PRODUCTION OF WINTER BARLEY HAPLOIDS BY *BULBOSUM* SYSTEM. 2. INFLUENCE OF BARLEY GENOTYPE ON *IN VITRO* HAPLOID REGENERATION

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ABSTRACT

Haploid induction in barley using biotechnological *bulbosum* system is influenced by numerous genetic and environmental factors. Thus, the reaction to *in vitro* culture, a compulsory stage of plant regeneration from haploid immature embryos, depends on: (I) the action of some specific genes, (ii) the interaction of these genes with the genome and (iii) the culture nutritive media, particularly their content in plant growth regulators (PGR). It is known that the auxine 2,4-D (2,4 - dichlorophenoxy acetic acid) regulates the initiation and development of embryogenesis, induces the callusing process (in larger concentrations), interfering in *in vitro* embryo growing and differentiation and in successive plant regeneration. Exogenous PGRs are applied in two essential stages of the *bulbosum* system: *in vivo* treatment, immediately after pollination and *in vitro* culture of haploid embryos. The paper presents the influence of genotype of winter barley on the response of haploid embryos to *in vitro* culture within *bulbosum* system. Combined treatments with GA3 (75 ppm) + 2,4-D (10 ppm, 20 ppm and 50 ppm), were applied in different ratios (1: 1; 4:1, 9:1), immediately after pollination. For *in vitro* culture of the haploid immature embryos, four nutritive media with different PGR contents (modified Gamborg, B II Norstog and modified variants of B5 and MS 1/2 media) were utilized. *In vitro* embryo germination and plant regeneration were estimated by several parameters: EC/E (cultivated embryos/100 developed embryos), EG/EC (germinated embryos/100 cultivated embryos), EG /EUND (germinated embryos/100 undifferentiated embryos), P/EC (regenerated plants/100 cultivated embryos) and P/EG (regenerated plants/100 germinated embryos). The results showed significant (χ^2) differences between the two cultivated types of barley, six - and two-rowed, as well as among the genotypes within each form concerning their reaction to *in vitro* culture of haploid embryos. Larger concentration of 2,4-D (50 ppm), applied *in vivo* affected negatively the seed development and embryo differentiation.

Key words: *in vitro*, haploid embryo germination, plant regeneration, *H. bulbosum, Hordeum vulgare*

INTRODUCTION

The reaction to *in vitro* culture of different crop species depends on the action of specific genes, the interaction of these genes with the respective genome and the nutritive media, particularly their content in plant growth regulators (PGR) (Lazar et al., 1983).

It is known that the auxine 2,4 - dichlorophenoxy acetic acid (2,4-D) regulates the initiation and development of embryogenesis, induces *in vitro* callusing process at higher concentration, interfering in embryo growing and differentiation and in plant regeneration.

Many studies have proved that the genes controlling *in vitro* initiation and development of the callus from wheat immature embryos are located on homoeologous chromosomes of the groups 2 and 4 (Mathias and Fukui, 1986; Ben Amer et al., 1992; Kaleikau et al., 1989).

Ganeva et al., (1995 a, b) studied *in vitro* effect of the barley (*Hordeum vulgare* L.) chromosomes 2H and 4H added to the wheat (*Triticum aestivum* L.) genome and of wheat chromosomes from the homoeologous groups 2 and 4 on immature embryo germination, callus formation and somatic embryogenesis. Overdoses of wheat chromosomes from the groups 2 and 4 (Chinese Spring tetrasomic lines) and addition of homoeologous barley chromosomes 2H and 4H (Chinese Spring / Betzes addition lines) modified significantly, depending on 2,4-D concentration, the rate of wheat immature embryo germination, somatic embryogenesis and plant regeneration. The influence of barley chromosome 2H and 4H on the response to *in vitro* culture was relatively differentiated and specific, much more connected to 2,4-D concentration of the nutritive media as compared to the homoeologous wheat chromosomes (Jensen and Linde-Laursen, 1992; Linde-Laursen et al., 1995; Pedersen et al., 1996).

Displaying of such differences demonstrated the presence of specific genes in barley genome for controlling the process switched on by *in vitro* immature embryo culture.

There is a well known hypothesis concerning a strong correlation between the genotypic reaction of plant species to the PGR exogenous application and its specific endogenous PGR contents (Mathias et al., 1988).

