International Journal of Agricultural and Natural Sciences
E-ISSN: 2651-3617 15(2): 212-220, 2022
Research Article

EFFECTS OF DIFFERENT PEPPER FRUIT TYPES ON NUMBER OF EMBRYOS TURNED INTO PLANT IN ANTHER CULTURE, EMBRYO FORMATION TIME AND HAPLOID / DOUBLE HAPLOID PLANT YIELD IN PEPPER

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(Received 23th June 2022; accepted 17th November 2022)

ABSTRACT. As it was in the other plants, there are various factors affecting the efficiency of *in vitro* anther culture used for "double haploidization" in pepper breeding. Among these factors, "genotype" is the leading factor. Pepper breeding companies need to know anther culture responds of different genotypes and plan accordingly in order to design their breeding programs. There are many different pepper fruit types, ranging from very small to large, and sweet to hot. It is imperative to predict for number of embryos that may turn into plants and the timing of the embryo formation in each pepper type. In present study, genotypes of California Wonder, Charleston, Bell, Jalapeno, Capia and Long green pepper types were used as plant material. The number of embryos (embryos/bud), the first embryo emergence time (days/genotype) and embryo emergence durations were determined in anther culture. The results of the study have the potential to shed light on the efficiency of anther culture technique integrated into pepper breeding programs and the relationships for response to anther culture among different pepper types.

Keywords: *Pepper, Double Haploid (DH) Technology, Tissue Culture*

INTRODUCTION

Pepper is one of the most important vegetables. It is produced intensively in the world, including Turkey. Turkey ranks third after China and Mexico for pepper production in the World [1]. Pepper is mostly produced in Asia with a share of 68%, nearly 2.625.000 tons in Turkey. Besides fresh consumption, it is used in various different forms including ground pepper, paste, roast, sauce, pickles and fresh in dishes [2].

Mexico and Central America, where *Capsicum annuum* shows a wide diversity, are known as the primary gene center of pepper; some parts of Southern and Central Europe, Africa, Asia and Latin America are known as the secondary gene centers [3]. The pepper belongs to the *Solanaceae* family and the cultivated species is *C. annuum* L. and there are 20-30 species in the genus *Capsicum*. *C. annuum, C. baccatum, C. pubescens, C. frutescens* and *C. chinense* species in the genus Capsicum have been economically cultured. Although majority of pepper species have 2n=24 chromosomes, it is possible to encounter 2n=48 chromosomes in wild peppers [4]. Capsicum genus has a large genome with 2C DNA coverage ranging from 7.65 pg/nucleus (*C. annuum*) to 9.72 pg/nucleus (*C. pubescens*) [5]. As comapred to tomato (*Solanum lycopersicum* L.) of the same family, pepper genome is 3-4 times larger [6]. Pepper is highly rich in nutritional

content. In 100g of fresh long green sweet pepper, there are 29 calories, 1.1 g protein, 0.2 g oil, 92.6 g water, 4.2 g carbohydrates and 1.4 g cellulose. Again, long green sweet peppers are rich in vitamins A, B1, B2, C and also contain P and K and alkaloids. The oil rate in pepper seeds is between 25-28% [7].

Pepper with several important characteristics also plays a significant role in plant breeding. Especially thanks to integration of anther culture technique into classical pepper breeding, significant advantages are gained both in domestic and international markets. Among the tissue culture techniques to shorten the classical breeding methods, haploidy technique has found a wide range of application in vegetable breeding.

As it was in the other plants, the "double haploidization" method, which is the method of obtaining 100% homozygous pure lines in the shortest time by doubling the chromosome numbers of haploid plants, is very important in pepper breeding [8]. This means that the parental lines of potential hybrids can be produced in a relatively shorter time. For the resistant pepper lines to be developed with this method, the crop can be protected from prevalent pest and diseases. Producers can produce uniform and high yielding varieties. However, with the inclusion of new pepper types in pepper breeding programs, a necessity for fruit type-based optimization of anther culture has arisen. It has been experienced that pepper anthers derived from different fruit types did not respond similarly to existing anther culture protocols.

