Sel'skokhozyaistvennaya Biologiya [Agricultural Biology], 2011, № 1, p. 44-53.

UDK 633.16:57.086.83

COMPARATIVE EVALUATION CALLUSOGENESIS AND REGENERATION IN DIFFERENT BARLEY VARIETIES

V.E. Chernov, G.I. Pendinen
N.I. Vavilov All-Russia Research and Development Institute of Plant Growing, RAAS, St.Petersburg 190000, Russia
e-mail: vechernov@mail.ru, pendinen@mail.ru

Received January 15, 2009

S u m m a r y

The authors studied the features of callusogenesis and regeneration (number, mass, morphological characteristics and efficiency of callus formation, morphological characteristics of regenerants and regeneration efficiency) in the species from different section of *Hordeum* L. genus. For induction of callusogenesis the three explants types were used (young blossom cluster, immature corcule, ripe corn seeds), and also the three nutrient media differed on mineral content (N6, B5L, Murashige-Skoog). It was established, that a formation of callus tissue in species of *Hordeum* section differs from the same in species of different section (*Anisolepis*, *Critesion*, *Stenostachys*). The callus type do not depends on medium content and used explants. Some of studied species didn't reveal the regeneration of plants from callus. The single regeneration type was specific for species from *Hordeum* section, but the multiple type — for species from *Anisolepis*, *Critesion*, *Stenostachys* sections. The type regeneration didn't depend on selected explants (young blossom cluster, immature corcule, and ripe corn seed).

Keywords: callusogenesis, regeneration, explants, in vitro culture, genus *Hordeum*, wild barley.

Barley (genus *Hordeum* L.) is widespread in nature and grows in different climatic conditions, which factors provide diverse physiological characteristics of its species (1, 2). However, this genus is poorly studied even today, especially the wild forms and their cultivation in vitro. Cultivated barley *H. vulgare* is frequently used in studies on culture of tissues and organs, there's the developed system for cultivation this crop and findings on its regeneration (3-6). Wild species are rarely used in such investigations, and the number of studied wild species is very small (7-12). At the same time, these close relatives of cultivated barley are the possible sources of valuable traits for introgression into the genome of *H. vulgare* (13). Knowledge of biological features of wild barley species cultivated in vitro is an extremely urgent task, since using their gene pool for breeding purposes is possible only at the use of in vitro methods.

The most common explants for embryogenic callus culture of barley are immature embryos (14). Microclonal propagation of individual unique plants (eg. sterile interspecific hybrids of cereals) can be performed using somatic tissues of young inflorescences (15-17), for microcloning and formation of somatic tissue culture of barley - apical meristem of growing shoots (18). Mature embryos can be used as explants providing a primary callus without any seasonal limitations (19). Investigations of callus formation and regeneration are usually carried out on the explant most suitable for experiments and effectively providing reliable results. Though, the comparative study of these processes in one genotype at the use of different explants hasn't been performed yet.

The purpose of this study was to determine in different barley species the phenotypic features of callus induction and regeneration, to evaluate regeneration potential of species and compare the features of in vitro cultivation of different explants.

