Chapter

Callus Induction from Unpollinated Ovary Explants of Beans

Asli Küçükrecep, Dilek Tekdal, İlknur Akça, Selim Çetiner and Rüştü Hatipoğlu

Abstract

Beans one of the essential plant protein sources for human and animal diets. Conventional breeding methods have been used to develop the cultivars of beans with high quality and high yield. However, conventional methods of plant breeding are time-consuming and laborious. Biotechnological methods can accelerate the breeding process in conventional plant breeding. However, the beans are thought to be a recalcitrant crop plant for applying biotechnological methods since plant regeneration under in vitro conditions in beans is not successful. Developing an appropriate method for in vitro bean regeneration remains a significant problem. The objective of this study was to develop a protocol for the culture of unfertilized ovaries of beans. Culture media and genotype are effective on the success of in vitro cultivation. For this reason, 12 genotypes of beans and some nutrient media such as MS and B5 with various 2,4-D/kinetin combinations were tested to obtain callus from unfertilized ovaries. The highest callus induction was obtained with a medium containing 2,4-D (0.5 mg L^{-1}) and Kinetin (2.5 mg L^{-1}). A literature review on beans indicates that no ovary culture has been carried out on tested varieties in this study to date.

Keywords: callus, common bean, 2,4-D, Kinetin, ovary culture

1. Introduction

Due to the increasing awareness of healthy nutrition globally, individuals obtain most of their daily calorie needs from plant-based foods. Legumes, which constitute the primary source of vegetable protein (22%), have an important place in human nutrition as an alternative to meat products. In addition to their rich nutritional values, legumes are also known as soil friendly due to their ability to bind the free nitrogen of the air to the soil [1]. Beans are one of the most grown edible legume plants in the world. Common bean (*Phaseolus vulgaris* L. 2n = 2x = 22) is a diploid species with a wide range of variability of phenotypic characteristics due to its tolerance to a variety of agroecological environments [2–4]. According to 2016 FAO data, the dry bean was grown on an area of 29.392.817 ha worldwide; fresh bean cultivation was carried out on an area of 1.557.233 ha [5]. Turkey is a significant producer of the economically valuable Fabaceae plant family. According to TÜİK data, the most cultivated crop after chickpea and lentil among legumes in Turkey is beans [6]. Although

Turkey could not rank in the top 10 worldwide in dry bean production, it is the third largest green bean producer after China and Indonesia in the world (**Figure 1**) [7].

One of the biggest problems encountered in breeding studies of beans with conventional breeding methods is that the breeding process is long. Different molecular marker systems have been developed to shorten this breeding process. In addition, bean growers often use local bean varieties that are available as a population. These populations used are not genetically and physically pure. This situation causes different problems in bean cultivation: (a) mechanized agriculture is complex because individuals in the population do not show uniform growth and development, and (b) problems occur in both cooking and storage of non-uniform products [8]. It is known that the first breeding studies of legumes in Turkey started in 1965 on fresh beans. The bean plant shows the feature of self-fertilization due to its flower structure, and foreign pollination by insects is also possible. There is a flag (vexillum) leaf on the outside of the flower, a fin (alea) at the bottom, and a boat (carina) in the middle of the flower. The flower has 10 stamens, and these are located in the carina.

It is possible to obtain doubled haploid plants by culturing ovaries under *in vitro* conditions and subsequent chromosome doubling [9]. Although there are many studies on this subject in some plant species, few studies are on obtaining haploid beans [2]. Haploid and doubled haploid plants are currently used in genetic mapping, QTL analysis, mutation breeding, and genomic studies. In addition, homozygosity is achieved in one generation by using doubled plants. Although selfing is possible in the bean plant, it takes a long time to reach homozygous. Crossbreeding can be difficult due to the flower structure. It is known that classical hybridization studies require a high labor force; selfing is required to obtain a pure line and takes a long time, such as 7–9 years. In the dihaploidization method, haploid plants are made doubled haploid as a result of chromosome folding using various chemicals. Each of these 100% homozygous lines obtained is a candidate of a variety.

In this chapter, a protocol for morphogenetic callus induction from ovary samples in beans is defined. This protocol is strongly repeatable for 11 different *P. vulgaris* genotypes and *Phaseolus* sp. (1 genotype).

