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Determination of Anther Culture Efficiency of Some Pepper Genotypes Originating from Kyrgyzstan

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Seda DEMİR[1], Hasan PINAR[1], Aydın UZUN[1], Halil TEKEREK[2]

[1]*Erciyes University, Department of Horticulture, Kayseri, Türkiye*

[2] *Teta Seed Co. Kahramanmaraş, Türkiye.*

***sorumlu yazar**: hpinarka@yahoo.com*

Seda DEMİR, ORCID No: 0009-0008-5812-6323, Hasan PINAR, ORCID No: 0000-0002-0811-8228,
Aydın UZUN, ORCID No: 0000-0001-9496-0640, Halil TEKEREK; ORCID No: 0000-0002-1707-2233

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Anahtar Kelimeler

Capsicum annuum, biber, Kırgızistan, anter kültürü

Özet

Orta Asya ülkeleri arasında, Kırgızistan biyolojik kaynakların zengin çeşitliliği açısından önemli ülkelerden biridir. Bu zengin çeşitlilik arasında, sebze ve meyve genetik kaynakları önemli bir yere sahiptir. Kırgızistan'ın ekolojisine benzer ülkelerde olduğu gibi, Kırgızistan'da da ekonomik açıdan önemli ürünler arasında biberin özel bir yeri vardır. Biber gibi genetik kaynakların, Kırgızistan'da ıslah programlarına dahil edilmesi önemlidir, tıpkı ülkemizde olduğu gibi. Genetik kaynakların çift haploid verimliliğini bilmek, ıslah programlarında avantaj sağlar. Bu çalışmada, Kırgızistan kökenli biber genotiplerinin anter kültürü verimliliğinin belirlenmesi amaçlanmıştır. Çalışmada, 25'i Kırgızistan kökenli ve 2'si anter kültürü verimliliği bilinen kontrol olarak kullanılan kapya tipi genetik materyal olmak üzere toplam 27 biber genotipinin anter kültürü verimliliği belirlenmiştir. Bulgulara göre, toplam 1336 tomurcuk kültüre alınmış ve 365 embriyo elde edilmiş, bu embriyolardan 264'ü sağlıklı bitkilere dönüşmüştür. En yüksek bitki dönüşümü BY-25 genotipinde belirlenmiştir. Yedi biber genotipinde bitki elde edilmezken, sadece 20 genotipte sağlıklı bitkiler elde edilmiştir. Tomurcuk başına embriyo oranı %14, bitki başına dönüşüm oranı ise %15,6 olarak belirlenmiştir. Sonuçlar, Kırgızistan kökenli biber genotiplerinde, diğer genotiplerde olduğu gibi anter kültürü verimliliği açısından varyasyon olduğunu göstermiştir.

Keywords

Capsicum annuum, pepper, Kyrgyzstan, anther culture

Determination of Anther Culture Efficiency of Some Pepper Genotypes Originating from Kyrgyzstan

Abstract

Among the Central Asian countries, Kyrgyzstan is one of the important countries in terms of the rich diversity of biological resources. Among this rich diversity, vegetable and fruit genetic resources have an important place. Pepper has an important place among economically important products in Kyrgyzstan, as in countries similar to Kyrgyzstan's ecology. It is important to include genetic resources such as pepper in breeding programs in Kyrgyzstan, as in our country. Knowing the double haploid efficiency of genetic resources is advantageous in breeding programs. In this study, it was aimed to determine the anther culture efficiency of pepper genotypes originating from Kyrgyzstan. In the study, the anther culture efficiency of a total of 27 pepper genotypes, 25 from Kyrgyzstan and 2 capia type genetic materials with known anther culture efficiency as controls, was determined. According to the findings, a total of 1336 buds were cultured and 365 embryos were obtained, while 264 of these embryos turned into healthy plants. The highest plant transformation was determined in genotype BY-25. While no plants were obtained in 7 pepper genotypes, healthy plants were obtained in only 20 genotypes. While the embryo rate per bud was 14%, the conversion rate to plant per bud was determined as 15.6. The results showed that there was variation in terms of anther culture efficiency in pepper genotypes originating from Kyrgyzstan, as in other genotypes.