Recent data confirmed indirectly this hypothesis: in barley Betzes variety (donor genome in wheat / barley addition lines) the PGR endogenous content in colleoptile is two times higher than in Chinese Spring (receptor genome) (Ganeva et al., 1995a). It seems that

the PGR endogenous content of barley genotypes determines a stronger intensity of reaction to the exogenous auxine supply.

Mathias and Atkinson (1988) suggested that the genes controlling the plant response to 2,4-D content of the culture media are implied in the regulation of PGR metabolism, and thus, determining a specific reaction to the exogenous auxin application.

The implication of the wheat chromosomes from homoeologous group 4 in the reaction to *in vitro* culture is also suggested by the location of some *Rht* genes (reduced height) and *D* genes (grass clump dwarfness) on these chromosomes which are implied in PGR metabolism (Gale and Youseffian, 1985).

Pickering (1983) and Pickering and Devaux (1992) made comprehensive studies concerning the influence of parental genotypes on haploid induction and doubled haploid lines (DH) production in barley. The authors showed that both parental genotypes, *H. vulgare* and *H. bulbosum* affected significantly seed formation, seed quality and the degree of haploid embryo differentiation. The reaction *in vitro* culture is preponderently controlled by female parent, strongly related to the PGR action.

In *bulbosum* system, PGR are applied exogenously in two essential stages: (I) *in vivo* treatment immediately after pollination (for embryo development and differentiation in the absence of the endosperm) and (ii) *in vitro* culture of immature haploid embryos (for germination and plant regeneration) (Mihăilescu et al., 1993, 1994, 1995, 1996).

This paper presents the genotypic differences of two - and six-rowed Romanian barleys regarding their reaction to *in vitro* culture of immature haploid embyos produced by *H. vulgare* x *H. bulbosum* crosses.

MATERIALS AND METHODS

A number of 8 Romanian varieties and lines of six-rowed barley (Miraj, Productiv, Precoce, Dana, Adi, Mădălin, F 208/88 and F 503/88) and 8 varieties and lines of two-rowed barley (Victoria, Grivița, Laura, Andra, F 1116/88, F 1167/89, F 1233/89 and F 2484/87) were crossed to four diploid cytotypes of *H. bulbosum*: the clones PBI- R 33 (Hb1) and Cb 2920/4 (Hb2) and two reciprocal hybrid selections from Cb 2929/1 x Cb 2920/4 (Hb3) and respectively Cb 2920/4 x Cb 2929/1 (Hb4).

The crosses were made in 1995 in both greenhouse (March 2 - May 9) and field (May 15 - June 19).

The detailed protocols used for the main stages of *bulbosum* system released by Research Institute for Cereals and Industrial Crops - Fundulea were described in previous papers (Mihăilescu et al., 1993, 1994, 1995, 1996).

Optimal *in vivo* PGR treatment variants, selected in our previous studies (Mihăilescu et al., 1994) were used: V4 (GA3 + 2,4-D, 75 ppm : 10 ppm, 1:1) and V7 (GA3 + 2,4-D, 75 ppm : 20 ppm, 4:1) for greenhouse conditions and V8 (GA3 + 2,4-D, 75 ppm : 20 ppm, 9:1) under field conditions. The treatment variant, V9 (GA3 + 2,4-D, 75 ppm : 50 ppm, 1:1), proposed by Pickering and Wallance (1994) was used under greenhouse conditions. *In vivo* PGR applications were made by dropping into the florets at 24 hours after pollination, followed by spike spraying after 48 hours.

Seeds for dissection were harvested at 12-14 days after pollination in greenhouse and 10-12 days in field (depending on the outside temperature).

Immature haploid embryos were cultivated on specific nutritive media in accordance with the differentiation stage as shown in table 1.

Differentiated embryos had a definite morphology, a size larger than 0.75 mm and a well developed scutellum, while undifferentiated embryos were variable in morphology, with frequently cuneiform aspect, flat, smaller than 0.75 mm and without scutellum primordium. The undifferentiated embryos were cultivated on the H1 and H2 media for an eventual accomplishment of growth and differentiation processes.

In these cases, the partially differentiated embyos or the callused ones were submitted to a second passage on B5 medium for germination and plant regeneration.

