultures of countries. Rwandan pepper production was recorded as 4750 tonnes in 698 ha area in 2017 according to FAO (2017) database. Pili-Pili belonging to *Capsicum chinense* is one of well-known and popular pepper variety in Rwandan cuisine. After one of authors of this study visited French markets in Paris, we have realized that there are different Pili-Pili varieties in the world (Figure 1). Therefore, we have decided to name ours as “Rwandan Pili-Pili”.

Figure 1. Different Pili-Pili pepper variety sold in a Paris-France market

There are many studies on anther culture of pepper (Buyukalaca et al. 2004; Taşkin et al. 2011; Irikova et al. 2011; Niklas-Nowak et al. 2012; Grozeva et al. 2013; Olszewska et al. 2014; Al Remi et al. 2014; Keleş et al. 2015; Arı et al. 2016; Ozsan and Onus 2017; Ata et al. 2019 etc.). However, the rapid progress of breeding projects carried out in pepper, a highly demanded crop in the world, justifies the continuation of studies aiming at the adaption of anther culture protocols to each high value genotype. Because, the parents of each newly developed variety need to be rapidly purified. Anther culture is a time-saving method at this stage. Therefore, the response of Rwandan Pili-Pili variety to anther culture was determined in comparison with two Turkish genotypes in this study. Turkish materials known their anther culture performances (one high and one low response) were selected for comparison. In simultaneous with this study, the breeding potential of Rwandan Pili-Pili in terms of different characteristics is also investigated in different studies.

2. Materials and Methods

This study was carried out in Prof. Dr. Saadet BÜYÜKALACA Tissue Culture Laboratory of Horticulture Department (Cukurova University, Adana, Turkey) and Alata Horticultural Research Institute (Erdemli-Mersin, Turkey) in 2018 spring season. Pepper plant materials used were presented in Table 1 and Figure 2.

Table 1. Pepper genotypes and varieties used in this study

Genotype-Variety Name	Species	Type	Origin
Pili-Pili	*C. chinense*	Scotch Bonnet	Rwanda
Alata 111 (A111)	*C. annuum*	Kahramanmaraş (Turkish type)	Turkey
Inan 3363	*C. annuum*	Urfa (Turkish type)	Turkey

Figure 2. Pepper plant materials used in anther culture studies (A) Rwandan Pili-Pili variety (B) Inan 3363 variety-Turkey (C) Alata 111 genotype-Turkey

Inan 3363 variety was developed via selection by GAP Agricultural Research Institute (Şanlıurfa, Turkey) in 2008. This variety is resistant to high temperature; suitable for open field cultivation in both spring and summer season and the average yield of Inan 3363 is 5-6 ton da^{-1}. Our previous studies (Ata et al. 2019) showed that it has high androgenesis capacity, especially in certain months such as August and September in Mediterranean conditions of Turkey. A111 genotype was obtained from pepper collection of Alata Horticultural Research Institute (ALATA). Anther culture capacity of this genotype was low in our previous researches. Pili-Pili variety was provided from Rwanda by one of authors of this study. Donor plants were grown in ALATA under greenhouse conditions and all necessary horticultural practices were implemented. The anther culture experiments were done during June. Proper anther culture stage of all materials was tested by staining with acetocarmine together with morphological markers described by different researchers focused in this subject (please see Ata et al. 2019 for detail). Surface sterilization was performed by following these steps: (i) waiting in 70% ethyl alcohol for 30 seconds (ii) rinsing at least five times using sterile water (iii) keeping in 15% sodium hypochlorite including 1 or 2 drops of Tween 20 (iiii) rinsing at least five times using sterile water. After surface sterilization, the anthers were extracted from flower buds using sterile forceps and scalpels in sterile bench and placed into petri dishes including nutrient medium. For the first 2 days, the cultured anthers were incubated at 35°C in the dark condition, then they were transferred to the growth chamber having 25°C temperature and 8/16 ours dark-light photoperiod. As nutrient medium, in first step, Murashige and Skoog (MS) medium (Murashige and Skoog, 1962)

including 30 g L^{-1} sucrose, 2.5 g L^{-1} activated charcoal, 15 mg L^{-1} $AgNO_3$, 4 mg L^{-1} NAA, 0.5 mg L^{-1} BAP, 6.5 g L-1 agar was used. Then, the anthers were transferred to MS medium containing 30 g L^{-1} sucrose and 6.5 g L^{-1} agar. The embryos obtained were cultured in the same nutrient medium (second one).