1. INTRODUCTİON

Among the Central Asian countries, Kyrgyzstan is of particular significance in terms of its rich diversity of biological resources. Such diversity is attributable to the country's diverse climatic and geographical factors. Additionally, the country possesses the potential to develop high-yielding and resistant cultural varieties, as well as decorative, medicinal, aromatic, and industrial plants. Among the country's rich biodiversity, vegetable and fruit genetic resources occupy a significant position. As is the case in countries with similar ecologies to Kyrgyzstan, the most economically important crops in Kyrgyzstan are pepper, melon, tomato, onion, and garlic. The global production of pepper, one of the species in question, is estimated to be 36 million tons per year. It is one of the five most consumed vegetables worldwide, in various forms, including in our country (FAO, 2022). In countries with similar climatic characteristics, such as Kyrgyzstan, pepper cultivation is considered an economically important crop. In addition to fresh consumption, pepper powder is utilized in various forms in the preparation of main dishes, including tomato paste, roasting, sauces, and pickles. The production of pepper for fresh consumption in Kyrgyzstan was 8,406.8 tons per year, while the production of dried pepper was 1,011.46 tons per year (FAO, 2022). The species of pepper native to the Solanaceae family is C. annuum L., which is commonly used. There are approximately 20 to 30 species in the genus Capsicum of the Solanaceae family. Although the majority of pepper species exhibit 2n=24 chromosomes, it is possible to identify instances where 2n=48 chromosomes are present in wild peppers (Krug, 1986).

However, as in other countries, local vegetable genotypes are facing extinction due to various reasons. It is of great importance to collect the genetic resources of the Central Asian Turkic Republics and to develop new disease-resistant, productive, and high-quality varieties with these materials. These varieties should then be presented to the region, contributing to the well-being of the local population while simultaneously being utilized in vegetable breeding programs. This will result in the incorporation of valuable characteristics into the varieties.

In recent years, there has been a rapid transition from the production of pepper varieties with standard characteristics to F1 hybrid varieties. In contrast, local pepper production with standard seed has remained in local areas. Nevertheless, as in the case of pepper and other vegetables, the production of F1 hybrid varieties, rather than local varieties, results in a failure to achieve the desired quality, despite an increase in yield. In parallel with the acceleration of local seed production in our country and globally, the use of local varieties for both standard seed and F1 hybrid seed has also accelerated. Prior to undertaking this work, it is first necessary to collect and characterise the local genetic resources. Subsequently, as a standard variety, it must be bred in order to eliminate any negative characteristics.

The application of biotechnological techniques, such as molecular marker technologies and tissue culture methods, is accelerating the pace of traditional breeding programs while simultaneously facilitating the overcoming of some genetic constraints. Tissue culture techniques offer a number of important tools that facilitate the shortening of classical breeding methods in vegetable breeding. In this context, the haploidy technique has found a wide range of applications in the breeding of vegetables. The use of androgenesis to obtain haploid plants through tissue culture could reduce the time needed to achieve this result to less than a year. Microspore embryogenesis represents a valuable and efficacious technique for the generation of homozygous lines from solely male gametes in pepper breeding (Comlekcioglu and Ellialtioglu, 2018). A multitude of researchers from diverse geographical locations have conducted investigations into the variables influencing anther culture, and have disseminated their findings. The optimal growing conditions for donor plants, the collection of donor buds at the optimal stage (Kim et al., 2004; Supena et al., 2006; Ari et al., 2016 a,b), the pre-treatment of buds (Dumas de Vaulx et al., 1981), and the culture media (Dumas de Vaulx et al., 1981; Dumas de Vaulx, 1990; Qin and Rotino, 1993; Comlekcioglu et al., 2001; Ozkum-Ciner and Tipirdamaz, 2002; Supena et al., 2006; Irikova et al., 2011), temperature treatment (Dolcet-Sanjuan et al., 1997; Koleva-Gudeva et al., In 2009, Gonzalez-Garcia, 2002; Buyukalaca et al., 2004; Ercan et al., 2006; Rodeva and Cholakov, 2006; Taskin et al., 2011) reported that anther culture success in pepper is affected by season, incubation conditions, plant

growth regulators, and the genotype of the donor plant. Wang and Zhang (2001), Ari et al. (2016a, b), and Buyukalaca et al. (2004) have all reported that these factors affect the success of anther culture in pepper.