Technique. The object of study were barley species from different sections of genus *Hordeum* preserved in the laboratory of biotechnology of N.I.Vaviolv All-Russia Research and Development Institute of Plant Industry (VIR). The section *Hordeum* was represented by the species *H. vulgare* L. (2×: cv Polyarnyi 14, Rannii 1, Betzes, Roland — from VIR collection), *H. vulgare* ssp. *spontaneum* (C. Koch) Thell. (2×: W 31 и W 485 — from VIR collection, I 0118914 — from VIR department of introduction), *H. vulgare* ssp. *agreochrithon* (Äberg) Bowd (2×: I 0118914 — from VIR department of introduction), *H. bulbosum* L. (2×: W 510 — from VIR collection 4×: W 4, W 121 p1, W 121 p2, W 121 p3 — from VIR collection), *H. murinum* L. (2×: W 20 — from VIR collection; 4×: I 0118916, I 0118919 — from VIR department of introduction, G 1 — from plants collected in expedition near the Gelendzhik city; 6×: W 277, W 278 — from VIR collection, I 491045 — from VIR department of introduction). The series of samples the section *Anisolepis* included only diploid (2×) species: *H. pusillum* Nutt. (I 491050), *H. intercedens* Nevski (H 1940 – the Swedish University of Agricultural Sciencis — SUAS, Svalov), *H. lexuosum* Steud. (H 1110, H 1112, H 1116 – SUAS, Svalov), *H. muticum* Pres. (H 958, H 1784, SUAS, Svalov), *H. cordobense* Bothmer et al. (H1702 – SUAS, Svalov). Species of the section *Critesion*: *H. jubatum* L. (4×: K 1 — plants collected in Krasnoyarsk region, Sh 2 и Sh 5 — plants collected in the Shortandy settlement, Kazakhstan, Ya 1 — plants collected in Yakutia), *H. procerum* Nevski (6×: W 356 — from VIR collection, I 491049 — from VIR department of introduction), *H. lechlery* (Steud) Schenck (6×: H 1496 – SUAS, Svalov). Species of the section *Stenostachys*: *H. marinum* Huds. (2×: H 299 – SUAS, Svalov; 4×: I 0118920 — from VIR department of introduction, W 493 — from VIR collectilon), *H. depressum* (Scribn. & Sm.) Rybd. (4×: I-538814 — from VIR department of introduction, W 309 — from VIR collection), *H. brachyantherum* ssp. *californicum* (Covas&Stebbins) Bothmer et al. (2×: H 559970 — from VIR department of introduction), *H. parodii* Covas (6×: H 1269 – SUAS, Svalov), *H. patagonicum* (Haumann) Covas (2×: H 1468 – SUAS, Svalov).

Callus tissue was obtained from three types of explants - young inflorescences (immature ear segments before meiosis), immature embryos (on the 14^{th} -16^{th} day after pollination) and mature caryopses (6 months after complete ripening, normally formed in vivo). Different modes of sterilization were applied. Shoots with young inflorescences were sterilized in 70% ethanol solution for 5 minutes. Caryopses before the isolation of immature embryos were sterilized in 70% ethanol for 4 minutes and then in 10% hydrogen peroxide solution for 15 minutes and finally washed three times with sterile water (1 minute in each change). Mature caryopses were sterilized similarly (exposure to hydrogen peroxide - 20 minutes). Before sterilization of caryopses, their glumes were removed. Mature sterilized caryopses were explanted on a nutrient medium notch up without isolation of the embryo.

Depending on a set task, all types of explants were planted in culture vessels on inducing media of different chemical composition: B5L (1920), N6 (21) and Murashige-Skoog (MS) (22). The only auxine hormone– 2,4-dihlorfenoksi-acetic acid (2,4-D) – was used at the concentration 2 mg/l constant in all the media. For organogenesis, N6 medium containing 1 mg/l kinetin was applied.

For callus induction, the explanted embryos and mature caryopses were cultured in an incubator at 27 °C in the dark. After the transfer of calli on the regeneration medium, the vessels were placed into a light chamber with 16-hour photoperiod at room tem-

perature. In variants with young inflorescences, the light exposure was applied during both callus induction and organogenesis.

Efficiency of callus induction and regeneration were recorded – respectively, after 15 and 30 days post explantation, and after 30 days following the transfer of calli on regeneration medium. Callusogenesis was assessed in all samples by the number of developed calli and their wet weight gain by the end of a passage. Efficiency of callus induction was expressed as a percentage of calli relative to a number of initial explants. Relative growth rate was determined by comparison with raw biomass measured after the 1st passage, considering the fact that this characteristic is below than detection threshold of weighing (scales Sartorius L 610D, Sartorius GmbH, Germany). External morphological features of calli were evaluated. At the stage of regeneration, normally developed plants having shoots and roots were accounted. Efficiency of regeneration was calculated as a percentage of calli resulted in normally developed plants relative to the total number of explants planted on organogenic medium. Along with it, morphological parameters of calli after formation of regenerants were controlled.

Efficiency of regeneration in calli obtained from different types of explants was compared using the χ^2 criterion for the 4-field table 2×2 (23). Reliability of the share (%) for alternatively diverse variants (presence / absence of callus induction and regeneration) was evaluated by its error (23).