Although there are many studies [9, 10, 11, 12, 13] about the efficacy of the anther culture of different pepper types/genotypes, most of them focused on the number of embryos obtained. However, studies on how many of the embryos turned into plants, and the timing of the first embryo emergence are quite limited. Therefore, objectives of the present study were to determine the effects of the criteria such as the number of embryos obtained from different types of pepper genotypes that are used extensively in commercial seed production (embryos/bud), the first embryo emergence times (days/genotype) and the duration of embryo formation on efficiency of anther culture. In this way, it is aimed to provide maximum benefit by type-based more efficient application of anther culture protocols available in literature. By revealing the responses of different pepper types to anther culture, results of the study are expected to shed light on possible modification of the protocols for each pepper type in the future.

MATERIALS AND METHOD

Experiments were conducted at the research long greenhouse of Areo Seed Company, Antalya, Turkey. As the plant materials; buds of 11 different pepper types including California Wonder (38 genotypes), Charleston (9 genotypes), bell pepper (29 genotypes), Jalapeno (17 genotypes), Capia (51 genotypes), Long green (7 genotypes) were used for anther culture.

The seeds of pepper genotypes were planted in 10 L pots containing peat and perlite (2:1 v/v) (Klasmann, Germany). Plants were irrigated with half-Hoagland solution, and the protection against diseases and pests was carried out similarly for all genotypes. The flower buds at the late uninucleate or early binucleate phase were collected in October and November, 2020 and checked by staining with acetocarmine to determine the suitable stage for anther culture. Flower buds were sterilized by 15% sodium hypochlorite solution including 1-2 drops of Tween 20 for 15 min, and then rinsed using sterile distilled water 3-4 times in the sterile bench. Then, the flower buds were dissected, the filaments were removed, and the anthers were placed on nutrient medium in 6 cm diameter glass Petri dishes using sterile forceps and scalpels. For each pepper genotypes, 150 anthers were plated. Cultured anthers were subjected to pretreatment by incubating at 35°C in the

dark for the first 2 d. Then, the anthers were transferred to the growth chamber at 25°C with 8 h dark and 16 h light photoperiod conditions. Embryos obtained from anthers were transferred to 15 cm glass tubes containing hormone-free MS nutrient medium. The anthers of all genotypes were used to determine the anther culture efficiency of different pepper genotypes. Treatments consisted of different number of anthers (15-85 anthers/genotype). Thus, anthers were cultured in MS media (15 $mg \cdot L^{-1}$ $AgNO_3$ + 4 $mg \cdot L^{-1}$ NAA + 0.5 $mg \cdot L^{-1}$ BAP + 100%MS + 30 g·L−1 sucrose) containing 0.25% activated charcoal (WAC medium). After observing the first embryogenesis in Petri dishes, the cultured anthers were transferred to the second medium. After the embryos appeared, they were transferred to hormone-free media for plant development [14]. The developed plants at 3-4 true-leaf stage were transferred to 3:1 peat:perlite media and grown in a plant growth chamber at 90% relative humidity for 1 week. Then, they were transferred to long greenhouse to obtain selfed seeds.

Following the anther culture of 151 pepper genotypes, the data were collected from the first to final embryo emergence days as well as the number of embryos at each embryo emergence day were determined.

RESULTS AND DISCUSSION

Statistical analyses revealed that first embryo emergence times varied among the pepper types. It was also observed that embryo emergence times varied with the fruit type/genotypes. In all pepper types, minimum (approximately) 15 days is required for the first embryo emergence. This time prolonged up to 85 d in Bell and California Wonder, 60 d in Capia, 35 d in Jalapeno and 25 d in Long green and Charleston. The first embryo emergence times of Charleston (mean 64.3 d), California Wonder (mean 64.78 d), Bell pepper (mean 63.7 days), Jalapeno (mean 61.5 days) and Capia (mean 64.6 d) varied between 62.7 - 65.1 days. The time was a few days shorter for Long green (mean 57.7 d) (Table 1).

Similar to differences in the first embryo emergence times in different pepper types, differences were also seen in the last embryo emergence times. For all pepper types, the time required to observe the last embryo emergence times ranged minimum between 36-51 days. Such a time prolonged up to 92 days in Long green type and the longest time was seen in California Wonder (111 days). While the average last emergence time was 85.1 days in California Wonder type, it was respectively followed by Bell pepper (79.1 d), Capia (74.2 d), Long green (70.7 d), Jalapeno (66.9 d) and Charleston (66 d). The greatest difference between the first and the last embryo emergence times was observed in California Wonder (20.3 days) and it was respectively followed by Bell pepper (15.4 d), Capia (9.5 d) and Jalapeno (5.5 d) and the least was observed in Charleston type (1.4 d) (Table 2).