Although successful results have been reported with anther culture in pepper, there is still no general protocol reported for all genotypes (Irikova et al., 2011). The genotype effect, which is one of the most significant factors influencing the efficacy of anther culture, continues to result in the absence of the desired outcome or a markedly low success rate in numerous pepper genotypes. In particular, there is a paucity of research on the anther culture efficiency of pepper genotypes originating from Kyrgyzstan. It would be advantageous to determine the anther culture efficiency of these genotypes and utilize them in breeding programs in Kyrgyzstan and Turkey. The objective of this study was to assess the anther culture efficiency of pepper genotypes originating from Kyrgyzstan.

2. MATERİEL AND METHODS

The present study utilized 25 pepper genotypes sourced from Kyrgyzstan in 2023 and 2 standard capia pepper genotypes as its material. Following their collection from Kyrgyzstan, the genotypes were subjected to self-pollination under controlled conditions in a greenhouse in Kahramanmaraş. This was done prior to their use in anther culture studies. The seeds of the genotypes were germinated in a 3:1 ratio of peat-perlite mixture. Subsequently, the seedlings were transferred to the greenhouse environment at the three-to-four-true-leaf stage. The plants were planted at a distance of 40 cm between rows and 60 cm between plants. A total of ten plants of each genotype were utilized as rotors. The drip irrigation method was employed for the irrigation process. Pesticides and fertilizers were applied as needed to support the growth of the plants. When it was necessary to string the plants, this was done, and pruning was carried out.

In another culture, bud collections were conducted at a stage where the petals do not completely overtake the sepals and are at the same level (Dumas de Vaulx et al., 1981; Çömleklioğlu et al., 1999; Bal et al., 2003; Büyükalaca et al., 2004). This stage is just before the microspore nucleus starts mitosis, the mitosis stage, or the post-division period following this stage, which is also reported as a morphological marker that is most suitable for bud collections. The stage just before the microspore nucleus begins mitosis (do Rego et al., 2016), the mitosis stage, or the post-division period following this stage The buds were collected in the morning or evening, when the weather was cool, on the day prior to the disinfection treatments to be applied to the buds. The buds were collected the day before planting for cold treatment and maintained at a temperature of 4 degrees Celsius for a period of 24 hours. The flower buds were initially treated with 75% ethyl alcohol for one minute, followed by a 10-minute immersion in a sterile pure water solution containing 10% commercial hypochlorite. This was then rinsed three times with sterile pure water. Subsequently, the buds were opened on sterile filter papers with the assistance of forceps and scalpels. The anthers were then separated from their filaments and placed on the nutrient media, with their dorsal surfaces in contact with the medium, in accordance with the methods of Buyukalaca et al. (2004) and Taskin et al. (2011) recommend the addition of 10 mg l-1 AgNO3, 4 mg l-1 NAA, 0.5 mg l-1 BAP, 0.05 mg l-1 biotin, 0.5 g l-1 maltose, 7.5 g agar, and 0.25% activated charcoal to Murashige and Skoog (MS) medium, which should be cultured in 60 mm sterile plates with 5-7 anthers per plate.

3.CULTURE CONDİTİONS, EMBRYO FORMATİON AND TRANSFORMATİON İNTO PLANTS

The anthers placed on MS nutrient medium were pretreated in the incubator at +35ºC and dark conditions for 8 days and then the petri dishes were kept in the climate chamber at 25±1°C for 35 days under 16 hours light and 8 hours dark light regime with a fluorescent lamp with 3600 lux power. After 35 days, the cultured anthers were transferred to hormone-free medium and after plantlet development was observed, they were transferred to tubes containing rooting medium containing MS+1mg/L IBA (Pınar et al., 2020). The cultured anthers were incubated with a 3600 lux fluorescent lamp in a climate chamber at a temperature of 25±1°C in a 16 h light-8 h dark light regime. When the plants reached a sufficient size, they were transferred to vials containing peat:perlite (3:1) and acclimatized in a climate chamber at 25±1°C with a fluorescent lamp with a power of 3600 lux in a 16 hours light-8 hours dark light regime. The acclimatized plants were transferred to the greenhouse for selfing. After the first appearance of the embryos, the total number of embryos obtained (number), the total number of embryos that developed into plants

(number) and the ratio of plants obtained per bud (%) were determined. The plants transferred to the greenhouse for selfing were first observed morphologically (leaf width, internode length, flower appearance and presence of pollen, etc.) and then selfing was performed and the plants that formed seeds were recorded as spontaneous double haploid.

