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EFFECT OF 2,4-DICHLORO-PHENOXYACETIC ACID ON BARLEY ANTHER CULTURE IN LIQUID AND AGAR MEDIA

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ABSTRACT

Anthers from two cultivars of barley (*Hordeum vulgare* L.), Chernigovski 5 and Dneprovski 435, were cultured on N_6 liquid and semisolid agar media with three different levels of 2,4-D (2.0, 4.0 and 6.0 mg/litre) and 0.5 mg/litre kinetin. Significant results were obtained for androgenic calli and embryoid production with increased 2,4-D level, especially in liquid medium preconditioned with isolated earheads. As a result, number of plantlets was also high although not much difference was observed among media supplemented with three levels of 2,4-D for the regeneration of green plantlets. Increased 2,4-D produced more androgenic calli than embryoids. The plantlets regenerated were mostly dihaploids (2n = 2x = 14) and ranged from 65.7% to 84.6% for both the cultivars.

Key words: *Hordeum vulgare*, 2,4-D, androgenic calli, embryoids, dihaploids.

Androgenesis in vitro enables to produce completely homozygous plants from microspores of a heterozygous population in one step. For anther culture, families Gramineae and Cruciferae generally require growth hormones, whereas plants of the family Solanaceae do not necessarily need these for androgenic calli production [1]. For anther and isolated microspore culture of cereals, three media are widely used: modified MS [2], N_6 [3] and potato II [4]. All three media contain combination of one auxin and one cytokinin at low levels. Liquid media preconditioned with isolated ovaries emplôyed for anther culture have also been supplemented with these hormones at low levels [5–7]. Use of 2,4-D at higher levels up to 8 mg/litre in Ficoll media is reported by Marsolais and Kasha [8] with increased androgenic response of barley anthers in culture.

In the investigation reported, combination of three levels of 2,4-D (2.0, 4.0 and 6.0 mg/litre) with 0.5 mg/litre kinetin was tested in semisolid agar and preconditioned liquid

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N_6 media for anther culture of barley.

MATERIALS AND METHODS

Two barley cultivars were used for anther culture: Chernigovski 5 and Dneprovski 435. The plants were raised in glasshouse with 16 h light and temperature range of 17–22°C. Tillers were harvested soon after the emergence of flag leaf ligule and stored for 6–10 days at 5 ± 1°C with their base dipped in plain water. The spikes enclosed in leaf sheath were surface sterilized with 70% ethanol before isolating anthers. After microscopic examination, anthers from only such spikes were selected for culturing which had middle to late stage of microspore development.

Three different levels of 2,4-D, viz. 2.0, 4.0 and 6.0 mg/litre and a constant quantity of 0.5 mg/litre kinetin were added to the N_6 basal medium in semisolid and liquid states. For preparation of semisolid media different combination of hormones were incorporated with 100 mg/litre meso-inositol, 90 g/litre sucrose and 7.5 g/litre agar. In the liquid media, the quantity of meso- inositol was increased to 1000 mg/litre and agar was not added but it was preconditioned with isolated earheads at mature pollen stage by taking 5 earheads per 50 ml medium and incubated in dark at 26°C for 5 days.

For culturing, 60 mm diameter glass Petri dishes for agar media and 40 mm diameter presterilized plastic Petri dishes for liquid media were employed. On agar media, 60 anthers per dish and in liquid media, 30 anthers per dish were cultured. In order to minimize variation within an earhead, anthers from a single floret were always distributed in media with three levels of 2,4- D. About 5800 (on an average 960) anthers per 2,4-D level per cultivar were cultured on agar media and 3400 (on an average 560) anthers per 2,4-D level per cultivar in liquid medium. The cultured dishes were incubated in dark at 26°C for 28 to 35 days.

The induced calli and embryoids attaining a size of about 2-3 mm were transferred for regeneration in MS medium [9] supplemented with 0.4 mg/litre benzyladenine (BA), 0.4 mg/litre indole-3-acetic (IAA), 20 g/litre sucrose, and 8 g/litre agar. The cultures were kept under light (fluorescent, 5000 lux intensity) and dark cycle 16/8 h at 20–22°C.

For cytological analysis, root tips of regenerated plantlets were fixed in Carnoy's solution (1 : 3, acetic acid : ethanol) and stored in refrigerator at +5°C. Root tips stained with Feulgen stain were squashed in 45% acetic acid (v/v).

