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The Effect Of Different 2,4-Dichlorophenoxyacetic Acid Doses On Chromosomal Structure Of Regenerants In Barley (*Hordeum vulgare* L.) Embryo Culture

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ABSTRACT

In this study, as a plant material, two-row barley (*Hordeum vulgare* L.) cultivars called 'Bülbül-89' and 'Tarm-92', which have great importance in Turkey's agricultural production, and as a synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) were used. This research, employed 2,4-D as an auxin for callus induction for mature barley embryos in different doses. In both cultivars, the highest values of callus induction, callus weight, regeneration capacity and culture efficiency were established when 3 mg l^{-1} 2,4-D auxin was used. In the cytological analysis of the root tips of regenerated plantlets, at 3 mg l^{-1} 2,4-D, no physical and numerical abnormality were observed in chromosomes, therefore the doses in question can be used confidently.

Keywords: *Hordeum*, callus, chromosome number, aneuploidy, mature embryo

INTRODUCTION

Barley is the most produced grain after wheat, corn and rice. Barley is an important type of fodder and its sustenance is 95% compared to corn. It is also an important raw material for the beer industry.

The increasing population, faulty agricultural activities and decreased productivity caused by erosion has resulted in production area to shrink, thus necessitates an increase in its unit area production. Therefore it is important to create plant breeding programs in order to produce high yielding, high quality plants that are resistant to biotic and abiotic stress factors.

Plant breeding programs today are conducted as classical or biotechnological techniques. It is widely known that classical breeding programs take many years to conduct and they require a high work force, therefore studies on biotechnological breeding programs have increased. Using biotechnological breeding programs, especially to compensate for the problems that are caused by classical breeding programs, will help to create new breeds [13].

The first necessity to be able to successfully apply biotechnological techniques is tissue culture. The main goal of acquiring tissue culture is to ensure healthy, high levels of plant regeneration and rapid multiplication. 2,4-D and many other chemicals have been used widely on field crops plant tissue culture in order to attain what has been mentioned above. 2,4-D is known to cause genetic changes in regenerative plants, especially chromosomal deviations, or in other words somaclonal variations, in plants regenerating from the callus [2,4].

Although there are many reports [1,3,8,9,10] on the commonly used herbicide 2,4-D's effect on cytological changes on field crops, there is very little research on the effect the substance has on regenerative plants gathered under *in vitro* conditions [15,18].

The aim of this research is to determine the 2,4-

D dose that best allows the highest rate of callus growth and plant regeneration in mature embryo cultures of the most widely used barley cultivars in Turkey 'Bülbül-89' and 'Tarm-92' and to see if there is any abnormality in the physical structure and number of chromosomes, when subjected to the determined dose.

MATERIAL AND METHODS

This research was conducted in Ankara University, Agriculture Faculty, Department of Field Crops Biotechnology Laboratory. In this experiment 'Bülbül-89' and 'Tarm-92' barley genotypes' mature embryos were subjected to four different doses of 2,4-D (3, 6, 9, 12 mg l^{-1}). The research was conducted in two phases, *in vitro* and cytological.

The mature seeds were stirred for 5 min in 70% (v/v) alcohol for surface sterilization and then rinsed 3 times with sterile distilled water. Later, the seeds were stirred in a 5% sodium hypochlorite (NaClO) solution for 30 min and then rinsed 7 times with sterile distilled water. The surface sterilized seeds were then imbibed in sterile water 33 ^{o}C for 2 h, allowing to easily separate the mature embryos from their endosperms. The embryos were placed with the scutellum upwards on a solid MS [11] medium in sterile Petri dishes containing 7 g l^{-1} agar and were subjected to 20 g l^{-1} sucrose and at different doses of 2,4-D (0, 3, 6, 9, 12 mg l^{-1}) they were left at 25±1 ^{o}C in the dark for 14 days for callus growth. At the end of the 14 days in order to initiate the development of the calli, roots and shoots, they were put in a hormone-free MS medium and kept at 25±1^{o}C for 16 h of light (27 μmol m^{-2} s^{-1}) darkness photoperiod conditions, for 4 weeks [14].