Table 1. Nutritive media for *in vitro* culture of barley haploid embryos

Culture media	Embryos	
	Differentiated	Undifferentiated
B5 (Gamborg, 1968 modified)	+	+
B II (Norstog, 1973)	+	+
H1 (B5 supplemented with PGR)	-	+
H2 (Murashige-Skoog 1/2, 1962, supplemented with PGR)	-	+

Crossability between *H. vulgare* and *H. bulbosum* was estimated by the following parameters: dissected seeds/100 pollinated florets (SD/F), dissected seeds /100 developed seeds (SD/S), developed embyos /100 pollinated florets (E/F), developed embryos/100 dissected seeds (E/SD) and differentiated embryos /100 developed embryos (ED/E).

The reaction to *in vitro* culture, expressed as embryo germination and haploid plant regeneration ability was determined on the basis of several parameters: cultivated embryos /100 developed embryos (EC/E), germinated embryos/100 cultivated embryos (EG/EC), germinated embryos/100 undifferentiated embryos (EG/EUND), regenerated plantlets/100 dissected seeds (P/SD), regenerated plantlets /100 cultivated embryos (P/EC) and regenerated plantlets /100 germinated embryos (P/EG). A final estimation of the haploid production efficiency (HPE) was computed after the relation proposed by Chen and Hayes (1989): HPE = SD/F x EC/SD x P/EC.

The test $\chi 2$ was applied to estimate the significance of the genotypic differences for the reaction to *in vitro* culture of immature haploid embryos, using the mean value of genotypes , averaged over nutritive media and experimental conditions.

RESULTS AND DISCUSSION

A number of 5209 florets (311 spikes) of six-rowed barley (8 genotypes) were pollinated and 2456 seeds were developed, of which 1394 seeds of quality class (submitted to dissection). Out of 839 developed embryos, 818 embryos were *in vitro* cultivated; a number of 329 embryos germinated and 270 haploid plants were regenerated (212 green, viable plants and 58 albinotic, lethal plants).

Similary, in two-rowed barley (8 genotypes) a number of 7731 florets (420 spikes) were pollinated, obtaining 4316 seeds, from which 2590 were of quality. 1596 embryos were developed, 1521 embryos were *in vitro* cultivated and 521 embryos have germinated. Out of these embryos, 427 haploid plants were regenerated (333 green, viable plants and 94 albinotic, lethal plants).

In a recently reported study differential genotypic effect of cultivated barley on the crossing compatibility with *H. bulbosum* has been evaluated (Mihăilescu et al., 1995).

The rates of crossability of barley genotypes included in 1995 hybridization cycle confirmed the differences between six and two - rowed types as well as variation among genotypes within each type (Table 2).

Table 2. Genotype reaction of winter barley in crosses with *H. bulbosum*

Lines / Varieties	Parameters			
	SD / F	SD / S	E / F	E / SD
SIX-ROWED				
Miraj	23.8	17.8	14.3	60.4
Productiv	26.7	54.4	15.6	58.3
Precoce	26.1	52.6	16.1	61.5
Dana	27.6	53.0	14.7	53.1
Adi	32.6	64.5	22.9	70.4
Mădălin	22.2	51.6	13.1	58.7
F 208/88	32.2	67.5	20.0	62.2
F 503/88	23.4	64.0	12.6	53.7
MEAN	26.8	56.8	16.1	60.2
TWO-ROWED				
Victoria	21.1	50.4	11.5	54.4
Griviţa	38.3	67.5	27.2	71.1
Laura	41.5	70.7	27.8	67.0
Andra	20.0	38.4	10.3	51.4
F 1116/88	24.2	41.9	12.8	53.0
F1167/89	42.3	67.2	23.6	55.9
F 1233/89	28.9	58.1	13.3	46.1
F 2484/87	36.9	57.2	30.7	83.2
MEAN	33.5	60.0	20.6	61.6

In six - rowed barley the values for SD/F varied from 22.2 (Mădălin) to 32.6 (Adi) with the average value of 26.8. The seed of quality proportion (SD/S) had the average value of 56.8 with the minimum in Miraj (17.8) and the maximum in F 208/88 (67.5). The average value for E/F was 16.1 with large variation limits from 12.6 (F503/88) to 22.9 (Adi). The values for embryos

formed per 100 dissected seeds (E/SD) ranged from 53.1 (Dana) to 70.4 (Adi) with the mean value of 60.2.

In two - rowed barley the mean value for SD/F was 33.5 (20.0 in Andra and 42.5 in F1167/89), SD/S varied from 38.4 (Andra) to 70.7 (Laura) with the average value of 60.0. E/F parameter had various values from 10.3 (Andra) to 30.7 (F2484/87) with the average of 20.6 and E/SD had the lowest value (46.1) in F1233/89 and the highest one (83.2) in F2484/87, with the mean of 61.6.