4. RESULTS AND DİSCUSSİON

According to the results obtained, a total of 1336 petri dishes were cultured and 365 embryos were obtained and 264 of these embryos were transformed into healthy plants. The highest plant transformation was determined in genotype B487-9-1 (standard capia) (96.8%), while embryos were obtained in 2 genotypes but did not transform into healthy plants. On the other hand, embryos could not be obtained in 5 genotypes. In addition, no plant was obtained in 7 pepper genotypes, while healthy plants were obtained in only 20 genotypes. While the embryo rate per bud was 18%, the transformation rate per bud to plant was 15.6%. Among the genotypes collected from Kyrgyzstan, BY-22 (53.3%) and BY-25 (55.1%) were determined as the genotypes with the highest anther culture efficiency. In addition, haploid and doble haploid plants were determined by morphological observation in the plantlets obtained by anther culture studies and the average spontaneous double haploid rate was determined as 43.2%, although it varied according to genotypes (Table 1).

Table 1. Number of buds cultured (number), number of anthers cultured (number), number of embryos obtained (number), number of embryos transformed into plants (number) in pepper genotypes originating from Kyrgyzstan

S.N.	Genotype	Collected region	Number of Cultivated Buds (pcs))	Number of Anthers Cultured (pcs)	Total Number of Embryos Obtained(pcs)	Total Number of Embryos Transformed into Plants(pcs)	Ratio of Plants Obtained per Bud (%)	Number of doubled haploid plants	Double haploid/Haploid Ratio (%)
1	BY1	Bişkek	50	245	0	0	0	0	0,0
2	BY2	Bişkek	50	248	11	5	9,1	3	60,0
3	BY3	Bişkek	50	249	21	3	5	2	66,7
4	BY4	Issık Göl	50	252	3	3	5,5	1	33,3
5	BY5	Issık Göl	50	252	3	1	1,7	0	0,0
6	BY6	Issık Göl	50	248	8	3	6,6	2	66,7
7	BY7	Issık Göl	50	252	8	2	3,2	1	50,0
8	BY8	Issık Göl	50	248	4	0	0	0	0,0
9	BY9	Issık Göl	50	252	12	4	8,5	1	25,0
10	BY10	Osh	50	250	20	18	35,9	11	61,1
11	BY11	Osh	50	249	24	19	38,9	10	52,6
12	BY12	Osh	50	250	2	1	1,6	1	100,0
13	BY14	Talas	50	252	2	0	0	0	0,0
14	BY15	Talas	50	247	2	2	3,1	2	100,0
15	BY17	Talas	50	249	3	1	2,2	1	100,0
16	BY18	Talas	50	258	0	0	0	0	0,0
17	BY19	Talas	50	275	0	0	0	0	0,0
18	BY20	Talas	50	250	0	0	0	0	0,0
19	BY21	Narın	50	251	9	6	12,5	4	66,7
20	BY22	Narın	50	249	32	28	55,3	13	46,4

21	BY23	Narın	50	247	2	2	3,3	2	100,0
22	BY24	Celalabad	50	249	2	2	4,9	0	0,0
23	BY25	Celalabad	50	250	28	28	55,1	17	60,7
24	BY26	Celalabad	50	258	0	0	0	0	0,0
25	BY30	Karakol	50	270	12	8	16,7	5	62,5
26	H309-3-8	Standart	50	250	29	28	55,6	19	67,9
27	B487-9-1	Standart	50	274	81	48	96,8	22	45,8
	Maximum	**50**		**275**	**81**	**48**	**97**	22	100,0
	Minumum	**50**		**245**	**0**	**0**	**0**	0	0,0
	Mean	**50**		**253**	**14**	**9**	**15,6**	4,3	43,2
	Total	**1500**		**7597**	**409**	**268**	**536**	**117**	

