

Cucumber Gynogenesis: Effects of 8 Different Media on Embryo and Plant Formation

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Abstract – Haploid and doubled haploid plants play a quite significant role in plant breeding programs. They allow obtaining pure lines from heterozygous parents in a short time. Present study was conducted to reveal the effects of different media on cucumber gynogenesis. The unfertilized cucumber ovaries of three genotypes (0701, 0702 and 0703) were collected one day before the anthesis. After surface sterilization, collected ovaries were sliced into 4 parts under sterile conditions, as pretreatment were placed on eight different modified cucumber basal media (CBM). All explants in different media were kept in dark conditions at 35°C for 3 days and then were transferred to 25°C in dark conditions for further 2 days. After the pretreatment at all cultured media were transferred to light conditions at 25°C in a photoperiod of 16/8 during 9 days. The effects of several factors such as genotypes and culture media were evaluated. And also callus, embryo formation and plantlets regeneration were recorded. Experiment results revealed that genotype 0703 and medium 5 (CBM with 0, 03 mg/L TDZ) combination gave the best results in terms of embryo formation and plantlet regeneration.

Keywords – *Cucumis Sativus* L., Cucumber, Gynogenesis, Haploidy.

I. INTRODUCTION

The *Cucurbitaceae* family contains several economical and especial garden crops. Cucumber (*Cucumis sativus* L.) is one of the most important economic vegetables in terms of in greenhouse and open field production and has a wide cultivation area in many parts of the world. Cucumber is an open-pollinated and monoecious crop. As it has monoecious nature, obtaining of hybrid cultivars via traditional breeding methods are both needed adequate facilities and time-consuming. Productivity can be enhanced through the use of hybrids in vegetable species. In this regard, pure lines are high-valued agents for plant breeding programs. However, to obtain pure lines require time, labor and space, alternative biotechnological approaches therefore, such as *in vitro* culture techniques can be suitable for traditional plant breeding. Thereby, alternatively, desired homozygous parental lines can be quickly and efficiently obtained through *in vitro* culture techniques such as androgenesis (pollen or anther culture) or gynogenesis (ovule or ovary culture). These alternative techniques have been used in many crops including cucumber. Haploid and doubled haploid plants play a quite significant role in plant breeding as they reduce the time needed to obtain pure lines. It is known that the development of homozygous parental lines using the traditional self-pollination method takes from 6 - 8 years on cucumber [1]. Haploid or doubled haploid plants are regenerated through either the formation of embryo-like structures (ELs) directly on ovules or the formation of

ELs or shoots on calli induced from ovules/ovaries [2]. Although haploid plants that developed through gynogenically can be obtained both in monocotyledonous and dicotyledonous species, only a few results have been published on haploid induction in *Cucurbitaceae* [1]. Towards the end of the 1950s the first haploid plants in the *Cucurbitaceae* family were obtained. To date, haploid and doubled haploid plants were obtained in widely grown and are of economic importance *Cucurbitaceae* species especially cucumber, squash, melon and watermelon through both androgenesis and gynogenesis techniques [1], [3]-[10]. Gynogenesis method is one of the approaches used to achieve homozygous pure lines for the commercial production of F₁ hybrid varieties and genetic studies [6].

So far, *in vitro* unfertilized ovule/ovary culture has been the most popular method of haploid induction in cucumber [11]. However, according to many researchers the major obstacle of gynogenesis especially uses of unfertilized ovules in breeding programs may show the low frequency in embryo induction, embryo development and regeneration. Nevertheless, *in vitro* unfertilized ovule/ovary culture method has been the most favourite method among haploid induction methods in cucumber, so far [11]. Various factors also effect *in vitro* gynogenesis, such as donor plants, pretreatments, developmental stages, plant growth regulators and additional constituents, culture conditions [1], [6], [10], [12]-[14]. Present study is, therefore, conducted to examine of the effects of different factors such as genotypes and culture media on callus, embryo formation and plantlet regeneration on cucumber gynogenesis.

II. MATERIALS AND METHODS

A. Plant Material

Three genotypes of cucumber, 0701, 0702 and 0703, belong Bati Akdeniz Agricultural Research Institute, were used as donor plants. Plants were grown in greenhouse of Bati Akdeniz Agricultural Research Institute and tissue culture techniques were carried out at Akdeniz University, Antalya, Turkey.