The fruit type-based differences in the first and the last emergence times also revealed the differences in length of emergence times. It was observed that embryo emergence times continued until different observation days in different pepper types. While embryo emergence times continued maximum 2 observation days in Charleston and Jalapeno types, such a time prolonged up to 5 observation days in Capia. Charleston and Jalapeno, that were respectively followed by Long green (3 observation days), California Wonder and Bell pepper (4 observation days) (Table 1).

These differences in embryo emergence times also influenced the total number of embryos obtained in the long run. Despite the relative decrease in the number of embryos with the progress of observation periods, a total of 4235 anthers and 446 embryos (10.5%) were obtained from 151

different genotypes. While the lowest number of embryos (6.9%) were obtained from Charleston (mean 19.4 anthers/genotypes), the highest number of embryos (17.9%) were obtained from the Long green type with relatively lower number of anthers per genotype (mean 20 anthers/genotypes). While 13.1% embryos were obtained in California Wonder (mean 32.2 anthers/genotype), 8.7% in Bell pepper type (mean 31.7 anthers/genotype), 8.6% in Jalapeno (mean 23.8 anthers/genotype) and 9.7% in Capia (mean 26.9 anthers/genotype).

Table 1. *Effect of different pepper genotypes on number of embryos turned into plants and embryo emergence times*

G.N.	TYPE	NA	FD	NEFD	SD	NESD	TD	NETD	FOD	NEFOD	FID	NEFID	TNE	LD
CL1	Charleston	15	62	2									2	62
CL2	Charleston	20	73	1									1	73
CL3	Charleston	15	60	1									1	60
CL4	Charleston	25	58	1									1	58
CL5	Charleston	20	70	1									1	70
CL6	Charleston	20	98	1									1	98
CL7	Charleston	20	38	2									2	38
CL8	Charleston	20	58	1									1	58
CL9	Charleston	20	62	1	75	1							2	75
CW1	California Wonder	25	36	1	70	6	71	3	91	3			13	91
CW10	California Wonder	25	69	2			105	1					3	105
CW11	California Wonder	30	55	6	105								6	105
CW12	California Wonder	25	105	1									1	105
CW13	California Wonder	50	69	1	70	2	105	1					4	105
CW14	California Wonder	50	97	1	105	2							3	105
CW15	California Wonder	20	53	2	67	1	103	1					4	103
CW16	California Wonder	85	67	2									2	67
CW17	California Wonder	40	53	7	67	2	95	1	103	5			15	103
CW18	California Wonder	30	53	1	67	9	95	1	103	2			13	103
CW19	California Wonder	30	55	3	105	1							4	105
CW2	California Wonder	15	70	1									1	70
CW20	California Wonder	60	67	1									1	67
CW21	California Wonder	45	53	1	67	2							3	67
CW22	California Wonder	50	53	2	67	2	95	1	103	4			9	103
CW23	California Wonder	25	95	1	103	1							2	103
CW24	California Wonder	20	36	2	68	5	71	1					8	71
CW25	California Wonder	35	67	1									1	67
CW26	California Wonder	45	67	1									1	67
CW27	California Wonder	40	67	3									3	67
CW28	California Wonder	35	53	6	67	1	103	3					10	103
CW29	California Wonder	40	67	8	103	1	111	1					10	111
CW3	California Wonder	25	70	1									1	70
CW30	California Wonder	20	55	2									2	55
CW31	California Wonder	25	65	6									6	65
CW32	California Wonder	20	53	1	61	1	71	2	89	1			5	89
CW33	California Wonder	20	51	2									2	51
CW34	California Wonder	25	55	2									2	55
CW35	California Wonder	20	69	4									4	69
CW36	California Wonder	30	68	1									1	68
CW37	California Wonder	25	51	1	101	1							2	101
CW38	California Wonder	55	97	1									1	97
CW4	California Wonder	25	68	1									1	68
CW5	California Wonder	25	70	1									1	70
CW6	California Wonder	20	55	2	97	1	105	2					5	105
CW7	California Wonder	20	68	2									2	68
CW8	California Wonder	25	105	1									1	105
CW9	California Wonder	25	55	3	69	2	105	3					8	105
D1	Bell Pepper	15	70	1	71	1							2	71
D10	Bell Pepper	30	98	1									1	98
D11	Bell Pepper	25	36	1	48	1							2	48
D12	Bell Pepper	25	62	2									2	62
D13	Bell Pepper	30	98	1									1	98
D14	Bell Pepper	25	48	3	62	3	98	1					7	98

