International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Androgenesis in Hot Pepper by Chilli Anther Culture

Komal P. Kharad[1], Sarosha Khan[2]

[1]Student, G H Raisoni University, Saikheda
[2]Head of Department, G H Raisoni University, Saikheda

ABSTRACT

In vitro androgenesis techniques play a key role in the quick generation of fully homozygous lines. This work's goal was to put the procedure for producing androgenic embryos in hot pepper populations into practice. A number of variables can affect androgenetic success, including the types and quantities of carbohydrate sources and the nature of the nutritional medium. Different sugar types were tested at different doses in anther culture of pepper to elucidate their influence on the yield of androgenesis. In this study, sucrose was used as a source of sugar, Murashige & Skoog (1962-MS) medium, silver nitrate (AgNO3), naphthaleneacetic acid (NAA), BAP and one breeding lines of pepper were used to know the genotypic effects on haploid embryo formation. It was found that one of the key elements influencing pepper anther and microspore culture success is genotype. Also, microspore stage and the cold treatment of flower bud responsible for the success of embryo formation. This embryo formation frequency has been calculated according to the results that were generated in one month and the frequency of embryo formation is 0.7%.

INTRODUCTION

Capsicum annuum L. is widely grown and produced all over the world. The domesticated pepper plant, which is a member of the Solanaceae family and the genus Capsicum, developed in the tropical and subtropical central region Due to their diverse range of sizes, colours, smells, fragrances, and levels of pungency, chili pepper fruits are valuable commodities in many parts of the world, used as components in a vast array of culinary preparations as well as in pickles, paprika, chili powder, curry powder, salad dressings, and pepper sauces.

Out of all the 27 species of capsicum, only five are domesticated and grown today: capsicum annuum Linnê, capsicum baccatum Linnê, capsicum Chinese Jacquin, capsicum frutescens Linnê, and capsicum pubescens Ruiz & Pavon. The substantial concentration of important nutrients and the elevated levels of vitamin C (ascorbic acid) and vitamin A in capsicum fruits determine their nutritious value. The B-vitamin complex can also be found in chili pepper fruits.

It has been discovered that capsicum fruits have antibacterial qualities in addition to physiological and pharmacological effects. It seems that the sole pharmacologic requirement for chili peppers to be used as a medication is pungency.

Chilli is the world's most widely grown and profitable crop due to its commercial value at international level. Fruits from chili peppers are among the most significant vegetables to be grown commercially in the tropics, second only to tomatoes. Dried, they are shipped to temperate nations where they are used as

a spice to season processed dishes and sauces. In India and throughout the world, pepper has a significant economic impact.

In breeding projects, breeders typically favour pepper. Nevertheless, F1 hybrid cultivars are chosen because they are more productive than standard varieties, have greater adaptability, and are resistant to a wider range of diseases—despite the fact that producing the seeds for these varieties is more challenging and expensive than for standard varieties used in vegetable growing.

the main goal of breeding is to develop varieties resistant to various stresses. Since developing pure homozygous lines through conventional methods requires at least six to seven generations of self-fertilization, breeding programs are primarily based on pure lines; however, in vitro biotechnological approaches can accelerate the breeding program by using various tissue culture approaches. Anther culture technique yields complete homozygous inbred lines in a single generation, it significantly lowers both the number of generations of selfings and the cost of production.

In pepper breeding, microspore embryogenesis is a valuable and significant culture technique that yields entire homozygous lines from solely the male gametes. Wang et al. (1973) did the first successful haploid embryo development from C. annuum anthers in China, while George and Narayanaswamy (1973) succeeded in India. Abak (1983) initiated the first in vitro androgenesis investigations on local pepper genotypes in Turkey. The induction of the microspores' sporophytic development path in anthers or isolated microspore cultures is a different, quicker technique. An immature gametophyte is forced to undergo sporophytic development during androgenesis, deviating from its usual course of development. The most widely documented technique for inducing embryogenesis from male gametophytic cells is the production of haploid chili plants through the growth of anthers or microspores, which are precursors of pollen. the large percentage of anther-derived embryos; yet, only a small percentage of these were able to grow into regular plant.