Concerning the genotypic reaction to the *in vitro* embryo culture (embryo germination and plant regeneration) the obtained results revealed significant differences between six - and two - rowed types of barley and among the genotypes. (Table 3).

In six - rowed barley the average value of cultivated embryos (EC/E) was 95.5 (93.6 in Mădălin and 100 in F208/88) and for germinated embryos (EG/EC) was 40.2 (7.9 in Dana and 56.7 in F 208/88).

The proportion of regenerated haploid plants from dissected seeds (P/SD) varied from 4.0 in Dana to 30.6 in F 208/88 (the mean value 19.4%). From cultivated embryos the mean value of regenerated haploid plants (P/EC) was of 33.0 (7.9 in Dana and 49.2 in F 208/88).

Generally the two-rowed barley showed great similarity with six-rowed barley in the reaction to *in vitro* culture of haploid embryos. There was a significant genotypic variation for *in vitro* behaviour of cultivated haploid immature embryos of two - rowed barley, too.

Table 3. Genotype reaction of winter barley for *in vitro* embryo germination and haploid plant regeneration

Lines / Varieties	Parameters			
	EC / E	EG / EC	P / SD	P / EC
SIX-ROWED				
Miraj	97.9	22.7	12.1	20.4
Productiv	97.5	38.8	19.6	34.5
Precoce	97.5	29.5	13.1	21.8
Dana	95.7	7.9	4.0	7.9
Adi	98.7	52.3	27.8	40.0
Mădălin	93.6	51.1	21.2	38.6
F 208/88	100.0	56.7	30.6	49.2
F 503/88	97.7	47.6	20.6	39.3
MEAN	95.5	40.2	19.4	33.0
TWO-ROWED				
Victoria	100.0	22.8	9.5	17.4
Grivița	87.5	30.5	14.0	22.5
Laura	96.0	31.4	16.7	26.0
Andra	98.6	52.9	24.6	48.6
F 1116/88	98.2	10.7	5.1	9.8
F1167/89	99.7	45.0	22.4	40.3
F 1233/89	96.3	46.6	16.8	37.9
F 2484/87	100.0	39.3	21.5	25.8
MEAN	95.3	34.2	16.5	28.1

The average value of cultivated embryos (EC/E) was of 95.3 (87.5 in Grivița and 100 in Victoria) and for germinated embryos of 34.2 (10.7 in F 1116 /88 and 52.9 in Andra). P/SD parameter had the mean value of 16.5 (5.1 in F1116/88 and 24.6 in Andra) and the average value for P/EC was of 28.1 (9.8 in F1116/88 and 48.6 in Andra). Statistical analysis carried out by χ^2 test revealed, without exceptions, significant differences ($P<0.05^*$, $P<0,01^{**}$ and $P<0.001^{***}$) in terms of *in vitro* embryo germination ability and

Table 4. Genotype differences for EG / EC among lines and varieties of barley

SIX-ROWED							
	Productiv	Precoce	Dana	Adi	Mădălin	F 208/88	F 503/88
Miraj	-16.06***	-6.76***	14.86**	-29.53***	-28.41**	-33.94***	-24.62***
Productiv		9.30***	30.92***	-13.47***	-12.35***	-17.88***	-8.83***
Precoce			21.62**	-22.77***	-21.65**	-27.18***	-18.13***
Dana				-44.39***	-43.27*	-48.80***	-39.75**
Adi					1.12***	-4.41***	4.64***
Mădălin						-5.53***	3.52***
F 208/88							9.05***
TWO-ROWED							
	Grivița	Laura	Andra	F 1116/88	F 1167/89	F 1233/89	F 2484/87
Victoria	-7.65***	-8.56***	-30.03***	12.12**	-22.22***	-23.77***	-16.50***
Grivița		-0.91***	-22.38***	19.77***	-14.57***	-16.12***	-8.85***
Laura			-21.47***	20.68***	-13.66***	-15.21***	-7.94***
Andra				42.15**	7.81***	6.26***	13.53**
F 1116/88					-34.34***	-35.89***	-28.62**
F 1167/89						-1.55***	5.72***
F 1233/89							7.27***