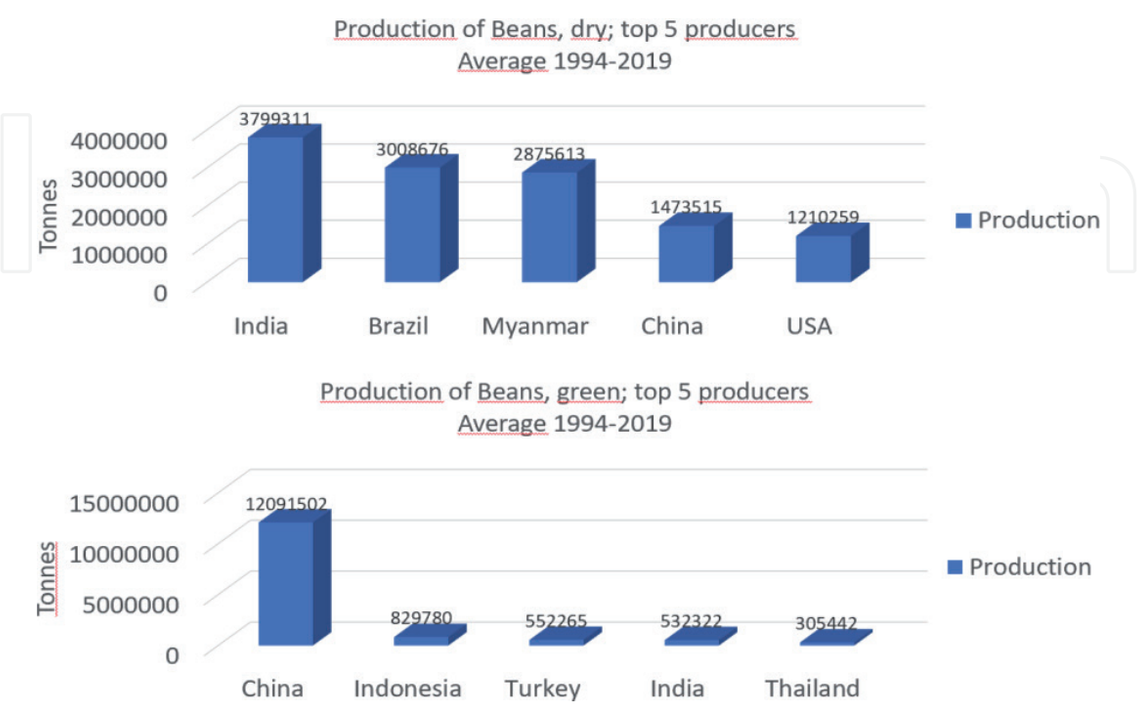

Figure 1.
*Statistical data of dry and green bean production of countries in the world. * From FAOSTAT database, May 2021 [7].*

2. Materials and methods

2.1 Plant materials

A total of 12 bean genotypes, seven genotypes selected from local lines and five commercial varieties, were used within the project's scope. Details on the total 12 bean genotypes are given in **Table 1**.

General views of seeds belonging to 12 bean genotypes are given in **Figure 2**.

2.2 Seed viability detection

The 2,3,5 triphenyl tetrazolium chloride method recommended by ISTA [10] was used for seed viability determination. The seeds of the genotypes tested were soaked in water for 24 h and peeled. Then, seeds were taken into 1 g L^{-1} 2,3,5 triphenyl tetrazolium chloride solution, and viability controls were carried out after 24 h.

2.3 Planting seeds and growing plants

Seeds of bean genotypes were sown in the greenhouse. Considering the weed reproduction situation, the seeds were first sown in viols containing peat and perlite (1:1) and allowed to germinate in order for the seeds to germinate easily. Plantlets

No	Genotype/variety name	Origin and characteristics
1	Akman	It is a variety registered by the Transitional Zone Agricultural Research Institute in 1998. It has a plant height of 60–70 cm. It is a variety with a harvest maturity period of 115–125 days
2	Bitlis-76	Local bean line, it was selfed three times and made homozygously
3	Bitlis-117	Local bean line, it was selfed three times and made homozygously
4	Göksun	It is a variety registered by the Transitional Zone Agricultural Research Institute in 2012. It has a plant height of 90–100 cm. It is a variety with a harvest maturity period of 104–124 days
5	Göynük	It is a variety registered by the Transitional Zone Agricultural Research Institute in 1998. It has a plant height of 45–55 cm. It is a variety with a harvest maturity period of 110–120 days
6	Hakkari-12	Local bean line, it was selfed three times and made homozygously
7	Karacaşehir	It is a variety registered by the Transitional Zone Agricultural Research Institute in 1990. It has a plant height of 50–55 cm. It is a variety with a harvest maturity period of 110–115 days
8	Önceler	It is a variety registered by the Transitional Zone Agricultural Research Institute in 1990. It has a plant height of 40–50 cm. It is a variety with a harvest maturity of 105–110 days
9	Tunceli-1	Local bean line, it was selfed three times and made homozygously
10	Van-59	Local bean line, it was selfed three times and made homozygously
11	Small reddish bean	It was obtained from the growers in the town of Elmalı in the province of Niğde in Turkey
12	Leklek	Local variety, it was obtained from the grower in the Gülnar district of Mersin Province in Turkey

Table 1.
Information on bean genotypes used in the study.

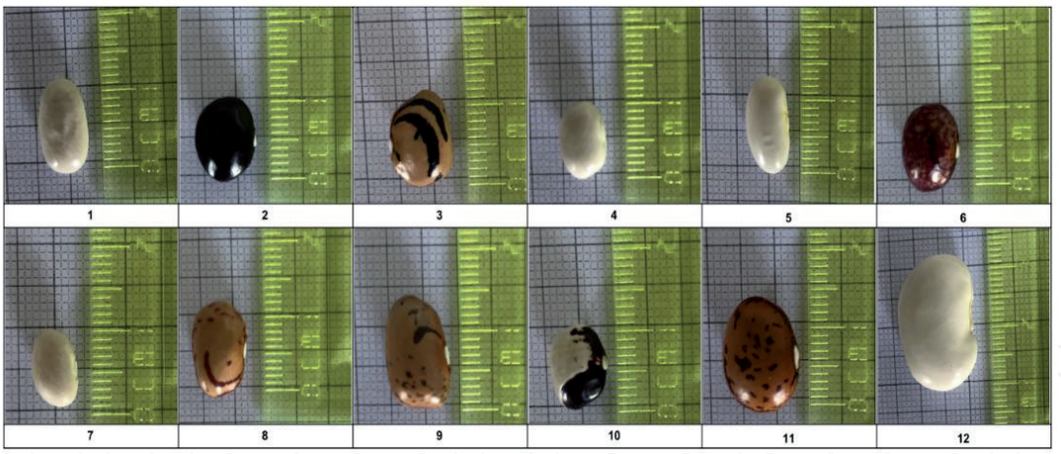

Figure 2.
*General images of seeds belonging to 12 bean genotypes (numbering of genotypes as indicated in **Table 1**).*

were developed in viols before they were transferred to pots. Humic fulvic acid was applied with life water in order to remove the initial stress and increase root activity while the plants were transferred to the pots. Plants growing in viols were transferred in large pots. Plants were planted in pots with 20 cm between the rows and plants and 10 plants in each pot.

