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Original article

Evaluation of CBM and Nitsch Media for Haploid Embryo Induction and Doubled Haploid Plant Production in Beith Alpha Type Cucumber (*Cucumis sativus* L.)

Ahmet Naci Onus *

Department of Garden Plants, Faculty of Agriculture, Akdeniz University, Antalya, Türkiye

Abstract

This study evaluated the efficiency of two culture media, CBM and Nitsch, for embryo induction and doubled haploid (DH) plant production in the Beith Alpha cucumber type, with particular emphasis on genotype-dependent responses. A total of 1,004 ovaries were cultured on CBM medium, resulting in embryo development in 439 ovaries, whereas only 79 embryos were obtained from 983 ovaries cultured on Nitsch medium. Colchicine treatment was applied to 231 and 13 healthy haploid plantlets derived from CBM and Nitsch media, respectively, leading to the successful development of 202 DH plants from CBM and 9 DH plants from Nitsch. Comparative analysis revealed that CBM medium was markedly more effective than Nitsch medium in terms of both embryo formation and DH plant regeneration. Additionally, the results demonstrated a strong genotype dependency in embryogenic response and DH plant production. Within the CBM medium, genotype BA20 exhibited the highest embryo induction capacity, while genotype BA64 showed superior performance in DH plant regeneration. Overall, the findings indicate that both culture medium composition and genotype play critical roles in determining the success of gynogenic embryo induction and DH plant production. These results provide valuable insights for optimizing haploid and DH production protocols in cucumber breeding programs.

Keywords: Gynogenesis, Pure Line, Speed Breeding, Cucumber, Homozygous Plants

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* **Corresponding author:**

Ahmet Naci Onus, Prof. Dr., Department of Garden Plants, Faculty of Agriculture, Akdeniz University, Antalya, Türkiye.
Email: onus@akdeniz.edu.tr

INTRODUCTION

When evaluated in terms of fruit, many different types of cucumber cultivation are practiced. The varieties contain different genetic structures according to fruit color, fruit shape, fruit length, number of fruits per node, fruit spines, how the fruit stem attaches to the fruit, fruit crispness, how the fruit is evaluated, and taste and aroma. In terms of length, cucumbers are classified into three different types: short (maximum 15 cm), medium (16-25 cm), and long (25 cm and above). When types are evaluated based on the number of fruits formed on a node, there are single (1 fruit), semi-multi (2-3 fruits), or multi (more than 3 fruits) varieties. Depending on how the fruit can be evaluated, there are table-ready slicing, table-ready cocktail, and pickling cucumber varieties. For table cucumbers, Beith Alpha type cucumbers that are sliceable, have no bitter taste, are dark in color, and have neckless fruit are preferred. In addition, these types of cucumbers must be resistant to diseases such as powdery mildew (PM), cucumber mosaic virus (CMV), and zucchini yellow mottle virus (ZYMV). For cucumber types produced for pickling, morphological characteristics such as long fruit stalks, dense spines, light green color, and short type are desired, while resistance to scab (Scap), powdery mildew (PM), and downy mildew (DM) is required. In addition to the desired characteristics for each type, the market to which the developed variety will appeal is also a decisive factor in determining breeding objectives. For example, while medium-sized table cucumber varieties, known as Beith-Alpha types, are more preferred in the domestic market, cucumber varieties with a longer fruit structure are more preferred in Asian countries. The Russian market, on the other hand, prefers prickly cucumber varieties. European countries demand varieties with a dark green fruit skin color. Responding to all these different demands and requests is possible through F_1 hybrid breeding studies.

Hybrid seed breeding is based on the principle of obtaining individuals with superior characteristics to their parents by crossing two homozygous individuals. Hybrid seed technology is considered the greatest achievement in plant genetics, particularly due to its contribution to plant yield. Hybrid seed breeding studies are conducted using both classical breeding methods and modern breeding methods. Breeding studies conducted using only classical breeding methods can take many years. The development of modern breeding techniques not only enables newly developed varieties to reach the market quickly but also greatly reduces the costs of breeding companies. Different techniques in particular, haploid plant production and the generation of double haploid (DH) plants enable the faster development of varieties that are being homozygosed through selfing. For this reason, research on haploid embryo and plant generation has been a major focus in the fields of horticultural and field crops in recent years. Appropriate protocols developed for different plants also facilitate the work of commercial companies in this field, helping them to develop commercial varieties. Through the application of certain chemicals to plant somatic tissues, plants with a haploid structure can now be converted into double haploid (2n=2x) plants. Somatic tissues containing n number of chromosomes