Table 5. Genotype differences for P / SD among lines and varieties of barley

	Productiv	Precoce	Dana	Adi	Mădălin	F 208/88	F 503/88
SIX-ROWED							
Miraj	-7.53***	-1.00***	8.08**	-15.72***	-9.17***	-18.49***	-8.55***
Productiv		6.53***	15.61***	-8.19***	-1.64***	-10.96***	-1.02***
Precoce			9.08*	-14.72***	-8.17***	-17.49***	-7.55***
Dana				-23.80**	-17.25***	-26.57**	-16.63**
Adi					6.55***	-2.77***	7.17***
Mădălin						-9.32***	0.62***
F 208/88							9.94***
TWO-ROWED							
	Griviţa	Laura	Andra	F 1116/88	F 1167/89	F 1233/89	F 2484/87
Victoria	-4.45***	-7.27***	-15.17***	4.35**	-12.96***	-7.34***	-12.03***
Griviţa		-2.73***	-10.63***	8.89***	-8.42***	-2.80***	-7.49***
Laura			-7.90***	11.62***	-5.69***	-0.10***	-4.76***
Andra				19.52***	2.21***	7.83***	3.14***
F 1116/88					-17.31***	-11.69***	-16.38**
F 1167/89						5.62***	0.93***
F 1233/89							-4.69***

Table 6. Genotype differences for P / EC among lines and varieties of barley

	Productiv	Precoce	Dana	Adi	Mădălin	F 208/88	F 503/88
SIX-ROWED							
Miraj	-14.03***	-1.34***	12.58**	-19.55***	-18.19***	-28.72***	-18.84***
Productiv		12.69***	26.61**	-5.52***	-4.16***	-14.69***	-4.81***
Precoce			13.92*	-18.21***	-16.85**	-27.38***	-17.50***
Dana				-32.13**	-30.77*	-41.30**	-31.42**
Adi					1.36***	-9.17***	0.71***
Mădălin						-10.53***	-0.65***
F 208/88							9.88***
TWO-ROWED							
	Griviţa	Laura	Andra	F 1116/88	F 1167/89	F 1233/89	F 2484/87
Victoria	-5.12***	-8.64***	-31.18***	7.57***	-22.88***	-20.47***	-8.45***
Griviţa		-3.52***	-26.06***	12.69***	-17.76***	-15.35***	-3.33***
Laura			-22.54***	16.21***	-14.24***	-11.83***	0.19***
Andra				38.75**	8.30***	10.71***	22.73**
F 1116/88					-30.45***	-28.04***	-16.02**
F 1167/89						2.41***	14.43***
F 1233/89							12.02***

Table 7. *In vitro* culture of haploid embryos on different nutritive media

Parameters		B5	B II	H1	H2
SIX-ROWED					
EG / EC		42.7	30.5	29.8	20.6
EG / E UND		8.3	91.7	90.0	28.6
Callused embryos		1.9	4.2	3.7	-
Regenerated plantlets		35.7	22.2	23.1	8.8
	-Green	76.7	80.8	87.1	66.7
	-albino	23.3	19.2	12.9	33.3
P / EC		27.4	17.8	20.1	5.9
P / EG		64.3	58.3	67.5	28.6
TWO-ROWED					
EG / EC		37.2	34.4	11.5	18.5
EG / E UND		7.4	36.4	22.2	60.0
Callused embryos		2.2	4.7	-	-
Regenerated plantlets		29.5	25.0	10.3	14.8
	-Green	80.3	75.0	75.0	100.0
	-Albino	19.7	25.0	25.0	-
P / EC		23.7	18.7	7.7	14.8
P / EG		63.7	54.5	66.7	80.0

haploid plant regeneration for both six- and two-rowed barley genotypes (Table 4, 5 and 6).

In order to enhance the efficiency of *in vitro* embryo germination and haploid plant regeneration several specific nutritive media were utilized, depending on the differentiation stage of haploid embryos.

In six - rowed barley, B5 medium both for differentiated and undifferentiated embryos, was the most efficient, this medium giving higher average values for embryo germination (42.7), for haploid plant regeneration (35.7) and for the frequency of green viable haploid plants (76.7). The callus induction on this medium was minimum, 1.9% (Table 7).

Among the nutritive media utilized only for *in vitro* culture of undifferentiated embryos, the most stimulating was H1 medium, followed by B II; on these media proportion of germinated embryos was of 29.8 and respectively 30.5 and of regenerated plants of 23.1 and respectively 22.0. The highest percent of green viable haploid plants (87.1) was achieved with H1 medium. The H2 medium was considered as inadequate to *in vitro* culture of undifferentiated embryos because of the lowest average values for both embryo germination (20.6) and plant regeneration (8.8). On this medium the negative effect of *embryo browning* occurred with high frequency.