The existence of both diploid and haploid plants among the androgenic regenerants is what distinguishes the capsicum plant. For plants that did not undergo spontaneous diploidization, the agent that causes chromosomal duplication must be applied in order to restore fertility. Studies on the colchicine treatment of haploid regenerants produced by androgenesis of Capsicum annuum genotypes are available in the literature. The efficiency and success of embryogenesis and subsequent development to complete plant are influenced by a number of factors, including the age of the donor plant, growing conditions, the growing season, growth regulators, and the donor plant's genotype. Ultimately, however, the genotype is the primary determinant of the successful embryogenesis of microspores from anther culture.

Genotype of donor plant

The genotype is the most important and often limiting factor in the pepper androgenic reaction. Some pepper genotypes are recalcitrant towards induction of androgenesis and formation of haploid regenerants.. Genotype has a significant effect on induction of embryogenesis and on subsequent development of embryos to plant regenerants but also influences the correlation between obtained haploid and spontaneously diploidized regenerants.

Fig1: Donor plant

Microspore stage

Late uninucleate and early binucleate stages of microspore development for effective embryogenesis to occur in C. annuum. Flower buds, anther morphology, and stages of development of chilli microspores: the collected flower buds were separated into three developmental stages. The anther colour is thought to be a reliable predictor of the microspore growth stage. Using a microscope, the stages of microspore formation in anthers were identified. Anthers with haploid microspores made up stage 1 flower buds; anthers with 80% haploid and 20% di haploid microspores made up stage 2 flower buds; and mature pollen grains made up stage 3 flower buds.

Fig 2: Bud stages

Materials and methods

- **Collection of plant material**

In the current study, Buds were taken from the first flush of flowering during morning hours we used unopened flower buds between uninucleate to early binucleated stage. Anther is a four lobed structure each bud contained 5 anthers. To check developmental stage of buds we deployed following staining method

- **Acetocarmine staining**

Anthers were placed on glass slides and crushed by adding 2, 3 drops of 1% acetocarmine stain. Coverslip then placed over it and let it stain for 30 mins, the examine under the microscope at 100x resolution.

- **Preparation, sterilization and storage of culture media**

MS Media- The stock solution for micronutrient, macronutrient, vitamins, iron and plant growth regulator were prepared with sterile water. To make one litre of medium, the required amount of each stock solution was added into glass beaker. A known quantity of distilled water was added into beaker and then required quantity of macronutrient and sucrose were weighed, added as a solids and dissolve fully and then volume was made up to one litre with distilled water. All the component of medium was mixed and final volume was made by adding double distilled water.

The culture medium was sterilized by autoclaving at 121° C at 15 psi pressure for 20 minutes. pH of the medium was set to 5.8 by using 1N HCl and NaOH solution before autoclaving. In order to prevent denaturation of hormones, thermolabile hormones were first filter sterilized by using 0.20-micron syringe filters and then added to the warm (35–40° C) autoclaved media before solidifying. AgNo3 (silver nitrate) is added as an antibacterial or antifungal component before media pouring. Then the media is poured in petri plates about 20 to 25 ml of media is poured in each plate. Then is labelled and wrap up with the parafilm tap.

- **Preparation and storage of stock solution of growth regulators**

Stock solutions of cytokinin (Kinetin, BAP, Zeatin) auxins (NAA, IBA, 2,4-D) and thidiozuron (TDZ) stock solutions were prepared. Stock solution was stored at 4 ^{o}C in refrigerator and used whenever needed. All stock solutions were used within one month of their preparation.