RESULTS AND DISCUSSION

The effect of different levels of 2,4-D on the formation of androgenic structures (embyroids + calli) and regeneration of plantlets from anther culture of two barley cultivars

in agar and liquid media can be seen from the data presented in Table 1. There was gradual increase in the number of androgenic structures with increase in the level of 2,4-D, but no significant difference was found in the formation of green plants. Preconditioned liquid medium was relatively more effective for in vitro androgenesis than the agar medium. Amongst the two cultivars of barley, Chernigovski 5 showed more androgenic potentiality in both liquid and agar media. There was not much difference in plantlet regeneration potentiality of the androgenic structures induced at different levels of 2,4-D. This regeneration capability was higher in Dneprovski 435 but the total plantlets produced were more in Chernigovski 5 because of production of a higher number of androgenic structures.

Table 1. Effect of 2,4-D concentration (mg/litre) on the formation of androgenic structures (embryoids + calli) and regeneration of plantlets in anther culture of two barley cultivars

Medium	Androgenic structures per 100 cultured anthers			Plantlets per 100 cultured anthers			Green plantlets per 100 cultured anthers		
	2.0	4.0	6.0	2.0	4.0	6.0	2.0	4.0	6.0
Cv. Chernigovski 5									
Semisolid	38.0^{a} ±1.52	46.4^{b} ±2.68	46.4^{b} ±2.22	17.9^{a} ±1.04	22.1^{ab} ±1.90	25.1^{b} ±1.63	2.55^{a} ±0.39	2.78^{a} ±0.67	2.99^{a} ±0.56
Liquid	156.0^{a} ±5.06	169.0^{a} ±5.66	253.0^{b} ±6.84	46.5^{a} ±2.77	52.2^{a} ±3.15	64.1^{b} ±3.44	4.61^{a} ±0.87	4.36^{a} ±0.91	6.01^{a} ±1.06
Cv. Dneprovski 435									
Semisolid	3.98^{a} ±0.59	4.87^{a} ±0.91	10.6^{b} ±1.12	3.12^{a} ±0.53	3.53^{a} ±0.77	8.08^{b} ±0.98	1.16^{a} ±0.32	2.18^{ab} ±0.61	3.32^{b} ±0.63
Liquid	55.0^{a} ±2.88	59.9^{a} ±3.63	78.0^{b} ±3.39	27.0^{a} ±2.02	27.8^{a} ±2.47	32.6^{a} ±2.20	1.06^{a} ±0.40	3.52^{b} ±0.88	3.39^{b} ±0.71

a–b) Data at the 2,4-D levels denoted by the same letters not significantly different ($P < 0.05$).

Table 2 shows that more calli tend to form with the increased levels of 2,4-D, especially in liquid medium where nearly 6 and 10 times increase was observed for cvs. Chernigovski 5 and Dneprovski 435, respectively. Although at lower levels of 2,4-D (2.0 mg/litre) less androgenic structures were produced, but among them embryoids were more and the calli produced were compact and embryogenic (Fig. 1: 1, 2). At higher level of 2,4-D (6.0 mg/litre), where higher production of androgenic structures was observed, the ratio of embryoids declined sharply and many calli produced were loose and nonembryogenic (Fig. 1: 3, 4). These results are in agreement with earlier reports that higher level of 2,4-D induces loose, nonembryogenic calli from anther culture in Ficoll media [8, 10].

Cytological analysis (Table 3) showed that the regenerated plantlets were mostly dihaploids ($2n = 2x = 14$). Similar results have been obtained by other workers [11–14]. The results obtained showed that the ploidy level of regenerated androgenic plants was generally not affected by 2,4-D level in the medium.