In order for the regenerated calli to develop better and so that the root count, that was going to be subjected to cytological examination increases, they were put in jars with the same culture medium. They were kept in the same photoperiod conditions for 4 weeks. During this period the regenerated calli numbers were counted and the regeneration capacity and culture efficiency parameters were designated.

A mitotic chromosome count was performed on root tip samples of regenerative plants that were subjected to the 2,4-D dose that produced the highest amount of callus numbers and regeneration in embryo cultures of 'Bülbül-89' and 'Tarm-92' barley genotypes.

Root tip samples were left in sterile water containing 6 ml l^{-1} α-monobromo-napthalene at +4 ^{o}C for 4 h, and then fixated for 30 min via glacial acetic acid. After being washed with distilled water for a few times the roots were put in a 1 N HC1 60 ^{o}C hot water bath for 10 min, then were stained with Feulgen stain for 2 h at room temperature, later to prepare the root tip samples by dropping acetic acid on them [12].

A completely randomized design with three replications per dose was used. Petri dishes containing 10 embryos were considered the units of replication. The collected data's statistical analysis was done through MSTAT-C and SPSS 20 programs, comparatively. An LSD test was conducted to specify differences between doses [17].

RESULTS AND DISCUSSION

The parameters of callus induction (%), callus weight (g), regeneration capacity (%) and culture efficiency (%) were determined. The callus induction (%) was measured by comparing the callus development in each Petri dish and the total number of embryos by the14th day. The callus weight (g) was measured by weighing the calli developed on the embryos on by the 14th day. The regeneration capacity (%) was measured by comparing the regenerated calli with the number of calli, while the culture efficiency (%) was measured by comparing the regenerated calli with the cultivated embryos. Chromosomes taken from root tips were evaluated in number and physical structure through cytological studies.

In Bülbül-89, the usage of different doses of 2,4-D was statistically significant ($P<0.05$) concerning callus development and callus weight. The LSD test used to determine dosage differences showed that the callus development of the Bülbül-89 formed 2 groups at 5%. The control group's (0 g l^{-1}) callus induction was at

93.3% whereas at different doses it went up to 100%. The callus weight formed into 4 different groups at 5 %. The heaviest callus weight was 0.091 g with a 3 mg l^{-1} 2,4-D dose, and the lightest callus weight was 0.404 g at a dose of 0 (Table 1).

Significant differences were observed in the calli of 'Tarm-92' at different doses ($P<0.01$). Accordingly, it formed 2 different groups at 5% level. Callus induction was the lowest with 0 and 12 mg l^{-1} doses at 83% whereas with a 3 mg l^{-1} callus induction was the highest at 100%. The callus weight parameter showed that the differences between doses were significant at $P<0.01$ and that they formed 4 different groups at 5%. Callus weight changed between 0.310 (0 mg l^{-1}) and 0.746 (3 mg l^{-1}) (Table 1).

The regenerated calli were counted 4 weeks after they were put in the regeneration media. 'Bülbül-89' showed significant different regeneration capacities at different doses of 2,4-D ($P<0.05$). Accordingly, 4 different groups formed at 5 %. Regeneration capacity was highest at 93.3% with a 3 mg l^{-1} 2,4-D dose whereas it was the lowest with a 12 mg l^{-1} dose at 46.7%. When considering the culture impact there was a significant difference at 5% level. A 3 mg l^{-1} dose showed the highest at 96.7% whereas a 12 mg l^{-1} dose showed the lowest at 46.7% (Table 1).

'Tarm-92' showed significant different regeneration capacities at different doses of 2,4-D ($P<0.05$). Doses formed 3 different groups. A 3 mg l^{-1} dose was the highest at 90% whereas a 12 mg l^{-1} dose was the lowest at 51.8%. The differences of doses were considered as important on culture impact ($P<0.01$). A 3 mg l^{-1} dose showed the highest at 90% whereas a 12 mg l^{-1} dose showed the lowest at 43.3% (Table 1).