3. Results and Discussion

Although this study was performed to determine androgenesis capacity of Rwandan Pili-Pili variety, no positive results were obtained from this variety. In fact, the negative anther culture response in Rwandan Pili-Pili did not surprise us. Because, androgenic response of *C. chinense* was too low in a previous study carried out by Denli (2019). While the androgenesis capacities were found to be 33.66% and 48% in two *C. annum* genotypes, it was determined as 0.66% in *C. chinense* genotype by Denli (2019). Similarly, in a study carried out by Nowaczyk et al. (2009), anther culture response of (*C. frutescens* × *C. chinense*) F2 plants was detected as 0.59%. Another possible explanation might be that Pili-Pili ecological requirements are not fulfilled in Turkey, although the study was carried out in Mediterranean part of Turkey known as the hottest and humid place of Turkey. Despite of our all efforts such as trying different flower bud sizes and some practices on light conditions in the greenhouse, we could not catch any embryo. In order to be sure of this, the response of Rwanda grown plants to anther culture should be examined. Currently, we do not have a publication to compare the results for this variety.

We also realized that the proper anther culture stage was not same in Rwandan material with Turkish types. Normally we used “the length of the corolla should be equal to that of the calyx or slightly longer, and almost half of or less the anthers have anthocyanin” as morphological markers in Turkish pepper types together with using staining acetocarmine one time at the beginning of study (Buyukalaca et al. 2004). At this morphological step, Rwandan variety was not in late uninucleate or early binucleate phase. Therefore, we tested different flower bud sizes for proper phase. And also we have realized that stage transitions were too fast in the flower buds due to high light and temperature. We tried to cover the greenhouse to reduce light violence. These were some of our efforts to increase the chance of success in this variety.

In terms of Turkish genotypes, as expected, Inan 3363 variety showed better performance than A111 genotype (Figure 3). The performance of this variety changed according to months and nutrient mediums in a study carried out by Ata et al. (2019). It was varied from 0 to 66.36% and the highest data was obtained in August. This study was performed in Alata Horticultural Research Institute conditions as ours. Although the climatic conditions change every year, we can compare the results of two studies for Inan 3363 variety. While anther culture experiments of our study were carried out during June, Ata et al. (2019) repeated their studies during one year every month. Therefore, we will just assess the results obtained from June. Ata et al. (2019) used two different nutrient media and recorded 5.14% and 8.80% anther culture performance from two media. In our study, we could calculate 19.4% success in Inan 3363 variety (Table 2). For genotype A111, the anther culture efficiency was 4.46% (Table 2). In previous studies from Turkey, green type genotypes have given similar results.

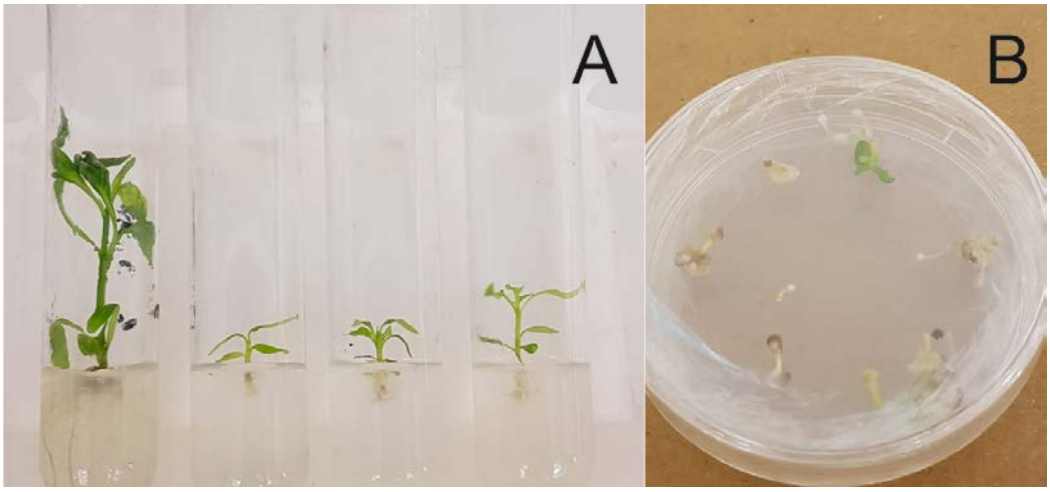

Figure 3. Germination of embryos obtained via anther culture method in pepper and *in vitro* plants (A) Inan 3363 variety (B) A 111 genotype.