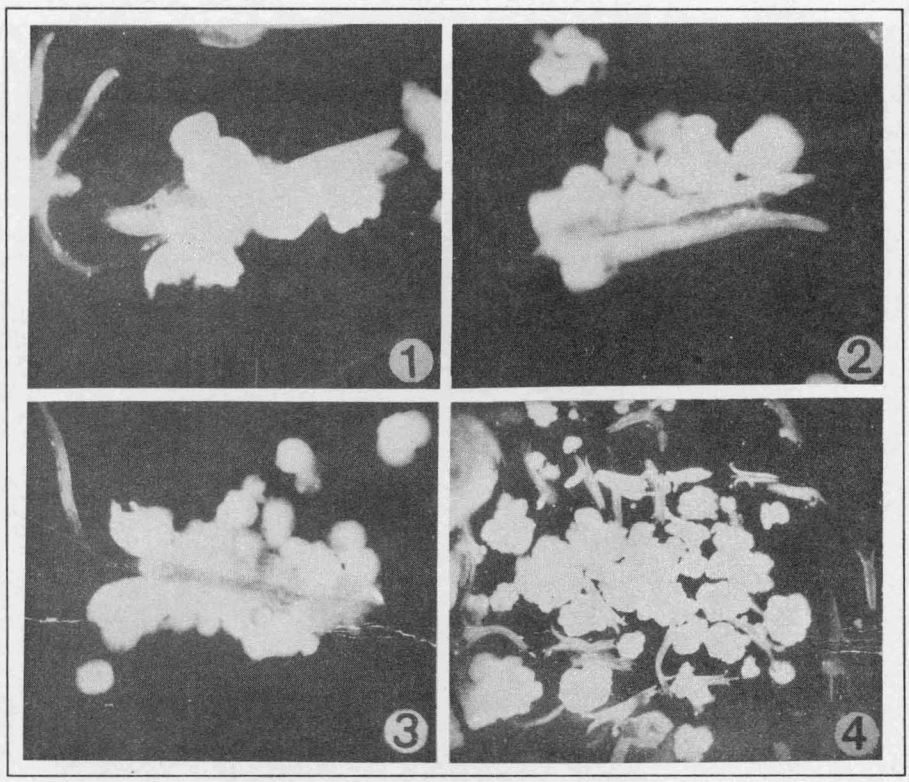

Fig. 1. Androgenic structures produced in two barley varieties the media containing 2.0 (1, 2) and 6.0 mg/litre (3, 4) 2, 4–D.

Both the cultivars under glasshouse conditions produced 96-98% microspores which were capable of germinating into fertile pollen grains. However, it is necessary that the microspores should switch on from gametophytic to sporophytic pathway during androgenesis if good results are to be obtained. The present study shows that addition of 2,4-D at higher level can be one of the factors for changing the pathway, since the number of androgenic structures increases

Table 2. Total number and ratio of embryoids : calli per 100 cultured anthers of two barley cultivars at different 2,4-D concentrations (mg/litre)

Medium	No. and ratio of embryoids : calli		
	2.0	4.0	6.0
	Cv. Chernigovski 5		
Semisolid	27.5 : 10.4 (2.63 : 1)	29.9 : 16.7 (1.81 : 1)	22.0 : 24.4 (1 : 1.11)
Liquid	40.6 : 115.3 (1 : 2.83)	34.5 : 134.3 (1 : 3.89)	34.4 : 218.5 (1 : 6.35)
	Cv. Dneprovski 435		
Semisolid	3.30 : 0.62 (5.29 : 1)	2.27 : 2.30 (1.23 : 1)	5.6 : 5.0 (1.12 : 1)
Liquid	24.6 : 30.7 (1 : 1.26)	11.4 : 48.5 (1 : 4.23)	6.9 : 71.0 (1 : 10.2)

to an appreciable degree while regeneration of green plantlets is not affected. To overcome this drawback, the two-step culture system may be employed. The anthers should be cultured in medium with higher level of 2,4-D for 10-15 days and then the level of hormone can be lowered.

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Table 3. Cytological analysis of the plantlets regenerated from anther culture of two barley cultivars at three concentrations of 2,4-D

Concentration of 2, 4-D (mg/litre)	Total No. of plantlets studied	Plantlets with different ploidy levels (%)			
		n	2n	4n	other*
Cv. Chernigovski 5					
2.0	268	12.7	76.1	7.8	3.4
4.0	180	6.1	74.4	10.0	9.4
6.0	143	7.0	84.6	4.2	4.2
Cv. Dneprovski 435					
2.0	105	20.0	65.7	11.4	2.9
4.0	58	8.6	72.4	6.9	12.1
6.0	88	15.9	68.2	13.6	2.3

*Includes 3n, 6n, 8n, and aneuploids.

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