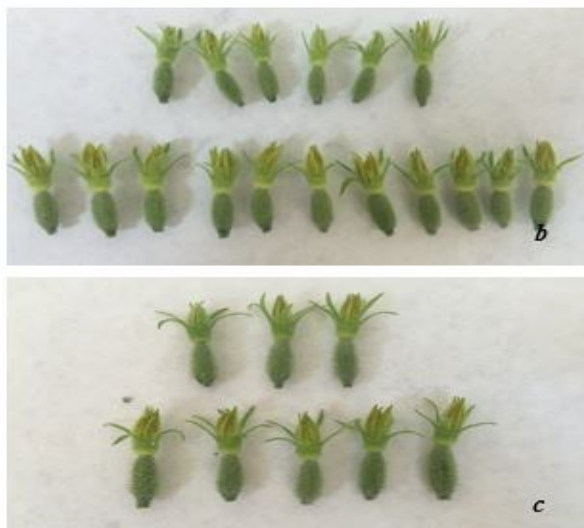


Fig. 1. Stages of genotypes (a. 0701, b. 0702, c. 0703) female flower developments

B. Culture Technique and Media Preparation

Ovaries of donor plants were used during the 2-3 weeks after the first female flowers appeared. According to the majority of researchers, the best time to excised the most suitable material for the gynogenesis technique is one day prior to anthesis [2], [3], [6], [15]-[20]. Thus, unfertilized ovaries were harvested 24 h before anthesis in present study. The stages of female flower development were assessed by observing the female flowers development morphologically (Fig. 1). Flower parts of female gametophyte were removed. Then, peeled ovaries kept under the tap water during the removing villus on the ovaries. After that, surface sterilization was carried out with 70% ethanol for 1 min, followed by a 20 min 1% sodium hypochlorite, and finally rinsed four times with sterile distilled water. Ovaries were peeled from external skin then sliced cross and longitudinally into four equal halves under sterile conditions and placed on eight different modified induction cucumber basal media (CBM) (Fig. 2). Several researchers have used CBM as basic medium via *in vitro* unfertilized ovule/ovary culture to obtain cucurbit haploids [1], [18], [21], [22]. All induction media that used in experiments were supplemented with 3% (w/v) sucrose and solidified with 0.8% agar (w/v). All media combinations differed in composition of growth regulators and FENAEDTA concentrations (Table I). The pH of the media was adjusted to 5.8 - 6.0 before autoclaving at 120 °C for 20 min. All cultured explants in eight different media were kept in dark conditions at 35°C for 3 days and then were transferred to 25°C again in dark conditions for 2 days. After the pretreatments all cultured media were transferred to light conditions in a photoperiod of 16/8 during 9 days. The ovule explants were sub-cultured onto fresh cucumber basal medium with 0.05 mg/L NAA + 0.2 mg/L benzylaminopurin (BAP) at the end of the fourteenth days of culture and incubation conditions were maintained at 25°C under a 16/8 h (light/dark) photoperiod for further development. The effects of several factors such as genotypes and culture media on cucumber gynogenesis were evaluated.

Media Codes	Culture Media	Kinetin (mg/L)	2,4-D (mg/L)	TDZ (mg/L)	FeNaEDTA (g/L)
M1	CBM	1.0	0.1	-	-
M2	CBM	-	-	-	0.037
M3	CBM	1.0	0.1	-	0.037
M4	CBM	-	-	0.02	-
M5	CBM	-	-	0.03	-
M6	CBM	-	-	0.04	-
M7	CBM	-	-	0.03	0.037
M8	CBM	-	-	-	-