2.4 Ovary culture

2.4.1 Surface sterilization

Flower buds of an appropriate size determined for the ovary culture experiment should be kept under tap water for 30 min to remove soil and/or dust residues. After then, it was washed several times with sterile distilled water. Explants taken in a sterile cabinet (Demair, class II A2 MSC 120) are rinsed in 70% EtOH for 1 min. Then, they were washed with pure water. Flower buds were kept in 25% NaOCl (sodium hypochlorite) for 15 min. The surface sterilization process of the samples was completed by washing 4–5 times with sterile distilled water. The unpollinated ovaries in the sterilized flower bud were isolated under a stereomicroscope (Olympus SZ61, Japan) and used as the explant in the tissue culture study.

2.4.2 Culture conditions

The sepals and petals of the flower buds, whose surface sterilization has been completed, were carefully removed. The isolated ovaries were then placed on the different basic media (MS and B5) [11, 12]. Different concentrations of 2,4-D (0, 0.5, 1.0, 2.0 mg L^{-1}) and Kinetin (Kin) (0, 0.5, 1.0, 2.0, 2.5, 3.0, 3.5, and 4.0 mg L^{-1}) and their combinations were added to the basic media (**Table 2**). The experiment was set up with three Petri dishes for each genotype and five ovaries in each petri dish (15 ovaries in total). The samples were kept in styrofoam until callus formation was observed and left to the culture in a climate cabinet (Miprolab, Ankara, Turkey) at 26 ± 1°C.

Calli were transferred to fresh MS media without PGR and MS supplemented with thidiazuron (TDZ; 0.4 mg L^{-1}) and salicylic acid (SA; 20 mg L^{-1}) for plant regeneration, which was previously described as a differentiation medium for *Phaseolus* embryos [13].

Experimental plan for ovary culture			
Application code	**Kin (mg L^{-1})**	**2,4-D (mg L^{-1})**	**Trial plan for each variety/genotype**
MS			
1	0	0	3 Petri dishes (5 ovary explants in each pet)
2	0.5	0.5	3 Petri dishes (5 ovary explants in each pet)
3	0.5	1	3 Petri dishes (5 ovary explants in each pet)
4	0.5	2	3 Petri dishes (5 ovary explants in each pet)
5	1	0.5	3 Petri dishes (5 ovary explants in each pet)
6	1	1	3 Petri dishes (5 ovary explants in each pet)
7	1	2	3 Petri dishes (5 ovary explants in each pet)
8	2	0.5	3 Petri dishes (5 ovary explants in each pet)
9	2	1	3 Petri dishes (5 ovary explants in each pet)
10	2	2	3 Petri dishes (5 ovary explants in each pet)
11	2.5	0.5	3 Petri dishes (5 ovary explants in each pet)
12	2.5	1	3 Petri dishes (5 ovary explants in each pet)
13	2.5	2	3 Petri dishes (5 ovary explants in each pet)
14	3	0.5	3 Petri dishes (5 ovary explants in each pet)
15	3	1	3 Petri dishes (5 ovary explants in each pet)
16	3	2	3 Petri dishes (5 ovary explants in each pet)
17	3	3	3 Petri dishes (5 ovary explants in each pet)
18	3.5	0.5	3 Petri dishes (5 ovary explants in each pet)
19	3.5	1	3 Petri dishes (5 ovary explants in each pet)
20	3.5	2	3 Petri dishes (5 ovary explants in each pet)
21	4	0.5	3 Petri dishes (5 ovary explants in each pet)
22	4	1	3 Petri dishes (5 ovary explants in each pet)
23	4	2	3 Petri dishes (5 ovary explants in each pet)
24	4	4	3 Petri dishes (5 ovary explants in each pet)
B5			
25	0	0	3 Petri dishes (5 ovary explants in each pet)
26	0.5	0.5	3 Petri dishes (5 ovary explants in each pet)
27	0.5	1	3 Petri dishes (5 ovary explants in each pet)
28	0.5	2	3 Petri dishes (5 ovary explants in each pet)
29	1	0.5	3 Petri dishes (5 ovary explants in each pet)
30	1	1	3 Petri dishes (5 ovary explants in each pet)
31	1	2	3 Petri dishes (5 ovary explants in each pet)
32	2	0.5	3 Petri dishes (5 ovary explants in each pet)
33	2	1	3 Petri dishes (5 ovary explants in each pet)
34	2	2	3 Petri dishes (5 ovary explants in each pet)
35	2.5	0.5	3 Petri dishes (5 ovary explants in each pet)
36	2.5	1	3 Petri dishes (5 ovary explants in each pet)
37	2.5	2	3 Petri dishes (5 ovary explants in each pet)

Experimental plan for ovary culture			
Application code	**Kin (mg L^{-1})**	**2,4-D (mg L^{-1})**	**Trial plan for each variety/genotype**
38	3	0.5	3 Petri dishes (5 ovary explants in each pet)
39	3	1	3 Petri dishes (5 ovary explants in each pet)
40	3	2	3 Petri dishes (5 ovary explants in each pet)
41	3	3	3 Petri dishes (5 ovary explants in each pet)
42	3.5	0.5	3 Petri dishes (5 ovary explants in each pet)
43	3.5	1	3 Petri dishes (5 ovary explants in each pet)
44	3.5	2	3 Petri dishes (5 ovary explants in each pet)
45	4	0.5	3 Petri dishes (5 ovary explants in each pet)
46	4	1	3 Petri dishes (5 ovary explants in each pet)
47	4	2	3 Petri dishes (5 ovary explants in each pet)
48	4	4	3 Petri dishes (5 ovary explants in each pet)

Table 2.
Medium variants used in ovary culture.