Many factors such as growing period and growing conditions of the donor plant, fertilization, pre-treatments, genotype affect anther culture. However, the most important factor in anther culture is genotype (Kristiansen and Andersen, 1993; Qin and Rotino, 1993; Rodeva et al., 2004; Lantos et al., 2009; Nowaczyk et al., 2009; Keleş et al., 2015; Arı et al., 2016; Durna, 2016; Atasoy, 2020). Previous studies have reported that genotype, environment and other factors affect embryo frequency success and transformation of embryos into plants in anther culture (Parra-Vega et al., 2013). Likewise, it has been determined that success is related to genotype, and anthers cultured in the same period show different responses according to genotypes (Karakullukçu and Abak, 1992; Çömlekcioglu et al., 1999; Çömlekcioglu et al., 2001; Çiner and Tıpırdamaz, 2002; Buyukalaca et al., 2004; Koleva-Gudeva et al., 2007; Taskin et al., 2011). In this study, it was found that in some genotypes, the rate of transformation of embryos into plants was either absent or low. In anther culture studies, although the rate of embryo formation in pepper was between 0.5% and 12.5%, some embryos could not transform into plants and the rate of transformation into plants was reported to be 0.5% (Çiner and Tipirdamaz, 2002). In a study conducted by (Ata, 2011) in which the effect of genotype on pepper anther culture was reported, low temperature sensitive genotype 195, low temperature resistant genotype 421, high temperature sensitive genotype 277A and high temperature resistant cultivar İnan 3363 were used. The highest embryo yield among the genotypes was obtained from the cultivar Inan 3363, which was identified as resistant to high temperatures. (Alremi, 2013) tested 3 different genotypes (B breeding line, genotypes 151 and 171), Alfajer cultivar from Syria and a total of 16 different nutrient media combinations in order to investigate the effects of anther culture on nutrient media and genotype in pepper plants. Among these nutrient media, 8 different combinations of the nutrient media recommended by (Dumas de Vaulx et al. 1981) (C series, C1-C8) and 8 different combinations of the basic nutrient media of (Murashige and Skoog 1962) (B series, B1-B8). In another study conducted by (Atasoy et al., 20201), anther culture performance was determined in 23 pepper genotypes of different types. According to the findings obtained, the average number of embryos per 100 anthers varied between 0.83 and 44.44. The highest rate was observed in genotype FT-509, followed by FT-508 (23.61 embryos/100 anthers), FT-1181 (23.37 embryos/100 anthers) and FT-905 (22.89 embryos/100 anthers). The genotype FT-1178 showed the lowest performance with 0.83 embryos/100 anthers. They stated that all of the embryos formed could develop into plants. The highest average number of plants was observed in genotype FT-508 with 20 plants followed by FT-509 (17.5 plants), FT-1181 (16 plants), FT-905 (12 plants), FT-263 (10.5 plants) and FT-507 (8.25 plants). The lowest number of plants was recorded in genotype FT-1178 with 0.5 plants. The findings obtained in this study show that the effect of genotype is important in anther culture success as in previous studies. In particular, some of the pepper genotypes collected from different regions of Kyrgyzstan (BY-22 and BY-25) were found to have high anther culture efficiency, while others did not respond to the anther culture protocol used in the present study. Although this result can be attributed to the genotype, it would be beneficial to determine the most appropriate protocol by investigating the effect

of different protocols for genotypes that do not respond or respond poorly to the protocol in question.

Kyrgyzstan has important advantages in pepper as in many vegetable species due to its climate and soil advantages. However, the lack of breeding of locally grown pepper genotypes in terms of yield, quality and resistance to diseases and pests is a disadvantage in production. The first stage of breeding programs is the development of pure lines. The use of homozygous genotypes obtained by double haploidy technique provides a great advantage in breeding programs for the development of new standard and hybrid varieties with superior properties. Plant regeneration from microspore-derived embryos is one of the most critical steps in pepper microspore culture. One of the most important factors affecting these steps is the genetic structure of the donor plant. As in previous studies, the findings obtained in this study showed that genotype is one of the main factors affecting the success of pepper anther culture. According to the findings, it was concluded that anther culture efficiency in Kyrgyzstan origin genotypes, as in previous studies, is genotype dependent and special protocols should be developed for specific genotypes.

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