Table1: Composition of plant growth regulators

Sr. no	Growth Regulator	Concentration
1	Auxins	
	2,4-dichloro Phenoxy Acetic Acid	0.1 mg/l
	L Naphthalene Acetic Acid	1 mg/l
2	Cytokinin	
	6 Benzyl Amino Purine	0.5 mg/l
	Kinetin	1 mg/l

Table2: Composition of MS media

Chemicals	concentration (mg/l)
Macronutrients	
$MgSO_4 \cdot 7H_2O$	1650
KH_2PO_4	170
KNO_3	1900
$CaCl_2 \cdot 2H_2O$	440
Micronutrients	
H_3BO_3	6.20
$MnSO_4 \cdot 4H_2O$	22.30
$ZnSO_4 \cdot 7H_2O$	8.60
$Na_2MoO_4 \cdot 2H_2O$	0.25
$CuSO_4 \cdot 5H_2O$	0.025

$CoCl_2 \cdot 6H_2O$	0.025
KI	0.83
$FeSO_4 \cdot 7H_2O$	27.80
$Na_2EDTA \cdot 2H_2O$	37.25
$AgNO_3$	10
Vitamins	
Nicotinic acid	0.5
Thiamine HCl	0.1
Pyridoxine HCl	0.5
Myo-inositol	100
Others	
Sucrose	0.5
Sorbitol	
Maltose	0.5
Glycine	2.0

Cold treatment

Buds from genotype selected plant were subjected to cold temperature pretreatment at 4° C for 24 h. To arrest the cell stage. treating flower buds to 4°C for two to four days produces superior results.

Explant surface sterilization

Samples were labelled (buds). Sample transferred to centrifuge tube. 0.01 % $HgCl_2$ were added and shaken for 7 min. Solution was drained & distilled water was added. Shaken for 5 min and drained. Take sterile tissue paper with forceps on Petri plate. Transfer sample on filter paper.

Anther inoculation

Anthers were taken out carefully from bud to avoid damage with the help of forceps (forceps must be sterilized with the ethanol and heat on heating beads). 15 to 20 Anthers were then placed on one petri plate containing MS (Murashige and Skoog) media. Purple tip anthers were preferred and brown coloured anthers were avoided. Plates were then labelled and wrapped using parafilm tape. The cultures were incubated in the dark conditions for 30 days at 20 to 25°C.

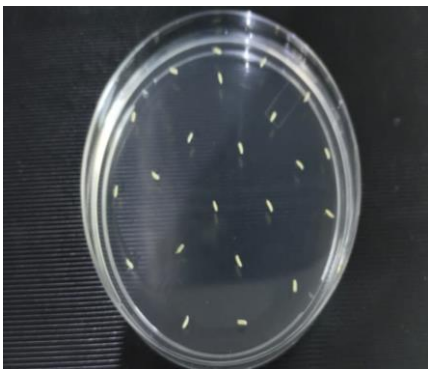


Fig3: Anther inoculation

- **Embryo culture**

The individual, healthy-looking embryos with two cotyledons and non-contiguous anthers were selected to be placed to the germination media. The embryos (semisolid anther cultures) from the previous two treatments were plated on MS media containing 0.8% plant agar and 3% sucrose. In the shed-microspore culture methodology, embryos were germinated in 90 mm diameter petri dishes with half-strength MS containing 0.1 µM BA, 2% sucrose, and 0.6% plant agar. Incubated in light room. After the formation of plantlets, they were subculture in half MS media containing glass tubes.