Results. The composition of nutrient media used in this research was revised to simplify and optimize conditions of in vitro experiments. To compare the indices of callus induction and regeneration in different *Hordeum* species, the authors selected most common and simple in composition nutrient media: Murashige-Skoog (MS), N6 (Chu medium) and B5L (modified Gamborg's B5-medium). These media have identical qualitative composition of macroelements, but they are distinct in contents of these elements in nutrient solution and the ratio of NO_3^- and NH_4^+ ions.

To study the induction and development of calli in different barley species depending on mineral composition of a medium, immature embryos were used as explants. Such explants were chosen owing to intense proliferation and competence of all embryo tissues when cultured in vitro (8, 24, 25), which prevents results of the experiments from the effects of reduced proliferative function in a specialized tissue. Explantation of immature embryos of different barley species revealed interspecific differences in both the efficiency of callus induction and growth rate of callus tissues.

In all studied samples of cultivated barley, immature embryos demonstrated a highest frequency of callus formation on the 2nd – 3rd day after explantation, and the active visible growth of calli – on the 4th – 7th day. A slight variation in timing of callus induction and formation of visible callus tissue was observed in different cultivated varieties and wild species. These distinctions were found to be species-specific rather than caused by mineral composition of a medium. All studied species showed no significant fluctuations in duration of the induction period depending on composition of a medium.

The obtained data on the efficiency of callus induction are represented in groups according to division of genus *Hordeum* into types and sections proposed by R. Bothmer et al. (2). Thus, immature embryos of *H. vulgare* after the explantation manifested high frequency of callus induction, while in different varieties of cultivated barley this rate was also high - from 53,4 to 96,4%. External morphological features of calli grown in all three media were most similar: immature embryos developed into large, semitransparent friable calli, i.e. I type callus (classification of C.S. Goldstein and W.E. Kronstadt, 1986) (25). By the end of the 1st period of in vitro cultivation, callus tissue transformed into compact and opaque II type callus (compact) (25). It is known that II type calli are more prone to regeneration, because development of dense tissue is associated with formation of meristematic zones de novo (25).

Immature embryos of wild subspecies *H. vulgare* ssp. *spontaneum* and ssp. *agreochrithon* were found to form calli of the same type and morphological structure as those in cultivated barley. *H. bulbosum* (2 ×, 4 ×) and *H. murinum* (2 ×, 4 ×, 6 ×) developed calli of the similar type, but of a smaller size than in cultivated barley. In the section *Hordeum*, I type callus prevailed during growth and development, which trait didn't depend on composition of different media (Table 1). In this section, the efficiency of callus induction dependant on medium composition was established only in *H. vulgare* and its wild subspecies, as well as in *H. bulbosum* (both diploid and tetraploid forms). In *H. murinum* (di-, tetra- and hexaploid cytotypes), composition of a medium caused no significant impact on efficiency of callus induction.

The studied species of the section *Hordeum* demonstrated regeneration of shoots and roots on calli re-explanted on organogenic medium. A crown type of roots (without formation of a shoot) was most frequently developed by almost all studied species. This fact was often observed in variants with calli formed on B5L-medium. The calli induced on MC-medium showed higher rates of tissue necrosis compared with two other media. Calli of the *Hordeum* section had unequal effectiveness of regeneration in organogenic medium. The absence of shoots regeneration was observed in diploid forms of *H. bulbosum* and *H. murinum*. Shoots were formed only on the calli grown from immature embryos of tetraploid and hexaploid *H. murinum* transplanted on organogenic medium N6 from the inducing media B5L and N6. In this case, efficiency of regeneration varied from 3,8-10,0% in tetraploid form up to 10,6-63,0% in a hexaploid.

In the section *Anisolepis*, callus induction and regeneration were assessed in *H. flexuosum* and *H. pusillum*. Immature embryos of these species were placed in the inducing media B5L, N6 and MC, and II type callus was formed fairly fast in all these variants. The observed efficiency of callus induction was 44,4-84,3% in *H. flexuosum* and 42,5-56,4% in *H. pusillum*; the maximum rate was established in the inducing medium N6. Regeneration of shoots was recorded only in the species *H. flexuosum* transplanted on the organogenic medium N6 from the inducing media B5L and N6.