In two - rowed barley genotypes B5 medium gave also the best results *in vitro* culture: 37.2 embryos germinated and 29.5 haploid plants were regenerated, of which 81.3% belonged to the green, viable type.

The obtained results suggested that in two-rowed barley the undifferentiated embryos had the best *in vitro* reactions on B II and H2 media.

Haploid production efficiency (HPE) in six - rowed barley ranged from 0.9 (Dana) to 7.5 (Adi) with the mean value of 4.1. In 7.5 (Adi) with the mean value of 4.1. In F208/88 line the highest value for HPE, 13.4 was attained under greenhouse condition. In two - rowed barley, HPE had the average value of 4.3 and exhibited large variation limits from 1.0 (F1116/88) to 8.0 (F1167/89) with the maximum of 8.8 in F1167/89 in greenhouse (Table 8). From the component parameters, SD/F factor sets an upper limit to HPE index (seed set in *H. vulgare* x *H. bulbosum* crosses is most probably controlled by a single dominant gene, of *Inc* locus located on 5H chromosome) (Hayes and Chen, 1989, Pickering et. al., 1992). SD/F parameter varied from 22.2 (Mădălin) to 32.6 (Adi) with the mean value of 26.8 in six - rowed barley, while in two - rowed genotypes SD/F had the average value of 33.5 with limits of variation from 20.0 (Andra) to 42.3 (F1167/89).

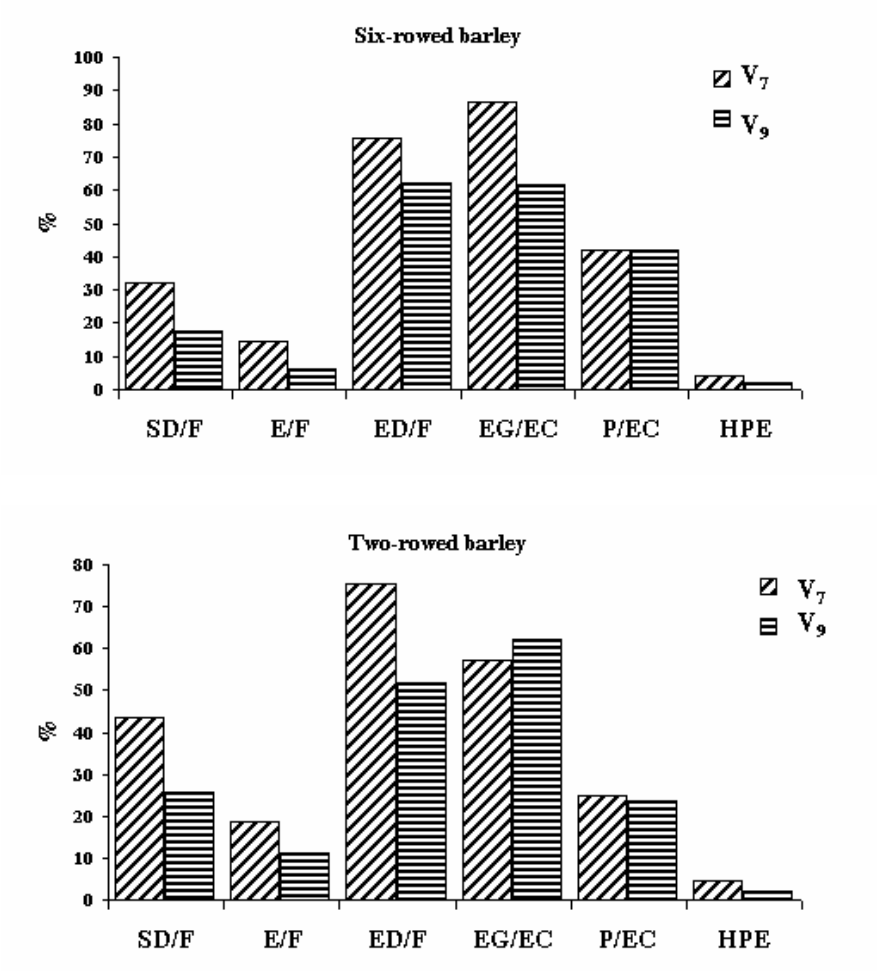
Table 8. Haploid production efficiency (HPE) and its component parameters in winter barley