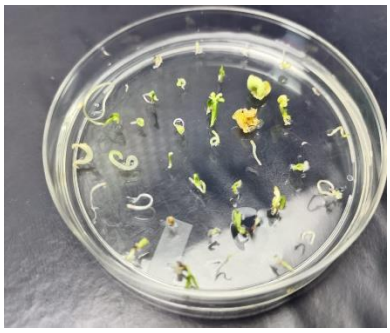
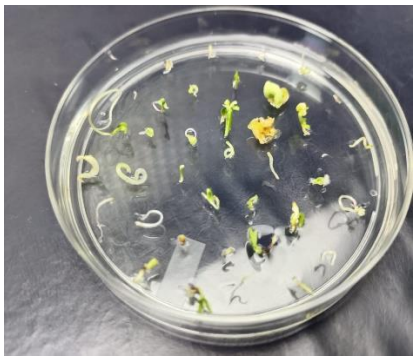


Fig4: Embryo culture

- **Sub-culture**

Take the cultured plates of grown embryo. Normally appearing plants separate and remove attached media with the help of sterile forceps. Then take the test tubes containing fresh media of half MS. Transfer separated plants on fresh media and incubated in light room at 27 degree Celsius.



(a)

(b)

(c)

Fig5: Plantlet conversion

Result and discussion

- **Percent of embryo formation frequency**

Percent response of anther to embryo formation at the end of 30 days of culture was assessed by calculating the number of anthers responded for embryo formation and expressed in percentage.

Frequency of embryo formation of genotype :

Response of anthers to embryo formation in 60 days is calculated by the formula

$$\% \text{ of embryo formation frequency} = \frac{\text{embryo formed anthers}}{\text{total no. of anther cultured}} \times 100$$

Table3: frequency of results

No. of anther inoculated	No. of embryo formation	Frequency (%)
20060	156	0.7

With the use of anther or microspore cultivation, haploid plants are stimulated and allowed to germinate. One of the most popular techniques is anther cultivation due to its excellent yield performance and the quantity of male gametes present in each bud. In pepper androgenesis, the genotype plays a major and frequently limiting role.

The bud flower used in this research, light violet anthers that were selected to contain microspores. The highest frequency of successful microspore cultures was obtained from unnucleated to early bi nucleated stage of anther.

In cytological study we examined the microspores which is in late unnucleated stage using acetocarmine stanning.

Four to six weeks after culture, the anthers responded to the induction of embryogenic development. The emergence of an embryo in the globular stage with a light green colour from weeks four and five of culture

in the pollen ducts of the anther was the first indication of androgenesis induction. The embryo then grew larger and started to differentiate; the root buds are the first structure to be identified; this process starts with the development of cells at one end of the embryo. The apical bud that gives rise to the cotyledons then differentiates on the side opposite the root bud's origin. Following anther separation, the embryos germinated and displayed the root, hypocotyl, and cotyledon characteristics.

Thermal shock stimulation has been essential, especially for genotypes that respond poorly. However, even for genotypes that do not respond, it will be useful to take into consideration various factors like the donor plant's growing conditions, the culture medium, regulators, supplements, genotype, and the stage at which the microspores are developing, A different way of differentiation has been observed through the generation of callus during anther culture in Murashige and Skoog medium.

Applying conditions intended to double the number of chromosomes in the cells is required for haploid plants. Colchicine is a frequently used c-mitotic agent that disrupts sister chromatid segregation and spindle apparatus architecture. Traditionally, young plants are treated with colchicine by being briefly submerged with a water solution , but it was unable to perform in these studies by us this will be the further step after sub culture before colchicine treatment ploidy analysis need to be apply to check whether the plants are haploid, diploid, or mixoploid. After that the colchicine treatment is given for 24 hours if the plants are haploid.

The low success rate of embryo germination in in vitro haploid methods with pepper is a common problem. We also ran into this issue in our research, which is likely connected to the occurrence of deviations in the apical shoot. The only embryos that could germinate at high frequencies into viable seedlings were those that appeared completely normal and had two symmetrical cotyledons.

- **Embryo regeneration from Microspore**

When the embryoids were separated from the anthers and cultivated separately in the presence of FeEDTA in the culture media, the percentage of growing embryoids increased. After being placed in either half MS or solid B5 basal medium containing 2% (w/v) sucrose and exposed to light, the typical cotyledonary embryos from the anthers became green and began actively growing roots in just five days, eventually developing into plantlets. Yet more research is required to fully understand growth regulators in androgenic pepper cultures, which are among the most significant elements. The best proliferation of the embryoids was obtained when they were transplanted to MS media.