2.5 Statistical analysis

Variance analysis was applied to the data on the rate of callus/embryo formation (reaction rate) of ovary explants according to the completely randomized design in split plots with three replications by using MSTAT-C Statistical Program.

3. Results and discussion

The first goal of developing a procedure for indirect regeneration of bean genotypes was to develop an optimum medium for morphogenetic calli induction. In this study, flower buds were used as an explant source. Seed germination occurred in all tested genotypes successfully. Unfertilized ovaries of the genotypes were picked on the day of anthesis. Isolated ovary samples were cultured on 48 different media. For callus induction from the explants, MS and B5 media, including different combinations of auxin (2,4-D) and cytokinin (Kin), were tested. Different concentrations and combinations of Kin (0, 0.5, 1.0, 2.0, 2.5, 3.0, 3.5, and 4.0 mg L^{-1}) and 2,4-D (0, 0.5, 1.0, and 2.0 mg L^{-1}) were investigated to optimize callus induction of 12 bean genotypes. Callus was obtained from all ovary samples studied. Non-morphogenic and morphogenic calli were generated in bean ovary cultures inoculated on different agar media. According to the microscope images of the calli developing from the ovary samples, it was observed that the calli mostly developed at the ends of the cultured ovary sample and had a light yellow-brown color scale (**Figures 3–6**).

On the other hand, no regeneration of calli was observed in the samples cultured on the medium free from PGR. The formation of embryos and embryogenic calli was an uncommon occurrence. However, callus was obtained from all 12 varieties tested in this study. Morphogenic calli in the presence of Kin and 2,4-D were characterized by cell proliferation. Nutritional medium with relatively high- and low-growth regulator concentration demonstrated only minor variations in the efficiency of morphogenic calli production. Some factors, such as stress factors and nutrient media composition, are thought to strongly influence the reprogramming

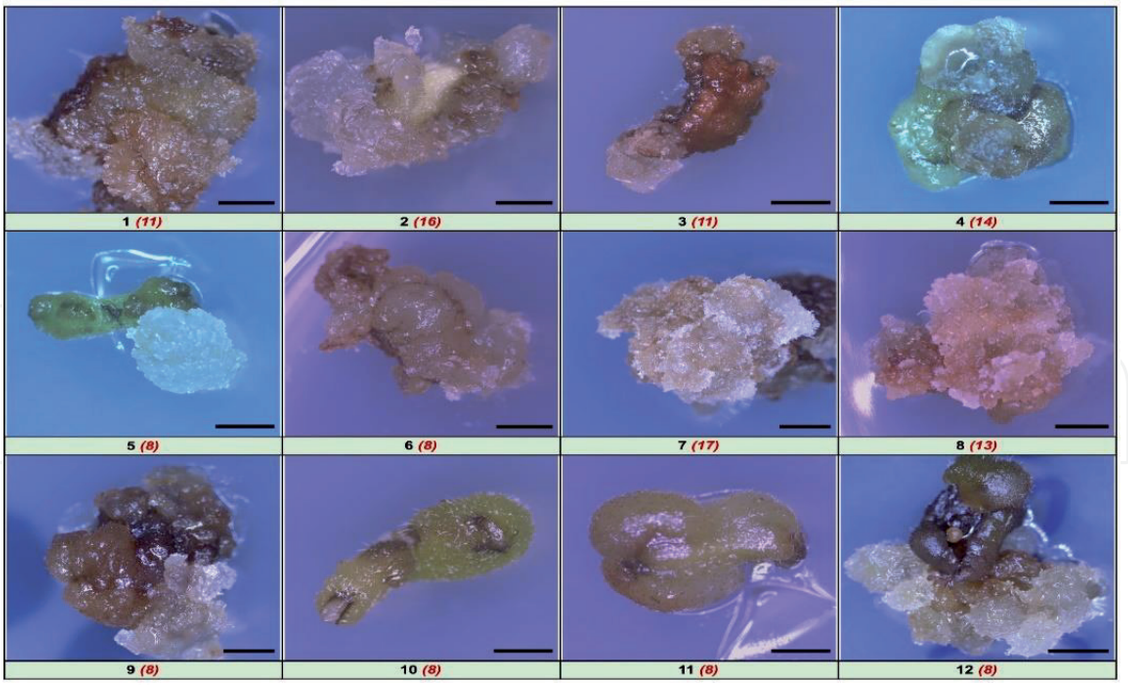

Figure 3.
Stereo-microscope images (Olympus SZ61, Japan) of callus growing from ovaries cultured in MS medium (black numbers indicate the genotype number and the detail is given in ***Table 1****; red numbers indicate the medium in which callus growth was observed; the detail is given in* ***Table 2****; magnification: 1.2×; scale bar 200 μm).*