undergo mitotic division, replicating during division to double the amount of DNA. In the prophase I stage, these chemicals prevent the formation of spindle fibers, preventing the chromosomes aligned on the equatorial plane in the subsequent stages from separating into two different cells in anaphase I and ensuring that the structure containing n number of chromosomes at the beginning in a single cell is converted to 2n. These new cells, formed as a result of the same DNA being copied, are homozygous. Therefore, pure lines with a homozygous structure are formed. Pure lines allow the homozygosis process, which takes years with classical breeding methods, to be carried out in a short time. It also increases the frequency of recessive traits appearing in individuals.

Gynogenesis (ovule and ovarium cultures) is the method to be used in haploid studies in cucumber. In that sense; numerous researchers have studied gynogenesis in cucumber have conducted studies using the gynogenesis technique and combining different culture media with varying hormone concentrations. Some of the basic culture media used in this context were Murashige and Skoog (MS, 1962), Nitsch basal medium, and cucumber basal medium (CBM) (Asadi et al. 2019, Chen et al. 2016, Domblides et al. 2019 a,b, Gemes-Juhasz et al. 1996, Golalabadi et al. 2017, Sorntip et al. 2017). This present study was conducted to evaluate the efficiency of two culture media, CBM and Nitsch, for embryo induction and doubled haploid (DH) plant production in the Beith Alpha cucumber type, with particular emphasis on genotype-dependent responses.

MATERIALS and METHODS

Six different cucumber genotypes obtained from a F_2 population of cucumber used as plant materials.

Seeds belonging to these genotypes were planted, donor plants were obtained, and transferred to the donor greenhouse. DH studies began approximately two weeks after the plants were planted in the greenhouse. The effect of different culture medium combinations, CBM and Nitsch culture media, on embryo and haploid plant formation via gynogenesis (ovary culture) was investigated. Ovaries collected 24 hours before anthesis, i.e., ovaries that had not yet flowered (anthesis) but had completed embryo sac formation, were selected as materials. The ovaries brought to laboratory conditions were first subjected to sterilization and then cultured. The primary objective in haploid studies is to determine the appropriate hormone concentration and culture medium for embryo induction. Therefore, Cucumber Basal Media (CBM) and Nitsch Media, which have the same hormone concentrations, will be used as culture media. The 0.04 mg/l TDZ hormone concentration used in previous studies was added to both culture media. The outer surfaces of the sterilized ovaries were peeled using sterile forceps and scalpels in a manner that would not damage the ovules. This was done to allow the ovaries to benefit more effectively from the nutrient medium. The ovaries were divided into 4 equal parts and sliced lengthwise without damaging the ovules. Each ovary was placed in a 60 mm diameter Petri dish containing the culture medium. The ovaries were first subjected to a heat shock pre-treatment at 35ºC in the dark and then

transferred to a climate chamber with a photoperiod of 24 ºC, 16 hours of light/8 hours of darkness. Immediately after the development of the ovules on the ovary, the ovaries were transferred to a regeneration medium. Approximately one month after the ovaries were transferred to the regeneration medium, haploid embryo formation occurred (see Figure 2). These haploid embryos were transferred to tubes containing hormone-free MS culture medium for germination (see Figure 3). Healthy haploid plantlets were treated with colchicine under *in vitro* conditions, and these plantlets were then kept in climate chambers for growth and development in large magenta containers.