Table 2. Anther culture response of pepper genotype and varieties used in this study

Genotype-Variety number	Total petri number	Total anther number	Total embryo number	Embryo number per 100 anther
Pili-Pili genotype	139	695	0	0
Genotype A111	112	560	25	4.46
Inan 3363 variety	100	500	97	19.4

As a result, we could not obtain any embryo from Rwandan Pili-Pili variety in Turkey. In order to ascertain that the anther culture response of this variety is weak, other trials should be repeated in Rwanda. If there is no response in Rwanda, it could be concluded that this variety does not respond to anther culture.

Acknowledgment

The authors want to thank the Cukurova University Scientific Research Projects Coordinating Office (FBA-2017-7761) for supporting this research.

References

Al Remi, F., Taşkin, H., Sönmez, K., Büyükalaca, S., & Ellialtioğlu, Ş. (2014). Effect of genotype and nutrient medium on anther culture of pepper (*Capsicum annuum* L). *Turkish Journal of Agricultural and Natural Sciences*, 1, 108-116.

Arı, E., Yıldırım, T., Mutlu, N., Büyükalaca, S., Gökmen, Ü., & Akman, E. (2016). Comparison of different androgenesis protocols for doubled haploid plant production in ornamental pepper (*Capsicum annuum* L.). *Turk. J. Biol*., 40, 944-954. doi:10.3906/biy-1509-36

Ata, A., Keleş, D., Taşkın, H., & Büyükalaca, S. (2019). Effects of season, genotype, and nutrient medium on pepper anther culture and microspore development. *Turk. J. Agric. For*., 43, 123-137. doi:10.3906/tar-1802-35.

Buyukalaca, S., Comlekcioglu, N., Abak, K., Ekbic, E., & Kilic, N. (2004). Effect of silver nitrate and donor plant growing conditions on production of pepper (*Capsicum annuum* L.) haploid embryos via anther culture. *Eur. J. Hortic. Sci*., 69, 206-209.

Denli (2019). *Expansion of genetic basis via interspecies crossing of pepper (Capsicum annuum) and determination the inheritance of androgenesis*. (PhD), Cukurova University, Institute of Natural and Applied Sciences, Adana, Turkey.

FAO (2017). Food and Agriculture Organization of the United Nations. http://www.fao.org/faostat/en/#data/QC

Grozeva, S., Todorova, V., Cholakov, T., & Rodeva, V. (2013). *Effect of temperature and growth period of donor plants on pepper anther culture*. 3rd International Conference: Research People and Actual Tasks on Multidisciplinary Sciences, Lozenec, Bulgaria.

Irikova, T., Grozeva, S., & Rodeva, V. (2011). Anther culture in pepper (*Capsicum annuum* L.) *in vitro*. *Acta Physiol. Plant*, 33, 1559-1570. doi: 10.1007/s11738-011-0736-6

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, 1-6. doi: https://doi.org/10.21273/HORTSCI.50.11.1671

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant*., 15, 473-497.

Niklas-Nowak, A., Olszewska, D., Kisiala, A., & Nowaczyk, P. (2012). Study of individual plant responsiveness in anther cultures of selected pepper (*Capsicum* spp.) genotypes. *Folia Horticulturae*, 24, 141-146. doi: https://doi.org/10.2478/v10245-012-0017-x

Nowaczyk, P., Olszewska, D., & Kisiala, A. (2009). Individual reaction of *Capsicum* F2 hybrid genotypes in anther cultures. *Euphytica*, 168, 225-233. doi: 10.1007/s10681-009-9909-4

Olszewska, D., Kisiala, A., Niklas-Nowak, A., & Nowaczyk, P. (2014). Study of *in vitro* anther culture in selected genotypes of genus *Capsicum*. *Turk. J. Biol*., 38, 118-124. doi:10.3906/biy-1307-50

Ozsan, T., & Onus, N. (2017). *In vitro* pepper (*Capsicum annuum* L.) anther culture: can be affected via vitamins B? *Biotech. J. Int*. 20, 1-13. doi: 10.9734/BJI/2017/37102

Taşkin, H., Büyükalaca, S., Keleş, D., & Ekbiç, E. (2011). Induction of microspore-derived embryos by anther culture in selected pepper genotypes. *Afr. J. Biotechnol*. 10, 17116-17121. doi: 10.5897/AJB11.2023