1. Characteristics of callus induction and regeneration in vitro in different species of *Hordeum* depending on composition of nutrient medium

Species (cultivar, sample)	Callusogenesis			Regeneration	
	medium	type of callus	efficiency, %	efficiency, %	type
H. vulgare (2×, Rolan, Betzes, Polyarnyi 14)	B5L	I	81,2±3,07	54,3±12,95	Single
	N6	I	66,0±3,83	40,1±13,40	Single
	MC	I	75,2±3,96	56,1±3,65	Single
H. spontaneum (2×, W 31, I 0118914, W 485)	B5L	I	88,8±2,94	40,1±6,30	Single
	N6	I	56,1±4,51	59,5±5,71	Single
	MC	I	84,1±3,72	42,3±4,43	Single
H. bulbosum (2×, W 510)	B5L	I	33,3±12,20	0	
	N6	I	6,3±6,07	0	
	MC	I	5,9±5,71	0	
H. bulbosum (4×, W 4, W 121)	B5L	I	66,7±10,28	10,2±3,24	Single
	N6	I	46,9±8,82	46,2±1,44	Single

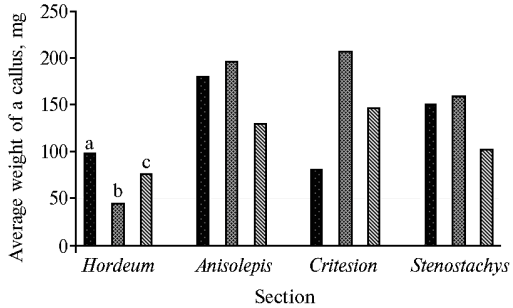
	MC	I	40,1±9,78	0	
H. murinum (2×, W 20)	B5L	I	25,0±6,45	0	
	N6	I	22,5±6,60	0	
	MC	I	20,0±6,32	0	
H. murinum (4×)	B5L	I	71,4±7,64	3,8±3,01	Single
	N6	I	57,1±5,40	10,0±2,05	Single
	MC	I	55,8±6,89	0	
H. murinum (6×, W 277, W 278)	B5L	I	25,0±2,17	10,6±3,24	Single
	N6	I	22,5±6,60	63,0±9,29	Single
	MC	I	11,1±5,24	0	Rhizogenesis
H. flexuosum (2×, H 1110,	B5L	II	44,4±8,78	51,0±8,84	Multiple
H 1112, H 1116)	N6	II	84,3±3,92	77,5±4,51	Necrosis
	MC	II	48,9±9,80	0	
H. pusillum (2×, I 491050)	B5L	II	42,5±7,82	0	
	N6	II	56,4±5,61	0	
	MC	II	46,7±7,98	0	Necrosis
H. jubatum (4×, K 1)	B5L	II	98,6±1,40	86,7±6,20	Multiple
	N6	II	96,7±3,26	87,5±5,90	Multiple
	MC	II	85,0±7,98	75,0±8,18	Multiple
H. procerum (6×, W 356,	B5L	II	77,5±6,36	41,7±6,37	Multiple
И 491049)	N6	II	64,7±6,69	51,0±6,11	Multiple
	MC	II	79,3±4,47	37,1±6,09	Multiple
H. lechlery (6×, H 1496)	B5L	II	96,0±3,92	40,1±8,94	Multiple with dominance
	N6	II	93,8±4,26	44,4±8,28	of one shoot
	MC	II	58,8±11,94	41,2±11,01	
H. marinum (4×, W 436, W 439)	B5L	II	97,8±2,17	20,0±8,94	Multiple
	N6	II	100	24,3±4,65	Multiple
	MC	II	93,6±2,34	27,8±8,32	Multiple
H. depressum (4×, W 309,	B5L	II	60,6±6,16	0	
И 538814)	N6	II	100	23,9±7,27	Multiple with necrosis of
	MC	II	86,8±4,33	8,6±8,09	callus

Note. In all variants, N6 without hormones was used as regeneration medium. Efficiency indices are given with their $X \pm x$ values (share and share of error expressed as percentage; see "Technique").