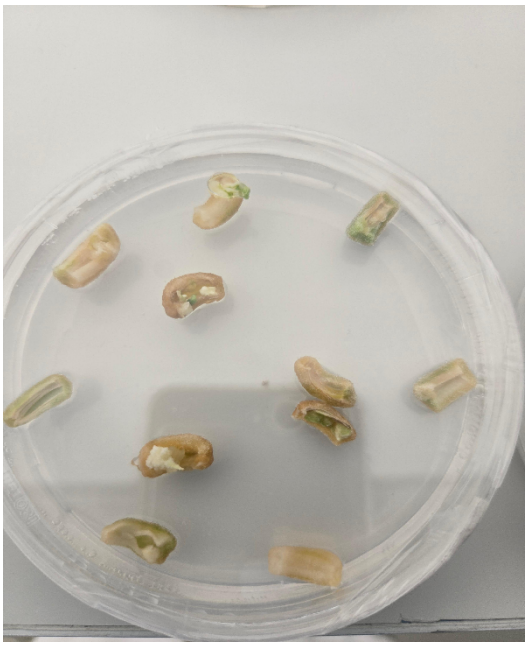


Figure 1. Placing sliced ovaries into petri dish and embryo formation

Figure 2. Further haploid embriyo development

Figure 3. Haploid plantlets in tubes

RESULTS and DISCUSSION

The embryos obtained were transferred to tubes to germinate. Plants grown in jars for approximately 10 days were treated with colchicine under *in vitro* conditions. A 0.5% colchicine solution was sterilized using a sterile syringe filter. The shoot tips of the plantlets were then immersed in the solution and left for 2 hours. After this process, the plantlets were rinsed three times with sterile water and then transferred back to tubes containing MS medium.

As can be seen from Table 1, a total of 1,004 ovaries were cultured on CBM medium of the Beith Alpha type. Embryo development was observed in 439 of these ovaries (Table 1). Colchicine treatment was applied to 231 haploid cucumber plantlets that exhibited healthy development, and 202 of these continued to grow healthily after treatment. Similarly, a total of 983 ovaries were cultured on Nitsch medium (Table 2). Embryo development was observed in 79 of these ovaries. Colchicine treatment was applied to 13 haploid cucumber plantlets showing healthy development, of which 9 continued to develop successfully.

Table 4. Beith Alpha type cucumber DH experiments - CBM medium

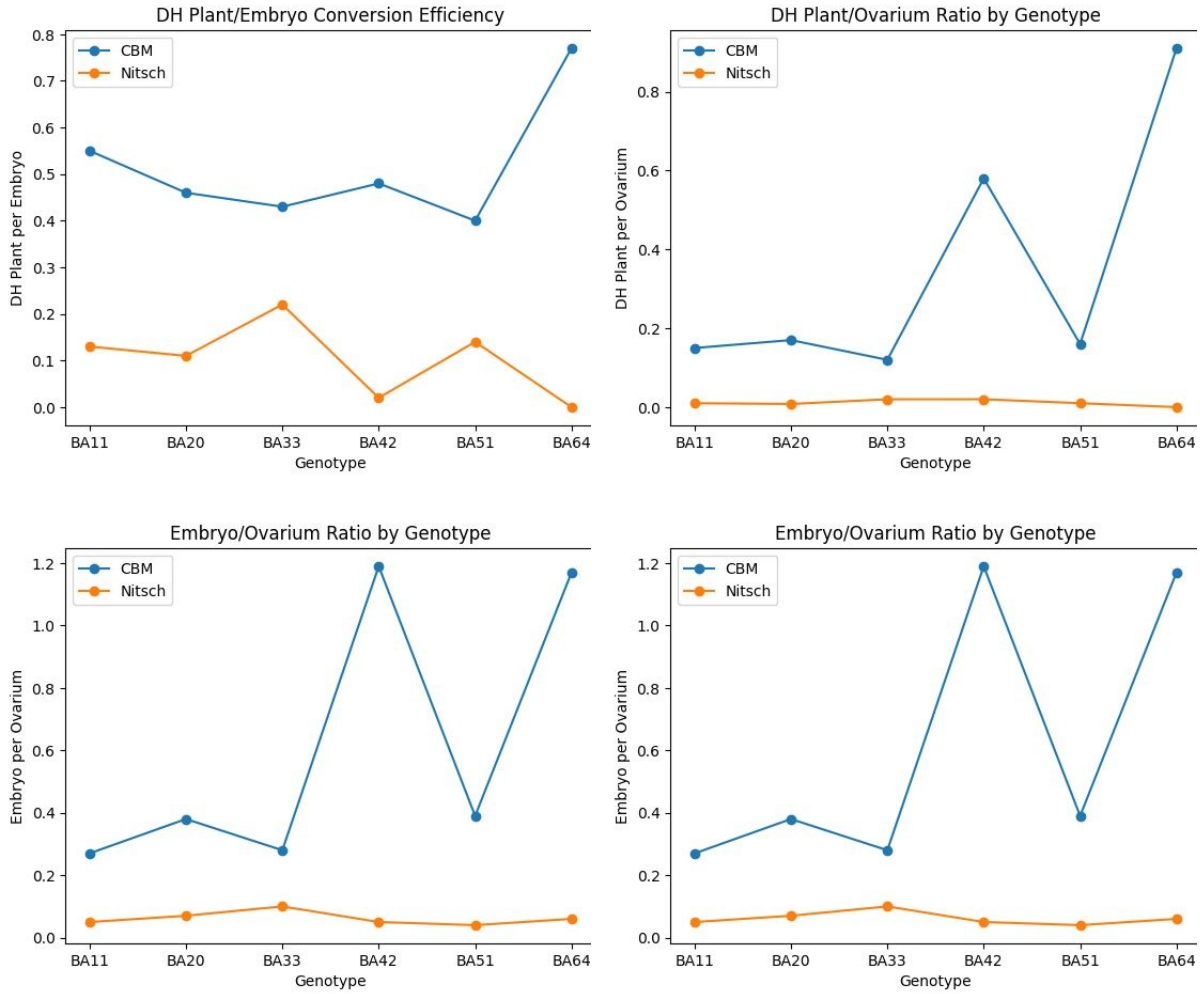
Genotype	Number of cultured ovarium	Total number of embryos	Embryo/ovarium	Number of DH plants	DH plant/ ovarium	DH plant/ embryo
BA11	252	68	0,27	38	0,15	0,55
BA20	233	89	0,38	41	0,17	0,46
BA33	222	62	0,28	27	0,12	0,43
BA42	62	74	1,19	36	0,58	0,48
BA51	167	66	0,39	27	0,16	0,40
BA64	68	80	1,17	62	0,91	0,77
TOTAL	**1004**	**439**	**3,68**	**231**	**2,09**	**3,09**