Lines / Varieties	SD / F	EC / SD	P / EC	HPE
SIX-ROWED				
Miraj	23.8	59.1	19.3	2.7
Productiv	26.7	56.9	27.6	4.2
Precoce	26.1	60.0	15.4	1.5
Dana	27.6	50.9	6.7	0.9
Adi	32.6	69.5	32.9	7.5
Mădălin	22.2	55.0	30.7	3.8
F 208/88	32.2	62.2	33.3	6.7
F 503/88	23.4	52.5	32.1	3.9
MEAN	26.8	58.7	25.9	4.1
TWO-ROWED				
Victoria	21.1	54.4	16.3	1.9
Griviţa	38.3	62.2	17.4	4.1
Laura	41.5	64.3	19.5	5.2
Andra	20.0	50.7	28.6	2.9
F 1116/88	24.2	52.1	8.0	1.0
F1167/89	42.3	55.7	33.8	8.0
F 1233/89	28.9	44.4	30.1	3.9
F 2484/87	36.9	83.2	20.2	6.2
MEAN	33.5	58.7	21.9	4.3

The results regarding the effect of 2,4-D higher concentration (V9) on *in vitro* culture of immature haploid embryos showed significant negative action on almost all the analysed parameters, compared with the lower 2,4-D concentration (V7) (Figure 1).

Larger concentration of 2,4-D (50 ppm) applied *in vivo* affected mainly the seed development (SD/F), embryo differentiation (E/F) and subsequent plant regeneration (HPE). HPE index had about two times lower mean values in both six- and two-rowed barley with V9 treatment as compared to V7 variant (2. 1 and respectively 2.2 in comparison with 3.9 and 4.5).

- variations in *H. vulgare* material (pure



V_7 = Ga3 (75 ppm) + 2,4-D (20 ppm) (4:1)
V_9 = GA3 (75 ppm) + 2,4-D (50 ppm) (1:1)

Figure 1. In vivo and *in vitro* efects of PGR treatment on barley haploid induction

These results are in obviously contrast with Pickering's data about the positive influence of 2,4-D higher concentration on ''quality seeds, survival of embryos for culture and plant regeneration'' (Pickering and Wallance, 1994).

The influence of genotypes on both crossing compatibility (seed setting, seed quality, embryo differentiation) and *in vitro* behaviour of haploid immature embryos (embryo germination, plant regeneration) in *H. vulgare* x *H. bulbosum* crosses have been reported by many workers (Mihăilescu et al., 1995, 1996, Pickering, 1983, Pickering and Devaux, 1992), but sometimes the conclusions have been inconsistents for the following reasons:

- substantial differences in environmental conditions and experimental techniques;
lines, varieties, hybrids);

- varied statistical analysis and evaluating parameters.

Anyway, our results confirm many previously studies (Hayes and Chen, 1989; Pickering and Devaux, 1992) concerning the influence of female partner on *in vitro* embryo culture and the differences between genotypes in the rates of embryo germination and haploid plant regeneration.

CONCLUSIONS

In vitro embryo germination and haploid plant regeneration in *H. vulgare* x *H. bulbosum* crosses appeared to depend only on *H. vulgare* genotype.

Barley genotype accounted for the greatest variation in the mean values of *in vitro* evaluating parameters.

In vitro behaviour of barley immature haploid embryos was influenced also by the exogenous post-pollination treatment with PGR.

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REFERENCES

Ben Amer I.M., Boerner A., Schlegel R., 1992 - The effect of the hybrid dwarfing gene D2 on tissue culture response of wheat (*Triticum aestivum*). Cereal Res. Comm. 20: 1-2, 87-93.

Chen F.Q., and Hayes P.M., 1989 - A comparison of *Hordeum bulbosum* - mediated haploid production efficiency in barley using *in vitro* floret and tiller culture. Theor. Appl. Genet. 77: 701-704.

Gale M.D. and Youseffian S., 1985 - Dwarfing genes in wheat. In: Progress in plant breeding (Russel G. ed.) Butterworth, London: 1-35.

Gamborg O.L., Miller R.A. A and Ojima K., 1968 - Nutrient requirements of suspension cultures of soybean root cells. Exper. Cell Research 50: 151-158.