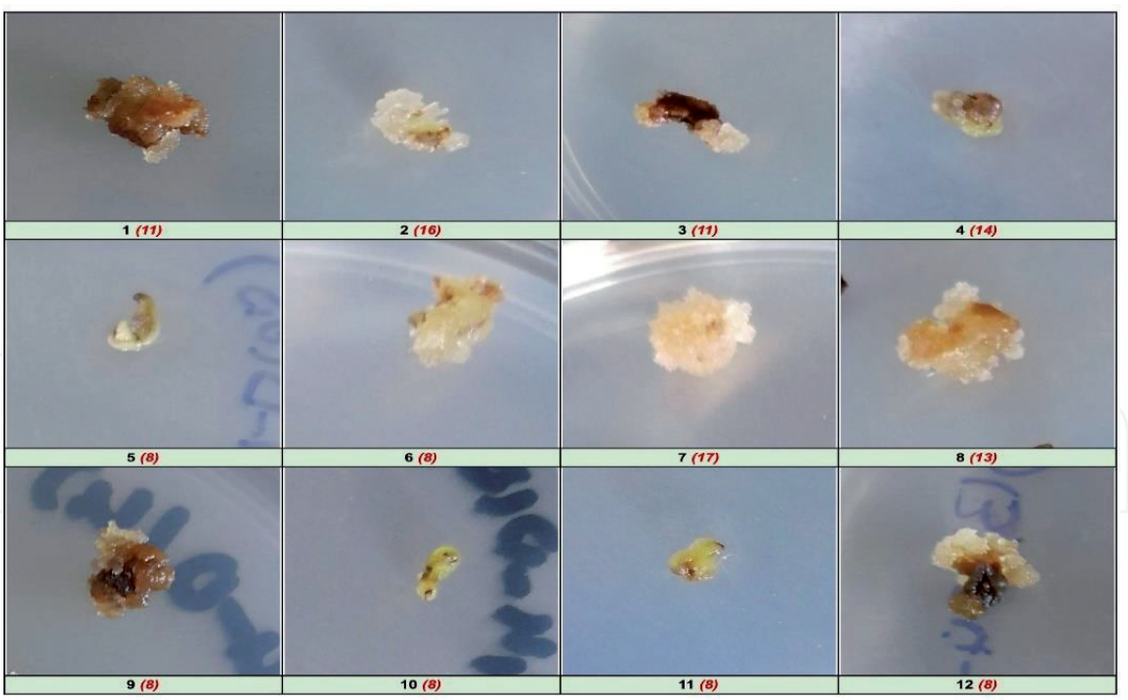

Figure 4.
Calli in petri dishes (black numbers indicate their genotype numbers, and the details are given in ***Table 1****) developed from ovaries cultured in MS medium and given microscopic images in* ***Figure 4****; red numbers indicate the medium in which callus development was observed; the detail is given in* ***Table 2****.*

of bean megaspore into the sporophytic developmental pathway. Kin was used in the presented study since cytokinins act on bud formation and plant cell division [14]. The effect of Kin and 2,4-D concentration on callus proliferation was observed, and calli increased in size and were nodular and compact (**Figures 3–6**).

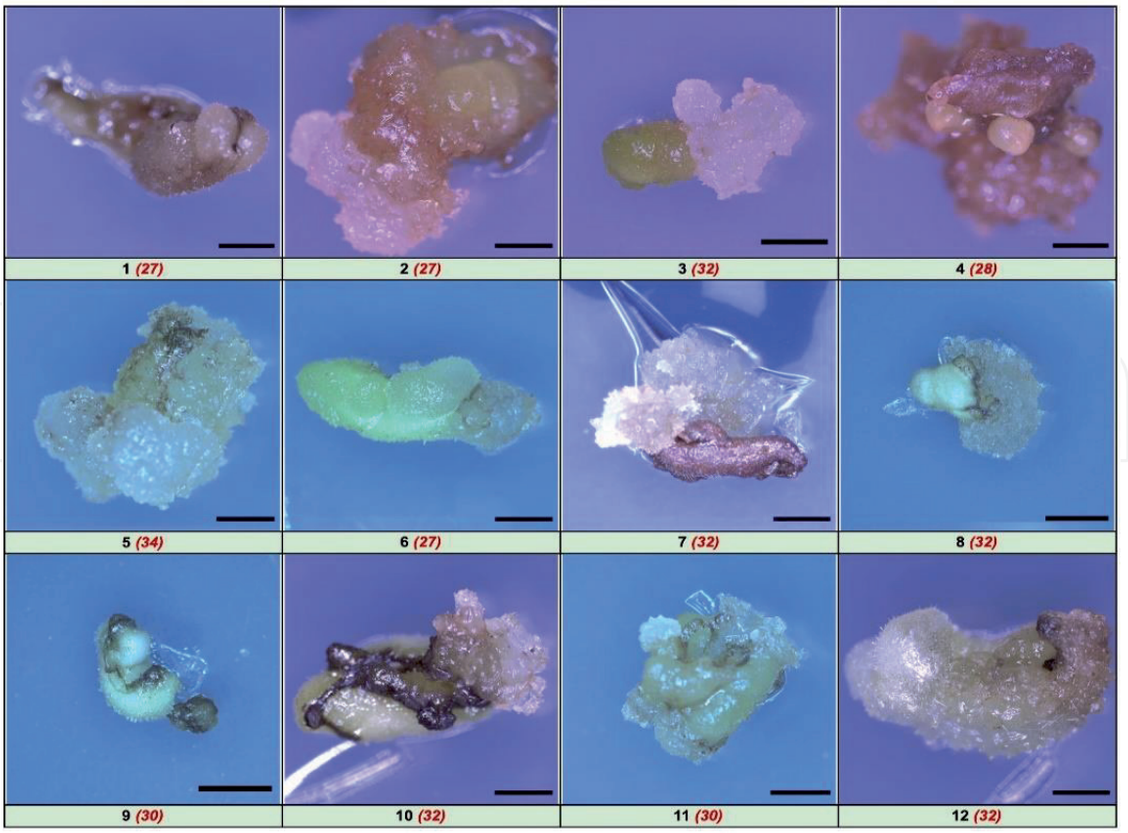

Figure 5.
Stereo-microscope images (Olympus SZ61, Japan) of callus growing from ovaries cultured in B5 medium (black numbers indicate the genotype number and the detail is given in ***Table 1****; red numbers indicate the medium in which callus growth was observed; the detail is given in* ***Table 2****; magnification: 1.2×; scale bar 200 μm).*