Fig6: Embryo formation

Conclusion

Di Haploid plant production would be highly beneficial to speed up the breeding program and thereby lower the cost of developing plant materials if the breeding cycle for chillies were shortened.

The study we conducted has shown that media culture and the stage of the microspores have a significant influence in direct embryogenesis in chillies. Compared to anthers in other stages, light violet-coloured, anthers microspores were superior for embryogenesis. The highest embryo frequency was noted, indicating that different growth regulator types and their variable concentrations in the MS medium played a major part.

Using anthers to create plants in vitro is a useful method for creating homozygous breeding lines. Donor plants should first be cultivated under extreme care in order to achieve a high haploidy frequency.

Success can be increased by maintaining the highest possible number of anthers in culture.

In this study 25 plates of anther culture are contaminated due to the bacteria, fungus and anther undergo callus formation and the frequency of embryo formation is 0.7%.

This embryo formation frequency has been calculated according to the results that were generated in one month. The complete project duration is 6 to 8 months which increase the embryo formation frequency 30% to 40%. but we conducted the project of 3 months and the results as above.

References

1. Y. A. Yazar Soyadı, M. Shidfar, N. Çömlekçioğlu, Ş. Ş. Ellialtıoğlu, and H. Bat, "In vitro androgenesis in pepper and the affecting factors on success: I. Carbon source and concentrations," *Tarla Bitk. Merk. Arastirma Enst.*, vol. 29, no. 2, pp. 62–68, Dec. 2020, doi: 10.38042/biost.2020.29.02.02.
2. S. Sanati, B. M. Razavi, and hossein hosseinzadeh, "A review of the effects of Capsicum annuum L. and its constituent, capsaicin, in metabolic syndrome," *Iran. J. Basic Med. Sci.*, vol. 21, no. 5, May 2018, doi: 10.22038/ijbms.2018.25200.6238.
3. J. Gry *et al.*, "EuroFIR-BASIS – a combined composition and biological activity database for bioactive compounds in plant-based foods," *Trends Food Sci. Technol.*, vol. 18, no. 8, pp. 434–444, Aug. 2007, doi: 10.1016/j.tifs.2007.05.008.
4. N. Baenas, M. Belović, N. Ilic, D. A. Moreno, and C. García-Viguera, "Industrial use of pepper (Capsicum annum L.) derived products: Technological benefits and biological advantages," *Food Chem.*, vol. 274, pp. 872–885, Feb. 2019, doi: 10.1016/j.foodchem.2018.09.047.
5. L. Guevara *et al.*, "Identification of Compounds with Potential Therapeutic Uses from Sweet Pepper (Capsicum annuum L.) Fruits and Their Modulation by Nitric Oxide (NO)." Mar. 26, 2021. doi: 10.20944/preprints202103.0675.v1.
6. M. Gómez-García and N. Ochoa-Alejo, "Biochemistry and Molecular Biology of Carotenoid Biosynthesis in Chili Peppers (Capsicum spp.)," *Int. J. Mol. Sci.*, vol. 14, no. 9, pp. 19025–19053, Sep. 2013, doi: 10.3390/ijms140919025.
7. J. L. Guil-Guerrero, C. Martínez-Guirado, M. Del Mar Rebolloso-Fuentes, and A. Carrique-Pérez, "Nutrient composition and antioxidant activity of 10 pepper (Capsicum annuun) varieties," *Eur. Food Res. Technol.*, vol. 224, no. 1, pp. 1–9, Oct. 2006, doi: 10.1007/s00217-006-0281-5.
8. M. Eggersdorfer and A. Wyss, "Carotenoids in human nutrition and health," *Arch. Biochem. Biophys.*, vol. 652, pp. 18–26, Aug. 2018, doi: 10.1016/j.abb.2018.06.001.
9. A. Azlan, S. Sultana, C. S. Huei, and M. R. Razman, "Antioxidant, Anti-Obesity, Nutritional and Other Beneficial Effects of Different Chili Pepper: A Review," *Molecules*, vol. 27, no. 3, p. 898, Jan. 2022,