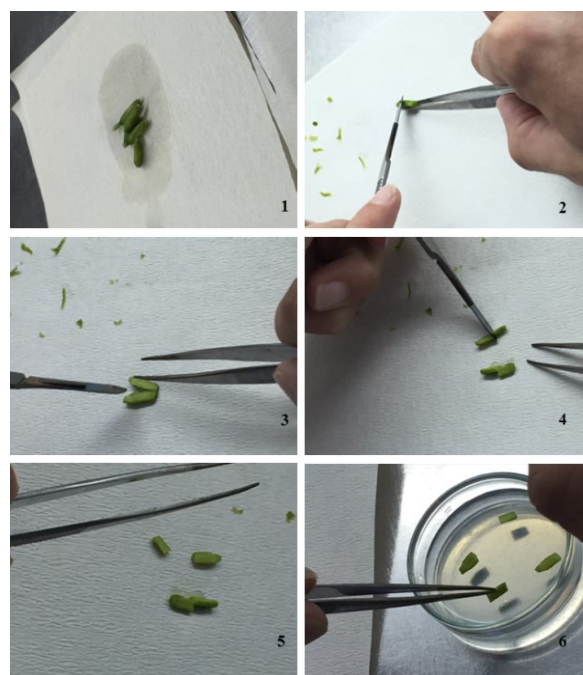


Fig. 2. Process of ovule/ovary culture

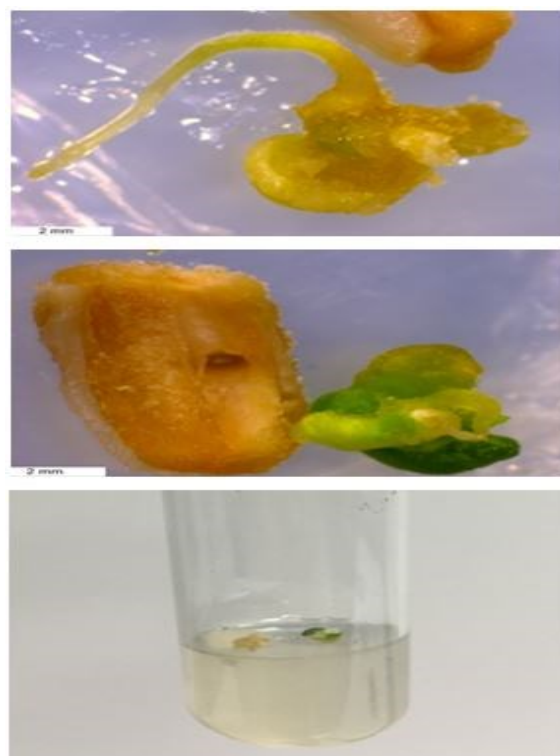


Fig. 3. Abnormal plantlet regenerations from gynogenic embryos with 0703 at medium 3

III. RESULTS AND DISCUSSION

All results are given in Table II. Cucumber ovule/ovary culture gets effected by various factors such as genotypes of donor plants, temperature shocks, female gametophyte developmental stages and media compositions [1], [6], [10], [12], [13]. In some studies [6], [17], [19], [20], the use of TDZ as a plant growth regulator at concentrations of 0.01 to 0.04 mg/l has been favorable for inducing gynogenesis. Especially, [6] and [19] obtained the highest embryo formations in their studies using 0.04 mg/l TDZ. This study is partially consistent with the results of other studies using different concentrations of TDZ for embryo and plant formation. Ovary slices cultured on different media were developed but notable structures were not observed except medium 5 and 3, respectively. In terms of embryo and plantlet formation medium 5 provided the best results although the plantlet formed on medium 5 could not survive further. Abnormal plantlets developments were observed on medium 3 (Fig. 3). According to many researchers, the selection of the appropriate genotype for the haploid embryo and plant formation by the gynogenesis technique is an important factor [7], [8], [16], [19], [23]-[27]. Our study also showed that genotypes of donor plants and culture media components effect on *in vitro* embryo formation abilities. With regard to the ovary culture

response of genotypes, 0703 was better than the others. It is likely that the induction of gynogenic competence, embryo formation and plantlets regeneration depend on genotype and culture media as reported in previous studies. Many researchers recommended thermal shocks (low or high temperature) to improve gynogenic response [1], [3], [6], [16], [19]. Our results demonstrated that the interaction between genotype and high temperature shock pretreatment with different media compositions on embryogenesis success was effected positively.

IV. CONCLUSION

In this study, gynogenesis technique, one of the haploid plant breeding techniques, was used. Among for three different genotypes used in the study, 0703 genotype gave the best results in terms of gynogenesis compared to the others. In terms of different media combinations used within present study medium number 5 (CBM + 0.03 mg/l TDZ) was the best. In conclusion, genotype and certain media supplements applied to *in vitro* unpollinated ovule/ovary culture have a quite potency on ratio of embryo and plant formation. Further studies are required for better results on cucumber gynogenesis, specifically on developing a culture media which can be suitable for a quite range of genotypes.

Table II. Evaluation of Genotypes

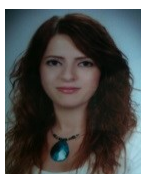
Media Codes	M1			M2			M3			M4			M5			M6			M7			M8		
	0701	0702	0703	0701	0702	0703	0701	0702	0703	0701	0702	0703	0701	0702	0703	0701	0702	0703	0701	0702	0703	0701	0702	0703
No. of Cultured Ovule/ Ovary	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
No. of Developed Ovule/ Ovary	15	9	17	3	5	10	8	15	15	10	10	15	17	12	17	16	9	15	5	8	17	5	17	17
Callus Formation (+/-)	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	+
No. of Embryo Formation	3	1	5	-	-	-	1	2	1	5	-	3	9	-	8	7	-	3	-	-	7	-	5	5
No. of Plantlet Formation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
No. of Abnormal Plantlet Formation	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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