Root samples taken from regenerative plantlets obtained from calli developed on mature embryos cultivated from species given a 0 and 3 mg l^{-1} dose of 2,4-D were subjected to cytological studies during the mitotic metaphase-1 period. These studies showed that there were no anomalies in chromosome numbers, that all chromosome numbers in the samples were type-specific ($2n=2x=14$) and that there were no physical changes. (Figure 1 and Figure 2).

Ziauddin et al. [13] has stated that plant tissue chromosome anomalies have increased with 2,4-D intensity and culture time length, under tissue culture conditions. Özgen et al. [13] used 8 mg l^{1} 2,4-D in winter bread wheat for callus

Table 1: Effect of different 2,4-D doses to tissue culture parameters in winter barley genotypes

Genotype and Doses (mg l^{-1})	Callus Induction (%)	Callus Weight (g)	Regeneration Capacity (%)[1]	Culture Efficiency (%)[2]
'Bülbül-89'				
0	93.3 b	0.404 d	56.5 b	46.7 c
3	100.0 a	0.901 a	90.0 a	90.0 a
6	100.0 a	0.776 b	82.9 a	80.0 ab
9	100.0 a	0.660 c	69.2 ab	66.7 b
12	100.0 a	0.604 c	51.8 b	43.3 c
0.05 LSD	4.6	0.103	22.3	17.7
'Tarm-92'				
0	83.3 b	0.310 d	74.0 ab	70.0 abc
3	100.0 a	0.746 a	93.3 a	96.7 a
6	96.0 a	0.660 b	80.0 a	80.0 ab
9	96.0 a	0.643 b	55.3 bc	53.3 bc
12	83.0 b	0.553 c	46.7 c	46.7 c
0.05 LSD	10.0	0.084	25.6	27.5

[a-c] Means followed by the same letter are not significantly different at P=0,05

[1] No. of regenerable cali/No. of cali induced x 100

[2] No. of regenerable cali/No. of embryos cultured x 100
(regenerable callus = nodular callus with green spot)

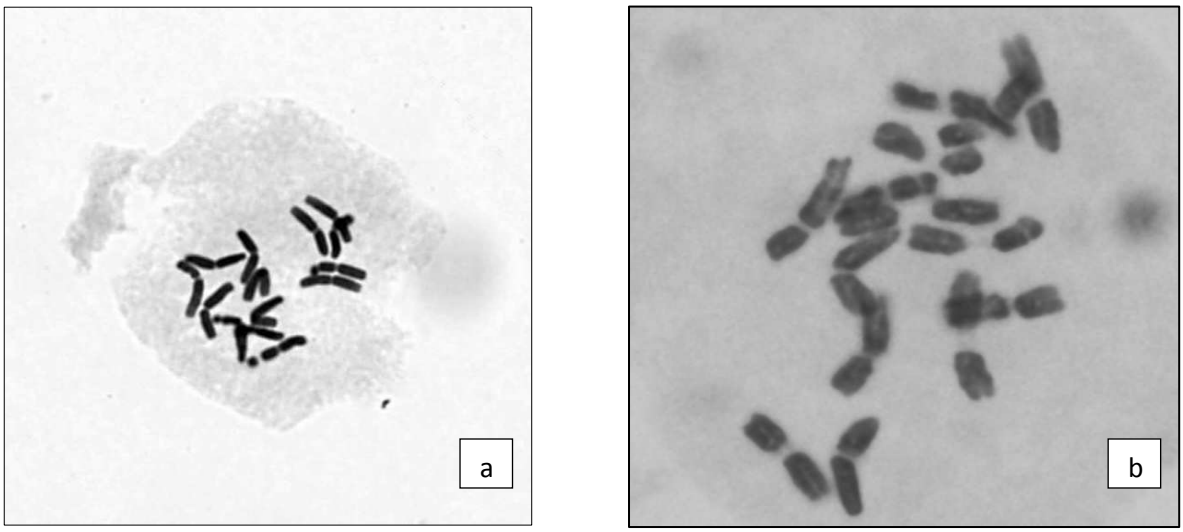

Figure 1: Mitotic metaphase-1 in 'Bülbül 89' (2n=2x=14) (a: Control group, b: 3 mg/l 2,4-D dose)