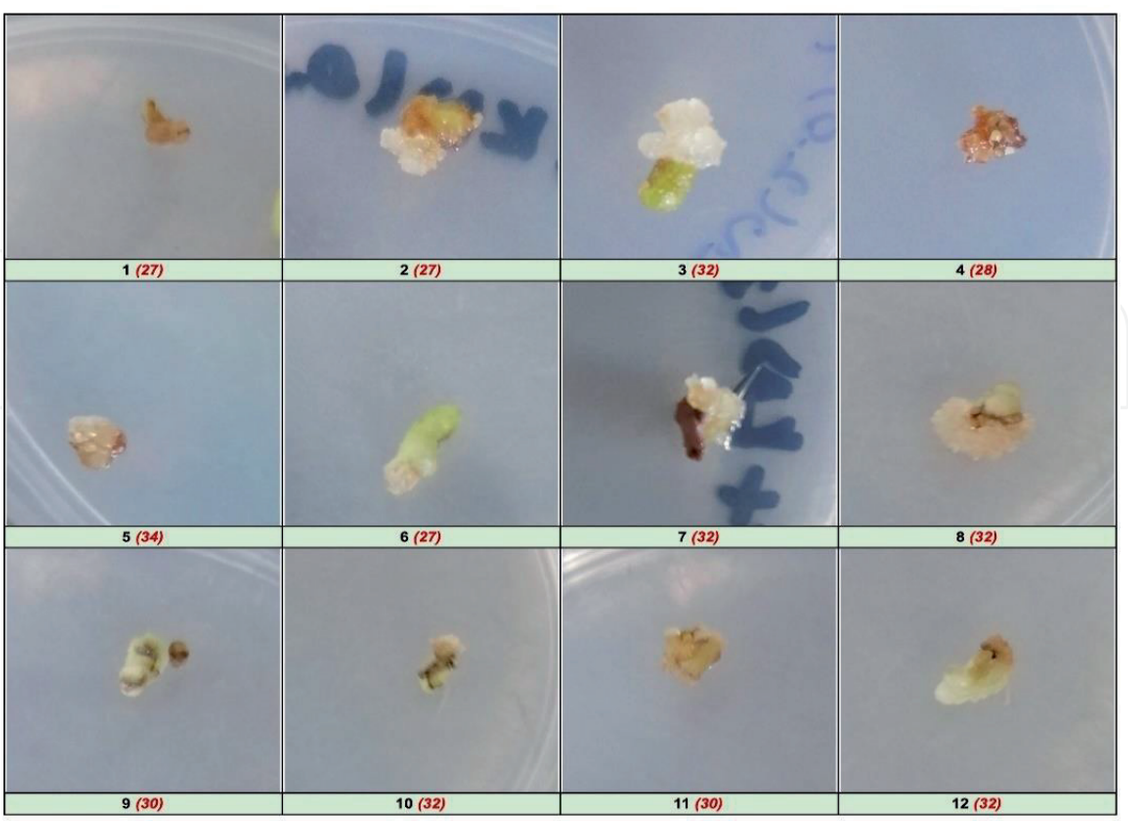

Figure 6.
Calli in petri dishes (black numbers indicate their genotype numbers, and the details are given in ***Table 1****) developed from ovaries cultured in B5 medium and given microscopic images in* ***Figure 6****; red numbers indicate the medium in which callus development was observed, and the detail is given in* ***Table 2****.*

Unpollinated ovaries/ovules or full flowers can be cultured to produce efficient gynogenesis methods that generate many embryos from female gametic cells. When the literature on haploidization studies conducted with the legume family was examined, very few studies were encountered. In a study on the *Cajanus cajan* plant, callus and immature embryos were obtained, but it was stated that callus cells initially had haploid and then a large variety of chromosome complements. Also, mature embryos and haploid plants were not obtained [15]. Grewal et al. [16] mentioned that members of the Fabaceae family are recalcitrant and, therefore, the difficulty of their development in culture.

In vitro regeneration and genetic transformation were difficult for *P. vulgaris* and other members of the *Phaseolus* genus since they are recalcitrant. While many *in vitro* regeneration protocols for *P. vulgaris* have been published, most of them were related to direct organogenesis or shoot production from meristematic cells [3]. Several reports have been on organogenesis in different cultured explants of *P. vulgaris* hypocotyls, cotyledonary nodes, and embryonic axes [3, 17]. However, no study exists on *in vitro* embryogenesis from the unpollinated ovary of *P. vulgaris.* Although plant regeneration is often genotype-specific in tissue culture, callus was successfully obtained from the ovaries of all 12 genotypes in this study. Some plant growth hormones may be stored in the ovary during plant development and may cause a different hormone balance *in vitro* culture with synthetically added hormones. This situation may also differ within each genotype and cause further growth or developmental problems in the culture. Studies indicate that successful shoot formation is observed in different bean explants cultured in nutrient media where TDZ and IAA are used together [18, 19]. In addition, success has been achieved in media containing a combination of TDZ and IAA in different *Phaseolus* species such as *P. acutifolius* A. Gray [20] and *P. polyanthus* Greenman [21]. Morphogenesis (roots) was induced from ovary samples in this study when the low PGR concentrations were used. Translucent embryos were obtained from ovaries when the calli were transferred to the medium with TDZ (0.4 mg L^{-1}) and SA (20 mg L^{-1}) (**Figure 7**). When the calli were transferred into the medium free from the PGR, no development was observed, and the calli began to darken. A previous study