D15	Bell Pepper	25	62	1									1	62
D16	Bell Pepper	25	48	2	98	1							3	98
D17	Bell Pepper	20	98	1									1	98
D18	Bell Pepper	40	90	1									1	90
D19	Bell Pepper	30	48	1									1	48
D2	Bell Pepper	30	62	3									3	62
D20	Bell Pepper	40	48	1	98	1							2	98
D21	Bell Pepper	30	48	1	62	5							6	62
D22	Bell Pepper	30	68	2									2	68
D23	Bell Pepper	45	62	1	98	1							2	98
D24	Bell Pepper	15	62	1	76	1							2	76
D25	Bell Pepper	15	38	2	75	1	78	1					4	78
D26	Bell Pepper	15	68	1									1	68
D27	Bell Pepper	85	48	2	62	3	98	2					7	98
D28	Bell Pepper	40	88	1									1	88
D29	Bell Pepper	25	48	2	62	2	98	2					6	98
D3	Bell Pepper	25	77	2									2	77
D4	Bell Pepper	25	62	1									1	62
D5	Bell Pepper	60	62	1									1	62
D6	Bell Pepper	30	62	1									1	62
D7	Bell Pepper	35	62	6	92	1	98	1	106	1			9	106
D8	Bell Pepper	30	62	5									5	62
D9	Bell Pepper	55	62	2	98	1							3	98
J1	Jalapeno	30	39	1									1	39
J10	Jalapeno	15	65	1	80	3							4	80
J11	Jalapeno	15	67	1	101	1							2	101
J12	Jalapeno	35	60	1									1	60
J13	Jalapeno	20	62	2									2	62
J14	Jalapeno	25	67	1									1	67
J15	Jalapeno	25	39	1									1	39
J16	Jalapeno	30	108	1									1	108
J17	Jalapeno	30	67	1									1	67
J2	Jalapeno	20	67	1									1	67
J3	Jalapeno	25	67	1									1	67
J4	Jalapeno	25	80	1	96	3							4	96
J5	Jalapeno	25	39	1									1	39
J6	Jalapeno	35	80	2									2	80
J7	Jalapeno	15	39	1									1	39
J8	Jalapeno	15	39	5	67	5							10	67
J9	Jalapeno	20	60	1									1	60
KP1	Capia	20	49	3	62	1	79	1					5	79
KP10	Capia	20	82	1	92	1							2	92
KP11	Capia	20	46	1									1	46
KP12	Capia	30	32	1	36	1	46	3					5	46
KP13	Capia	20	54	2									2	54
KP14	Capia	30	54	1	76	1	82	4	92	1			7	92
KP15	Capia	30	75	1	81	6	88	6	91	1	109	2	16	109
KP16	Capia	25	82	3									3	82
KP17	Capia	40	46	1									1	46
KP18	Capia	30	82	1	92	2							3	92
KP19	Capia	40	32	1	36	1							2	36
KP2	Capia	35	84	1									1	84
KP20	Capia	20	40	2	81	2							4	81
KP21	Capia	20	46	8	82	2							10	82
KP22	Capia	25	40	1	75	2							3	75
KP23	Capia	20	75	1									1	75
KP24	Capia	25	40	1									1	40
KP25	Capia	25	74	1	75	1							2	75
KP26	Capia	30	82	1									1	82
KP27	Capia	25	40	1	75	2							3	75
KP28	Capia	20	82	1	88	1							2	88
KP29	Capia	25	75	1									1	75
KP3	Capia	25	84	1									1	84
KP30	Capia	25	60	1									1	60
KP31	Capia	15	60	1									1	60
KP32	Capia	20	60	4	80	1							5	80