doi: 10.3390/molecules27030898.
10. Y.-H. Zhou *et al.*, "Effect of Anti-Obesity Drug on Cardiovascular Risk Factors: A Systematic Review and Meta-Analysis of Randomized Controlled Trials," *PLoS ONE*, vol. 7, no. 6, p. e39062, Jun. 2012, doi: 10.1371/journal.pone.0039062.
11. N. Bourne, D. I. Bernstein, and L. R. Stanberry, "Civamide (*cis* -Capsaicin) for Treatment of Primary or Recurrent Experimental Genital Herpes," *Antimicrob. Agents Chemother.*, vol. 43, no. 11, pp. 2685–2688, Nov. 1999, doi: 10.1128/AAC.43.11.2685.
12. "socio-economic_chilli.pdf."
13. E. A. M. de Swart, *Potential for breeding sweet pepper adapted to cooler growing conditions: a physiological and genetic analysis of growth traits in Capsicum*. S.l.: s.n., 2007.
14. M. Mangal *et al.*, "Haploid induction through microspore embryogenesis in Bell pepper genotypes." Feb. 15, 2023. doi: 10.21203/rs.3.rs-2571870/v1.
15. G. Gulyas, K. Pakozdi, J.-S. Lee, and Y. Hirata, "Analysis of Fertility Restoration by Using Cytoplasmic Male-sterile Red Pepper (Capsicum annuum L.) Lines," *Breed. Sci.*, vol. 56, no. 3, pp. 331–334, 2006, doi: 10.1270/jsbbs.56.331.
16. Y. Van De Peer, J. A. Fawcett, S. Proost, L. Sterck, and K. Vandepoele, "The flowering world: a tale of duplications," *Trends Plant Sci.*, vol. 14, no. 12, pp. 680–688, Dec. 2009, doi: 10.1016/j.tplants.2009.09.001.
17. Investigations on the Anther Culture in Vitro of Nicotiana tabacum L. and Capsicum Annuum L..pdf."
18. T. Popova Irikova, S. Kintzios, S. Grozeva, and V. Rodeva, "Pepper (Capsicum annuum L.) anther culture: fundamentalresearch and practical applications," *Turk. J. Biol.*, vol. 40, pp. 719–726, 2016, doi: 10.3906/biy-1506-79.
19. E. D. J. Supena, S. Suharsono, E. Jacobsen, and J. B. M. Custers, "Successful development of a shed-microspore culture protocol for doubled haploid production in Indonesian hot pepper (Capsicum annuum L.)," *Plant Cell Rep.*, vol. 25, no. 1, pp. 1–10, Feb. 2006, doi: 10.1007/s00299-005-0028-y.
20. N. T. D. Phuong, "Direct embryogenesis from anther culture of hot chilli Capsicum annuum L.," *Eng. Technol.*, vol. 11, no. 1, pp. 3–10, Mar. 2021, doi: 10.46223/HCMCOUJS.tech.en.11.1.560.2021.
21. M. İlhan and E. S. Kurtar, "Doublehaploidization Efficiency of Selected Pepper Genotypes Via in Vitro Anther Culture," *Selcuk J. Agric. Food Sci.*, p. 2, Aug. 2022, doi: 10.15316/SJAFS.2022.033.
22. D. Olszewska, A. Kisiała, and P. Nowaczyk, "The assessment of doubled haploid lines obtained in pepper (*Capsicum annuum* L.) anther culture," *Folia Hortic.*, vol. 23, no. 2, pp. 93–99, Mar. 2012, doi: 10.2478/v10245-011-0014-5.
23. S. L. Shrestha, B. P. Luitel, T. J. Lee, and W. H. Kang, "Agronomic Variation in Anther Derived Plants of Sweet Pepper (Capsicum annuum L.) Genotypes," 2010.
24. T. Popova Irikova, S. Kintzios, S. Grozeva, and V. Rodeva, "Pepper (Capsicum annuum L.) anther culture: fundamentalresearch and practical applications," *Turk. J. Biol.*, vol. 40, pp. 719–726, 2016, doi: 10.3906/biy-1506-79.
25. S. Misal and A. Das, "Successful development of a unique anther culture protocol for the production of doubled haploids in Hot Pepper." Sep. 01, 2023. doi: 10.21203/rs.3.rs-3294675/v1.
26. N. Çömlekçioğlu and Ş. Ş. Ellialtıoğlu, "Review on the Research carried out on in vitro Androgenesis of Peppers (Capsicum annuum L.) in Turkey," *Res. J. Biotechnol.*, vol. 13, 2018.
27. Department of Horticulture, Kangwon National University, W. H. Kang, S. L. Shrestha, P. B. Adhikari, and B. P. Luitel, "Morphological Characterization of Anther Derived Plants in Minipaprika (Capsicum