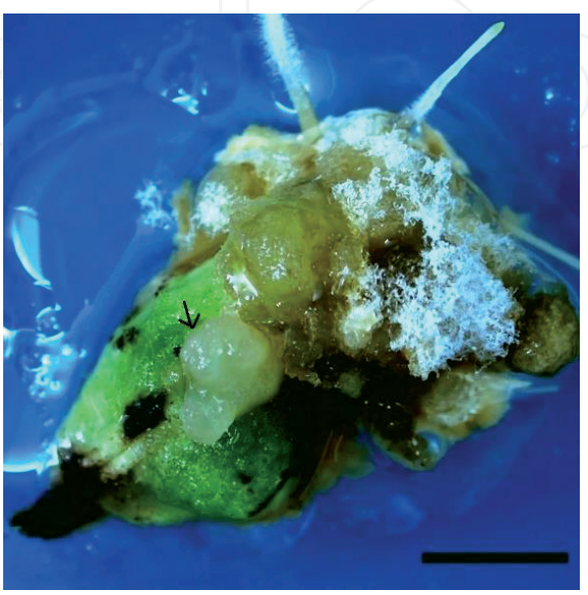

Figure 7.
Development of heart-shaped embryo on MS medium containing TDZ (0.4 mg L^{-1}) and SA (20 mg L^{-1}) in two weeks (black arrow indicates embryogenic formation; scale bar 200 μm).

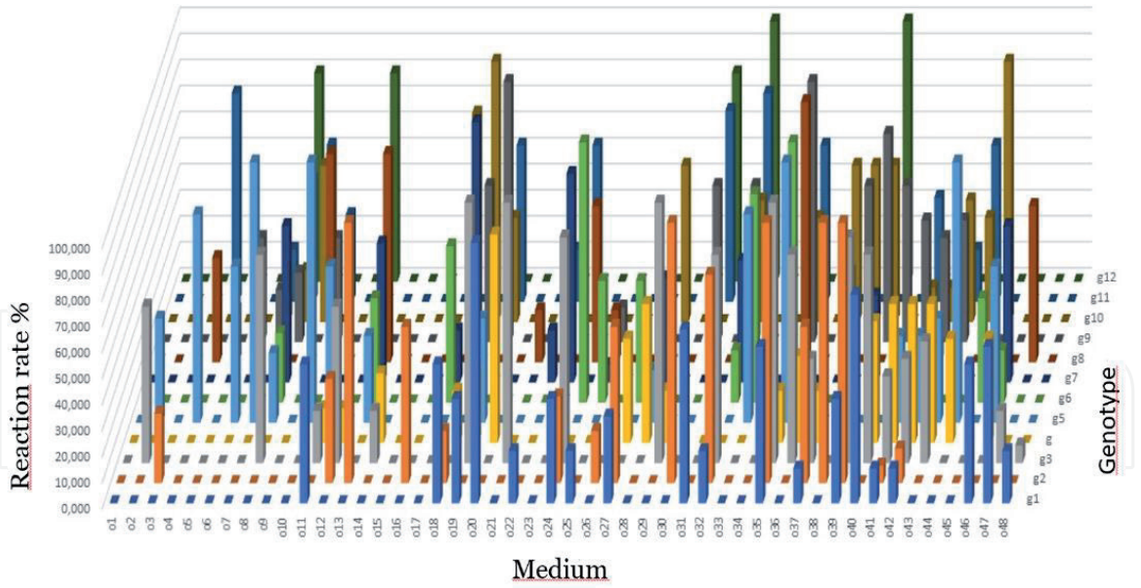

Figure 8.
Callus/embryoid formation rates of ovaries of different genotypes in different nutrient media (%).

of *Cucumis anguria* L. showed that unpollinated ovaries cultured *in vitro* did not enlarge [22].

According to a literature review, it is known that the B5 [12] medium is more effective in the tissue culture of some Fabaceae family members than the MS medium [2, 23]. Ovary samples taken in culture in MS and B5 media specified in **Table 2** were analyzed comparatively for each genotype and each tested medium. The statistically significant interaction of the nutrient medium × genotype revealed that the effect of the nutrient medium on the reaction rate differs depending on the genotypes (**Figure 8**).

The medium, including 2.5 mg L^{--1} Kin, provided a significantly higher reaction rate than all other media studied. The reaction rate of the explants differed significantly depending on the genotypes. According to the analysis results, the best callus yield was obtained from B5 media containing 2.5 mg L^{-1} Kin and 0.5, 1, and 2 mg L^{-1} 2,4-D in the ovary culture experiment. MS medium free from plant growth regulators never triggered callus induction in all tested genotypes, whereas B5 without plant growth regulators resulted in callus induction only in two genotypes (g1 and g6, given in **Table 1**).

In the future, these findings might act as a clue in generating the whole plants *in vitro* conditions. The future applications of these bean genotypes hold a great promise as a management tool for obtaining the plants against climatic conditions.

4. Conclusions

The most successful medium for callus induction in ovary culture of *P. vulgaris* was B5, and the influence of different stages of female gametophyte should be investigated for higher callus induction and plant regeneration in common beans. As a result, the technique we describe has the potential to enhance indirect organogenesis in the future and may serve as the foundation for developing a procedure for *P. vulgaris*. We believe that the research results discussed here contribute to further studies on *in vitro* regeneration of common beans. Understanding the role of growth regulators for selective bean genotypes has greatly aided bean regeneration under controlled conditions.

Acknowledgements

The study described here was carried out within the Project (No. 119O003) funded by the Scientific and Technological Research Council of Turkey (TÜBİTAK). The authors are grateful to the Republic of Turkey Ministry of Agriculture and Forestry Mersin Directorate of Provincial Agriculture and Forestry for providing Leklek's seeds. We would like to thank also Assoc. Prof. Dr. Faheem Shahzad Baloch for graciously supplying common bean seeds indicated by numbers 1-11 in **Table 1**.

Conflict of interest

The authors declare no conflict of interest.