KP33	Capia	20	66	3									3	66
KP34	Capia	15	67	1									1	67
KP35	Capia	25	66	1									1	66
KP36	Capia	20	66	3									3	66
KP37	Capia	25	67	2									2	67
KP38	Capia	20	66	1									1	66
KP39	Capia	30	91	1									1	91
KP4	Capia	20	46	1	82	2							3	82
KP40	Capia	35	55	8	99	1							9	99
KP41	Capia	60	85	2									2	85
KP42	Capia	35	55	1									1	55
KP43	Capia	40	55	1									1	55
KP44	Capia	25	55	1									1	55
KP45	Capia	35	55	2									2	55
KP46	Capia	30	85	1									1	85
KP47	Capia	25	85	1									1	85
KP48	Capia	35	55	1									1	55
KP49	Capia	25	95	1									1	95
KP5	Capia	25	82	1									1	82
KP50	Capia	20	62	1	78	1							2	78
KP51	Capia	25	67	1	94	1							2	94
KP6	Capia	40	46	1	76	1	89	1	92	1	95	1	5	95
KP7	Capia	30	82	2									2	82
KP8	Capia	20	92	1									1	92
KP9	Capia	30	75	1									1	75
S1	Long Green	25	36	4	70	7	71	1					12	71
S2	Long Green	20	36	1	70	1	70	3					5	70
S3	Long Green	15	70	1									1	70
S4	Long Green	25	40	1	76	2							3	76
S5	Long Green	20	40	2									2	40
S6	Long Green	20	76	1									1	76
S7	Long Green	15	92	1									1	92
	Max.	85	108	8	105	9	111	6	106	5	109	2	16	111
	Min.	15	32	1	36	1	46	1	89	1	95	1	1	36
	Mean	284	63,8	1,8	78,3	2,1	88,4	2	96,8	2,3	102	1,5	3	76,4
	Total	4235		262		115		47		19			446	

NA: Number of anthers, FD: First embryo emergence day, NEFD: Number of embryos at the first embryo emergence day, SD: Second embryo emergence day, NESD: Number of embryos at the second embryo emergence day, TD: Third embryo emergence day, NETD: Number of embryos at the third embryo emergence day, FOD: Fourth embryo emergence day, NEFOD: Number of embryos at the fourth embryo emergence day, FID: Fifth embryo emrgence day, NEFID: Number of embryos at fifth embryo emergence day, TNE: Total number of embryos, LD: Last embryo emergence day.

Table 2. *Effect of different pepper types on number of embryos turned into plants and embryo emergence times*

G.N.	TYPE		NA	FD	NE FD	SD	NE SD	TD	NE TD	FOD	NEFO D	FID	NE FID	TNE	LD
1	Charleston	Max.	25	98	2	75	1	0	0	0	0	0	0	2	98
		Min.	15	38	1	75	1	0	0	0	0	0	0	1	38
		Mean	19,44	64,33	1,3	75	1	0	0	0	0	0	0	1,4	65,77
		Total	175		11		1	0	0	0	0	0	0	12	
		Standard Deviation (SD)	30	15,98	0,44	0	0	0	0	0	0	0	0	0,5	16,33
2	California Wonder	Max.	85	105	8	105	9	111	3	103	5	0	0	15	111
		Min.	15	36	1	61	1	71	1	89	1	0	0	1	51
		Mean	32,24	64,78	2,4	81,3	2,6	94,5	1,7	97,3	3	0	0	4,4	85,10
		Total	1225		85		40		21		15	0	0	161	
		SD	14.41	16,44	1,9	17,95	2,23	14,46	0,87	7,15	1,58	0	0	3,86	19,42
3	Bell Pepper	Max.	85	98	6	98	5	98	2	106	1	0	0	9	106
		Min.	14	36	1	48	1	78	1	106	1	0	0	1	48
		Mean	31,72	63,69	1,8	76,5	1,9	92,3	1,4	106	1	0	0	2,9	79,10
		Total	920		50		22		7		1			80	
		SD	14,89	17,16	1,22	17,72	1,25	8,94	0,54	0	0	0	0	2,24	182
4	Jalapeno	Max.	35	108	5	101	5	0	0	0	0	0	0	10	108

		Min.	15	39	1	67	1	0	0	0	0	0	0	1	39
		Mean	23,82	61,47	1,5	85,3	3	0	0	0	0	0	0	2,4	66,94
		Total	405		23		12							35	
		SD	6,73	18,61	0,99	15,51	1,63	0	0	0	0	0	0	2,27	21,32
5	Capia	Max.	60	95	8	99	6	89	6	92	1	109	2	16	109
		Min.	15	32	1	36	1	46	1	91	1	95	1	1	36
		Mean	26,86	64,64	1,7	76	1,8	74,1	3,1	91,6	1	102	1,5	2,8	74,17
		Total	1370		82		30		14		3		3	133	
		SD	85	170	1,48	16,72	1,17	17,71	2,12	0,57	0	9,90	0,70	2,78	16,7
6	Long Green	Max.	25	92	4	76	7	71	3	0	0	0	0	12	92
		Min.	15	36	1	70	1	70	1	0	0	0	0	1	40
		Mean	20	57,71	1,8	72,4	3,6	70,5	2	0	0	0	0	4,2	70,71
		Total	140		11		10		4	0	0	0		25	
		SD	48	23,10	1,13	3,46	3,21	0,7	1,41	0	0	0	0	3,99	15,56