Ganeva G., Karadimova M., Djambova G., 1995a - Effects of 4H *Hordeum vulgare* L., 4A and 4D *Triticum aestivum* L. chromosomes on the genetic control of *in vitro* and *in v*ivo plant response to exogenous auxins. Cereal Res. Comm.23, 1-2: 27-33.

Ganeva G., Karadimova M., Bocheva N., Zozikova E., Djambova G., Bochev B., 1995b - Effects of *Hordeum vulgare* L. 2H and of second homoeologous *Triticum aestivum* L. group chromosomes on in vitro plant response to exogenous auxins. Cereal Res. Comm. 23, 1-2: 35-43.

Hayes, P.M., and Chen, F.Q., 1989 - Genotypic variation for *Hordeum bulbosum* L. mediated haploid production in winter and facultative barley. Crop Sci. 29: 1184-1188.

Jensen J. and Linde - Laursen I., 1992 - Statistical evaluation of length measurements on barley chromosomes with a proposal for a new nomenclature for symbols and positions of cytological markers. Hereditas 117: 51-59.

Kaleikau E.K., Sears R.G. and Gill B.S., 1989. Monosomic analysis of tissue culture response in wheat (*Triticum aestivum* L.).Theor. Appl. Genet. 78: 625-632.

Lazar M.D., Collins G.B and Vian W.S., 1983 - Genetic and environmental effects on the growth and differentiation of wheat somatic cultures. J. Hered. 74: 353-357.

Linde - Laursen I., Bothmer R. Von, and Jacobsen N., 1995 - Karyotype differentiation and evoluation in the genus *Hordeum* (*Poaceae*) In: Kew Chromosome Conference IV (eds Brandham and MO Bennett), R. Bot. Gard, Kew: 233-247.

Mathias R.J., and Fukui K., 1986 - The effect of specific chromosome and cytoplasmic substitutions on the tissue culture response of wheat (*Triticum aestivum* L.) callus. Theor Appl. Genet. 71:797-800.

Mathias R.J., and Atkinson E., 1988 - *In vitro* expression of genes affecting whole phenotype - the effect of Rht/Gai alleles on the callus culture response of wheat (*Triticum aestivum* L.). Theor. Appl. Genet 75: 474-479.

Mathias R.J., Higgins P. and Atkinson E., 1988 - Genetic control of wheat (*Triticum aestivum*) tissue culture response. The 8th Intern. Wheat Genetics Symp., Beijing, China: 763-768.

Mihăilescu A., Bude A., and Giura A., 1993 - Reconsiderarea metodei *bulbosum*, alternativă de homozigotare rapidă a orzului cultivat. Probl. Genet. Teor. Aplic. XXV (1) 1-26.

Mihăilescu A., Giura A., and Bude A., 1994 - Increasing efficiency of barley haploid production by combined treatments with plant growth regulators applied *in vivo*. Rom. Agric. Res. 2: 25-33.

Mihăilescu A., Giura A., and Bude A., 1995 - Production of winter barley haploid by *bulbosum* system. 1. Crossing compatibility between winter barley Romanian varieties and *H. bulbosum* for *in vitro* haploid regeneration. Rom Agric. Res. 4: 1-9.

Mihăilescu A., Giura A., and Bude A., 1996 - Progrese în obținerea de haploizi și linii homozigote, dublu haploide (DH) la orzul și orzoaica de toamnă. Cercet. Genet. Veget. Anim. IV: 49-63.

Murashige T., and Skoog F., 1962 - A revised medium for rapid growth and bio-assays with tabacco tissue cultures. Physiologia Plantarum 15: 473-497.

Norstog K., 1972 - Factors relating to precocious germination in cultured barley embryos. Phytomorphology 22/34: 139.

Pedersen C., Rasmussen S.K., and Linde - Laursen I., 1996 - Genome and chromosome identification in cultivated barley and related species of the *Triticeae* (*Poaceae*) by *in situ* hybridization with the GAA - satellite sequence. Genome 39: 93-104.

Pickering R.A., 1983 - The influence of genotype on doubled haploid barley production. Euphytica 32: 863 - 876.

Pickering R.A. and Devaux P., 1992 - Haploid production approaches and use in plant breeding. In: Barley genetics, biochemistry, molecular biology and biotehnology. C.A.B. Intern: 519 - 547.

Pickering R.A. and Wallance A.R., 1994 - Gibberellic Acid + 2,4-D improve seed quality in *Hordeum vulgare* . x *Hordeum bulbosum* L. crosses. Plant breeding 113: 174 - 176.