Appendices and nomenclature

MS	Murashige and Skoog medium
B5	Gamborg's medium
2,4-D	2,4-dichlorophenoxyacetic acid
Kin	Kinetin
P. vulgaris	*Phaseolus vulgaris* L.

Author details

Asli Küçükrecep[1†], Dilek Tekdal[1†*], İlknur Akça[1], Selim Çetiner[2] and Rüştü Hatipoğlu[3]

1 Department of Biotechnology, Institute of Science, Mersin University, Mersin, Turkey

2 Faculty of Engineering and Natural Sciences,Biological Sciences and Bioengineering Program, Sabanci University, Istanbul, Turkey

3 Faculty of Agriculture, Department of Field Crops, Çukurova University, Adana, Turkey

*Address all correspondence to: dilektekdal@mersin.edu.tr

† Asli Kucukrecep and Dilek Tekdal equally contributing authors.

References

[1] Gülümser A. Dünyada ve Türkiye'de Yemeklik Dane Baklagillerin Durumu. Tarla Bitki Merkezi Araştırma Enstitüsü Derg. 2016;**25**(ÖZEL SAYI-1):292-292

[2] Croser JS, Lülsdorf MM, Davies PA, Clarke HJ, Bayliss KL, Mallikarjuna N, et al. Toward doubled haploid production in the fabaceae: Progress, constraints, and opportunities. Critical Reviews in Plant Sciences. 2006;**25**(2): 139-157

[3] Arellano J, Fuentes SI, Castillo-España P, Hernández G. Regeneration of different cultivars of common bean (*Phaseolus vulgaris* L.) via indirect organogenesis. Plant Cell, Tissue and Organ Culture. 2009;**96**(1): 11-18

[4] Crop A, Society S, Okii D, Tukamuhabwa P, Odong T, Namayanja A, et al. Morphological diversity of tropical common bean germplasm. African Crop Science Journal. 2014;**22**(1):59-68

[5] FAOSTAT. Crops [Internet]. 2016. Available from: http://www.fao.org/faostat/en/#data/QC

[6] TÜİK. Dry Pulses, 1988-2020 [Internet]. 2021. Available from: https://data.tuik.gov.tr/Search/Search?text=dry%20pulses&dil=1

[7] FAOSTAT. Crops [Internet]. 2019. Available from: http://www.fao.org/faostat/en/#data/QC

[8] Anonymous. Ulusal Bilim ve Teknoloji Politikaları 2003-2023 Strateji Belgeleri [Internet]. 2014. Available from: https://www.tubitak.gov.tr/tubitak_content_files/vizyon2023/Vizyon2023_Strateji_Belgesi.pdf.

[9] Van Der Knaap E, Tanksley SD. Identification and characterization of a novel locus controlling early fruit development in tomato. Theoretical and Applied Genetics. 2001;**103**(2-3): 353-358

[10] ISTA. International rules for seed testing. Seed Science and Technology. 1996;**13**:299-513

[11] Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum. 1962;**15**:473-497

[12] Gamborg OL, Murashige T, Thorpe TA. Vasil IK 1976. Plant tissue culture media. In Vitro Cellular & Developmental Biology. 1976;**12**(7): 473-478

[13] Sadeghipour O, Aghaei P. Response of common bean (*Phaseolus vulgaris* L.) to exogenous application of salicylic acid (SA) under water stress conditions. Advances in Environmental Biology. 2012;**6**(3):1160-1168

[14] Gatica Arias AM, Valverde JM, Fonseca PR, Melara MV. In vitro plant regeneration system for common bean (*Phaseolus vulgaris*): Effect of N6-benzylaminopurine and adenine sulphate. Electronic Journal of Biotechnology. 2010;**13**(1):1-8

[15] Bajaj YPS, Singh H, Gosal SS. Haploid embryogenesis in anther cultures of pigeon-pea (*Cajanus cajan*). Theoretical and Applied Genetics. 1980;**58**(3-4):157-159

[16] Grewal RK, Lulsdorf M, Croser J, Ochatt S, Vandenberg A, Warkentin TD. Doubled-haploid production in chickpea (*Cicer arietinum* L.): Role of stress treatments. Plant Cell Reports. 2009;**28**(8):1289-1299

[17] Mohamed MF, Coyne DP, Read PE. Shoot organogenesis in callus induced from pedicel explants of common bean (*Phaseolus vulgaris* L.). Journal of the

American Society for Horticultural Science. 2019;**118**(1):158-162

[18] Kwapata K, Sabzikar R, Sticklen MB, Kelly JD. In vitro regeneration and morphogenesis studies in common bean. Plant Cell, Tissue and Organ Culture. 2010;**100**(1):97-105

[19] Mukeshimana G, Ma Y, Walworth AE, Song GQ, Kelly JD. Factors influencing regeneration and *Agrobacterium tumefaciens*-mediated transformation of common bean (*Phaseolus vulgaris* L.). Plant Biotechnology Reports. 2013;**7**(1):59-70

[20] Dillen W, De Clercq J, Goossens A, Van Montagu M, Angenon G. *Agrobacterium*-mediated transformation of *Phaseolus acutifolius* A. Gray. Theoretical and Applied Genetics. 1997;**94**(2):151-158

[21] Zambre M, Geerts P, Maquet A, Van Montagu M, Dillen W, Angenon G. Regeneration of fertile plants from callus in *Phaseolus polyanthus* Greenman (year bean). Annals of Botany. 2001;**88**(3):371-377

[22] Nitsch JP. The role of plant hormones [PhD thesis]. 1951

[23] Lulsdorf MM, Croser JS, Ochatt S. Androgenesis and doubled-haploid production in food legumes. Biology and Breeding of Food Legumes. 2011;**2016**(May):159-177