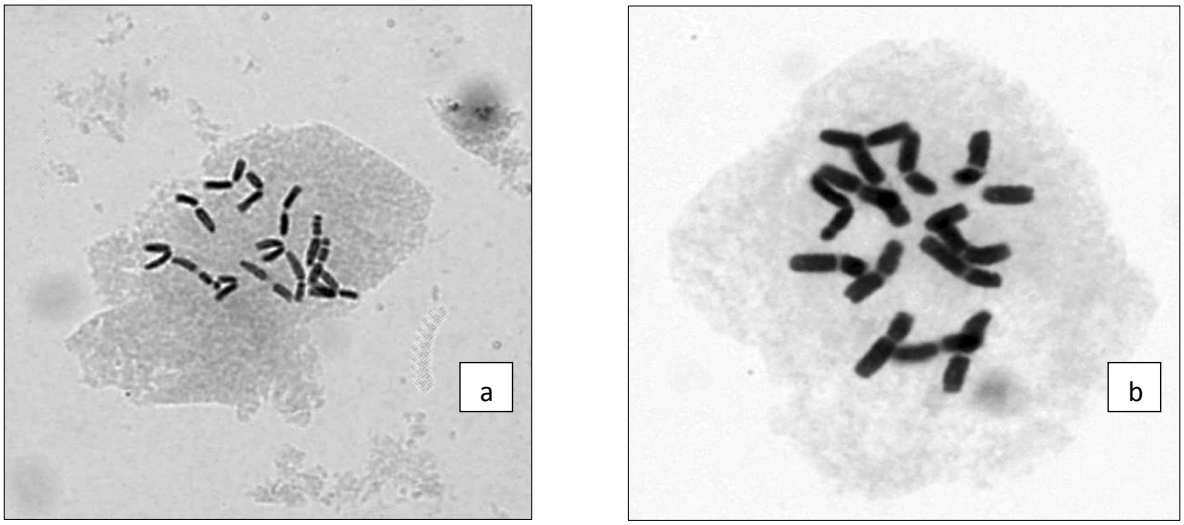

Figure 2: Mitotic metaphase-1 in 'Tarm 92' (2n=2x=14) (a: Control group, b: 3 mg/l 2,4-D dose)

development. After said researches, it was concluded that wheat species had 2n=6x=42 somatic chromosomes. Preztakiewicz et al. [16] used three different auxin types (2,4-D, dicamba, picloram) at 3 mg 1^{-1} doses and their combinations to observe their effects on immature embryos of wheat, barley and triticale to observe callus induction and plant regeneration. The research showed that wheat genotypes showed the highest callus induction rate when 2,4-D or dicamba was utilized into the culture medium by themselves. Zapata [19] states that an increased dose of 2,4-D in mature wheat embryo decreases callus induction and regeneration. Choi et al. [5,6] states that their research on wheat and oat transgenic callus cell karyotype anomalies have shown to be much more than non-transgenic calli cell anomalies. Truta [18] subjected wheat seeds to a pretreatment of different solutions that contained 2,4-D and observed that there were chromosome anomalies in both the primary

seedling phase and in cell division. In conclusion, the research showed that that chromosomal changes caused solely by 2,4-D were statistically insignificant. Doğan [7] used the growth regulator auxins 2,4-D and picloram to determine the callus development and callus formation in durum wheat by using the mature embryo culture method. Doğan [7] concluded that the highest callus formation and callus weight was caused by a 3 mg l^{-1} dose of 2,4-D and picloram. Cytological studies showed that there were no changes in the chromosomal structure. Patel and Patel [15] studied the sugar cane's leaf and stem, cultivating them with different doses of 2,4-D and observed their chromosome anomalies in callus cells and reported that increased doses of 2,4-D caused chromosomal deviations.

CONCLUSION

The study performed on barley tissue culture shows that 3 mg l^{-1} doses of 2,4-D do not cause chromosomal anomalies while attaining the highest tissue culture parameters.

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