Pepper is a recalcitrant plant concerning the in vitro organ, tissue, and cell culture [15]. The in vitro growth of pepper is relatively slower than the other members of the Solanaceae family due to its high genotypic dependence and recalcitrant nature [12, 16, 17].

The present anther culture study was conducted to determine number of embryos (embryo/bud), the first embryo emergence times (days/genotype), and duration of embryo emergence of different pepper types. The pepper lines showed different responses to androgenesis. The results from this study are largely consistent with the outputs of previous studies using the anther culture technique. Similar with the present findings, it was also reported that many pepper genotypes were recalcitrant towards the formation of haploid regenerants and androgenesis induction [18, 19]. Furthermore, present findings were similar with the results of Haque and Ghosh [18] who demonstrated that ten cultivars of *Capsicum spp.* differed in response to regeneration. Results from a study of Mitykó et al. [9] and Rodeva et al. [10] showed that anthers of different lines, cultivars and hybrids differed greatly in their in vitro response. As a result of the study conducted by Shrestha and Kang [11] with 9 different hybrids of sweet pepper, it was determined that the success of anther culture varies depending on the genotype of the donor plant.

Seven Charleston, six Bell, eight Capia and seven long green pepper genotypes were tested to determine the effects of pepper types on obtaining spontaneous doubled haploid plants via anther culture. Seven different genotypes were used to determine the rate of spontaneous doubled haploidy for long green peppers. For each genotype, 300 anthers were examined. The highest number of plants obtained from anther culture was found to be 6.7 plants per 100 anthers in long green type 2. This genotype was followed by types -4, -5, -6, and -3 with 5.7, 5.3, 4, and 3.7 plants per 100 anthers, respectively. In Charleston pepper type, seven different genotypes were tested. The fewest plants were obtained from Charleston type-4 with 7 plants from 300 anthers; however, three of these plants were spontaneous doubled haploids. Number of plants per 100 anthers varied with the values of 6 (type-1), 4.7 (type-2), 7 (type-3), 2.3 (type-4), 6.7 (type-5), 6.7 (type-6), and 10.3 (type-7) depending on the genotype [8]. Previous studies, efficiency of anther culture was assessed over the number of embryos obtained and number of embryos turned into plant, but in present study, efficiency was assessed over the first embryo emergence day and embryo emergence periods.

Present findings revealed that embryo emergence durations varied with the pepper types and even with the genotypes. For instance, an average of 20.3 days should pass from the first embryo emergence to the last embryo emergence in California Wonder type, while this period was less than 20 days on average for the other types tested. This indicates that while the last embryo emergence was relatively shorter for one type, it may take longer for another type. Similarly, total

number of embryos to be obtained will increase in relation to time specific to that type and then efficiency of anther culture will also increase at the same rate. The results obtained by Irikova et al. [12] also emphasize that it is important to determine the growing period of anthers as well as the nutrient media for the effectiveness of pepper anthers culture.

CONCLUSION

In conclusion, although the total amount of embryos obtained varied with initial number of anthers obtained, different results were observed for total number of embryos obtained (%) in different pepper types. Such a case indicated that a single standard anther culture protocol did not produce the same efficacy in different pepper types and protocols should be optimized/modified on the basis of type/genotype. With this study, it was determined that the response times of the genotypes in the culture environment may be different, and emphasized that the genotypes need optimization on the basis of time to get the most optimum result when planning the studies.

Consent to Publish (Ethics)

In this study, data belonging to Feride Ardic's unpublished master's study titled “Farklı Biber Tiplerinin Anter Kültüründe Bitkiye Dönüşen Embriyo Sayısı, Embriyo Oluşum Zamanı ve Spontan Double Haploid Oranına Etkilerinin Belirlenmesi” were used.

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