In species of the section *Critesion* (*H. jubatum, H. procerum, H. lechlery*), it was observed the high efficiency of callus induction - from 98,6% (*H. jubatum* on B5L-medium) to 58,8% (*H. lechlery* on MC-medium) (Table 1). In all callus-inducing media used in the experiment, a loose white opaque callus was formed and quickly transformed into the II type callus with nodulous structure. The highest efficiency of regeneration was observed in the species *H. jubatum*, the intermediate position - *H. procerum*, and the lower value was recorded in *H. lechlery*.

The reaction on media with different composition was assessed in the section *Stenostachys* upon its two tetraploid species - *H. marinum* and *H. depressum*. They manifested high efficiency of callus induction on all used inducing media: immature embryos developed into II type callus. Regeneration occurred with relatively low efficiency - from 20,0-27,8% in *H. marinum* to 8,6-23,9% in *H. depressum*.

In various *Hordeum* species, cultivation of calli in different inducing media and further transfer to a single organogenic medium contributed to common features – callus types and types of regeneration specific for certain groups of species. So, I type calli were most frequently found in species of the section *Hordeum*, II type – in sections *Anisolepis, Critesion* and *Stenostachys*; prevailed types of regeneration - single (mainly detected in the section *Hordeum*) and multiple (peculiar to most species of sections *Anisolepis, Critesion* and *Stenostachys*). *H. lechlery* exhibited multiple regeneration with dominance of one shoot, *H. depressum* – a multiple regeneration with necrosis of callus tissue. An equally sample-specific type of regeneration was manifested on all the three media. Composition of a medium provided small effect on regeneration of calli, but calli induced on B5L-medium more often developed rhizogenesis a crown type after transplantation on the organogenic medium, while the inducing medium MC used to result in necrosis of a callus.



Growth rates of calli in species of different sections the genera *Hordeum* depending on composition of nutrient medium: a, b and c – respectively, B5L, N6 and MC media. Description of the media – see "Technique".

Mineral composition of nutrient medium affected growth rates of callus biomass. Total mass of calli was determined as an integral characteristic of their growth in species of different sections the genus *Hordeum*: samples of the section *Hordeum* demonstrated more intense growth in media MC and B5L, less intense - in N6 medium. In other sections (*Anisolepis, Critesion* and *Stenostachys*), N6 medium caused better growth of calli than two others (see figure). The calli formed on N6 medium had greater number of meristematic zones than those on media B5L and MC, which fact was observed in both wild and cultivated barley. N6 medium also provided higher efficiency of regeneration in majority of wild species and the minimum number of callus necroses. In this regard, N6 medium was considered as optimum for in vitro cultivation of all *Hordeum* species and interspecific hybrids, so only this medium was used during a comparative assessment of micropropagation efficiency in explants of different types (young inflorescences, immature embryos and mature caryopsis).

Samples of the section *Hordeum* demonstrated callus formation in all types of explants (Table 2). Primary calli of a similar structure (loose transparent – I type) were formed by all types of explants. In samples of *H. vulgare* different subspecies and *H. bulbosum* cytotypes, regeneration from young inflorescences didn't occur. Regeneration was observed in tetraploid samples of *H. murinum*. One of these samples (*H. murinum* I 0118916) showed the efficiency of regeneration much lower in the variant with immature embryos than that with young inflorescences ($P_{\chi 2} = 0{,}015$), and the same order indices were recorded when using caryopses. All tested samples of the section *Hordeum* demonstrated a single type of regeneration from calli of different origin despite their differences in regeneration efficiency. Multiple regeneration with dominance of one shoot was found in only tetraploid sample G 1 of *H. murinum*.

2. Characteristics of callus induction and regeneration in vitro in different species of the section *Hordeum* depending on type of explant