Table 2. Beith Alpha type cucumber DH experiments - Nitsch medium

Genotype	Number of cultured ovarium	Total number of embryos	Embryo/ovarium	Number of DH plants	DH plant/ ovarium	DH plant/ embryo
BA11	261	13	0,05	4	0,01	0,13
BA20	235	18	0,07	2	0,008	0,11
BA33	233	24	0,10	5	0,02	0,22
BA42	40	2	0,05	1	0,02	0,02
BA51	162	7	0,04	1	0,01	0,14
BA64	72	5	0,06	0	-	-
TOTAL	**983**	**79**	**0,37**	**13**	**0,07**	**0,62**

When the two media were compared in terms of embryo and doubled haploid (DH) plant production for the Beith Alpha type, the CBM medium clearly outperformed the Nitsch medium. Another key finding of the study was that embryo and DH plant formation rates varied depending on genotype.

Evaluation of the results obtained from the CBM medium indicated that genotype BA20 showed superiority in terms of embryo formation, whereas genotype BA64 was superior in terms of DH plant production. Therefore, genotype dependency another important outcome of this study can significantly influence the efficiency of embryo induction and DH plant regeneration rates.



For the Beith Alpha type, CBM medium showed a superior performance compared to Nitsch medium in terms of both embryo formation and DH plant production, This result strongly emphasize the necessity of medium optimization in gynogenic haploid induction protocols. In addition, the reported variation among genotypes regarding embryo and DH plant formation reveals that haploid and DH responses are genotype-dependent, and that is why there is no single protocol optimal for different cucumber genetic backgrounds. The superior embryo induction observed in genotype BA20 and the higher DH plant recovery in genotype BA64 underline the need for genotype-specific optimization strategies in breeding programs. The results clearly indicate that both culture medium composition and genotype play decisive roles in embryo induction efficiency and subsequent DH plant regeneration.

Overall, the findings emphasize that successful DH production relies on a multifactorial interaction between genotype and culture medium. The application of colchicine proved effective for chromosome doubling, yielding a high proportion of viable DH plants, which are of direct value for use as pure parental lines in hybrid breeding.

Conclusion

In conclusion, the combined use of optimized *in vitro* haploid induction systems and advanced breeding tools provides a powerful platform for accelerating cultivar development. The outcomes of this study contribute valuable practical insights for breeding programs aiming to enhance efficiency, reduce breeding cycles, and strengthen the competitiveness of domestic seed industries in both national and international markets.

Author Contributions

In this study, theoretical framework of the study was created by the author, the data collection, analysis process and writing were also carried out by the same author.

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Responsible Artificial Intelligence Statement

In this study, artificial intelligence tools were used in language editing, and to correct language errors.

Conflicts of Interest

The author declare that there are no conflicts of interest related to the publication of this study.

Ethics Approval

This study does not require ethics committee approval as it does not involve any direct application on human or animal subjects.

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