annuum L.)," *Korean J. Breed. Sci.*, vol. 44, no. 4, pp. 450–461, Dec. 2012, doi: 10.9787/KJBS.2012.44.4.450.

28. M. M. González-Chavira, B. Z. Guerrero-Aguilar, J. L. Pons-Hernández, M. D. L. Á. Escobedo-Landín, J. F. García-Reyna, and M. A. Mora-Avilés, "Inducción de embriones androgénicos y regeneración de plantas haploides en genotipos experimentales de chile poblano a través de cultivo de anteras," *Rev. Mex. Cienc. Agríc.*, vol. 14, no. 2, pp. 277–287, Mar. 2023, doi: 10.29312/remexca.v14i2.3054.
29. K. Jha, P. Kumar Choudhary, and A. Agarwal, "DOUBLED HAPLOID PRODUCTION IN CAPSICUM ANNUUM L. USING ANTHER CULTURE : A REVIEW," *PLANT Arch.*, vol. 21, no. Suppliment-1, pp. 168–173, Jan. 2021, doi: 10.51470/PLANTARCHIVES.2021.v21.S1.031.
30. S. Grozeva, V. Todorova, and A. N. Nankar, "Creation of pepper doubled haploids and morphological characterization of androgenic plants," *Euphytica*, vol. 217, no. 6, p. 113, Jun. 2021, doi: 10.1007/s10681-021-02840-w.
31. D. Keleş, H. Pınar, A. Ata, H. Taşkın, S. Yıldız, and S. Büyükalaca, "Effect of Pepper Types on Obtaining Spontaneous Doubled Haploid Plants via Anther Culture," *HortScience*, vol. 50, no. 11, pp. 1671–1676, Nov. 2015, doi: 10.21273/HORTSCI.50.11.1671.
32. T. Irikova, S. Grozeva, and V. Rodeva, "Anther culture in pepper (Capsicum annuum L.) in vitro," *Acta Physiol. Plant.*, vol. 33, no. 5, pp. 1559–1570, Sep. 2011, doi: 10.1007/s11738-011-0736-6.
33. S. Grozeva, G. Pasev, V. Radeva-Ivanova, V. Todorova, V. Ivanova, and A. N. Nankar, "Double Haploid Development and Assessment of Androgenic Competence of Balkan Pepper Core Collection in Bulgaria," *Plants*, vol. 10, no. 11, p. 2414, Nov. 2021, doi: 10.3390/plants10112414.
34. K. P. Sahana *et al.*, "Anther-derived microspore embryogenesis in pepper hybrids orobelle and Bomby," *Bot. Stud.*, vol. 65, no. 1, p. 1, Jan. 2024, doi: 10.1186/s40529-023-00408-6.