		Efficiency, %	

	plant	callusogenesis	regeneration	
H. vulgare:				
Roland	EM	71,7±5,81	16,7±3,40	Single
	CR	82,0±5,41	25,0±6,85	Single
	I	75,0±4,84	0	
Betzes	EM	85,0±4,61	70,0±6,42	Single
	CR	80,0±8,00	0	
	I	76,7±5,48	0	
Polyarnyi 14	EM	83,6±3,13	0	
	CR	98,0±1,98	37,5±7,65	Single
	I	93,3±4,56	0	
Rannii 1	EM	73,9±5,29	2,0±1,96	Single
	CR	90,2±4,64	19,0±6,05	Single
	I	12,5±5,23	0	
ssp. *spontaneum* W 31	EM	59,5±8,07	0	
	I	28,8±5,03	0	
ssp. *spontaneum* I 0118914	EM	58,3±8,22	20,0±8,94	Single
ssp. *agriocrithon* I 0118914	EM	70,0±5,92	33,8±9,28	Single
H. bulbosum (2×):				
W 510	EM	6,3±6,07	0	
	CR	10,0±9,49	0	
	I	43,5±10,34	0	
H. bulbosum (4×):				
W 4	EM	37,5±8,56	46,2±9,78	Single
	CR	46,7±12,88	30,0±14,49	Single
	I	29,4±5,52	0	
W 121 p1	I	81,0±6,05	0	
W 121 p2	I	29,4±5,52	0	
W 121 p3	I	38,1±7,49	0	
H. murinum (2×) W 20	EM	11,2±2,93	0	
	CR	50,0±5,48	47,5±7,53	Single
H. murinum (4×) I 0118916	EM	62,5±7,65	2,1±2,05	Single
	CR	85,0±4,61	30,0±2,65	Single
	I	74,3±7,39	22,2±9,80	Single
H. murinum (4×) I 0118919	I	88,2±5,53	22,7±8,93	Single
H. murinum (4×) G 1	I	71,4±9,22	13,3±8,77	Multiple with dominance of one shoot
H. murinum (6×):				
W 278	EM	84,4±6,41	63,0±9,29	Single
	CR	44,1±8,52	66,7±12,17	Single
	I	28,0±4,61	0	
I 491045	I	61,5±7,79	0	

Note. EM, CR and I – respectively, immature embryos, mature caryopses and young inflorescences. Efficiency indices are given with their $X \pm x$ values (share and share of error expressed as percentage; see "Technique").

The section *Anisolepis* performed callusogenesis in all types of explants (Table 3) with formation of a dense nodulous callus (II type). Regeneration wasn't observed in several studied samples. Thus, *H. pusillum* I 491050 didn't exhibit regeneration from calli regardless the type of explant. In *H. flexuosum* H 1116, the rate of regeneration from inflorescences was significantly lower than that from embryos and caryopses. The comparison by χ^2-criterion revealed statistically reliable differences of these variants: for calli derived from inflorescences and embryos - at p=0,001 (χ^2= 30,07), from mature caryopses and inflorescences - at p=0,01 ($P_{\chi 2}$ = 0,002). In *H. muticum* H 958, regeneration efficiency in calluses developed from caryopses was significantly higher than in two other types of explants. The section *Anisolepis* (*H. muticum* H 958, *H. flexuosum* H 1116) showed varying efficiency of regeneration from calli of different origin, while the type of regeneration didn't depend on the used explants (Table 3). Species of this section developed the following types of regeneration: single (*H. cordobense* H 1702), multiple (*H. intersedens* H 1940), multiple with dominance of one shoot (*H. muticum* H 958, H 1784) and multiple with necrosis of callus (*H. flexuosum* H 1116, H 1112; *H. chilense* I 559966).

3. Characteristics of callus induction and regeneration in vitro in different species of *Hordeum* sections *Anisolepis*, *Stenostachys* and *Critesion* depending on type of explant

Species, cultivar, sample	Type of explant	Efficiency, %		Type of regeneration
		callusogenesis	callusogenesis	
		section *Anisolepis*		
H. pusillum I 491050	EM	56,4±5,61	0	
	CR	84,0±7,33	0	
	I	33,3±7,85	0	
H. muticum:				
H 958	EM	83,6±4,53	6,8±3,80	Multiple with dominance of one shoot
	CR	32,1±8,82	65,0±10,67	
	I	48,3±9,28	11,5±6,26	
H 1784	EM	61,5±9,54	26,7±11,42	Multiple with dominance of one shoot
H. intercedens H 1940	EM	86,4±4,22	54,5±10,61	Multiple
H. flexuosum:				
H 1116	EM	80,0±5,16	77,5±6,60	Multiple with necrosis of callus
	CR	95,0±3,45	70,0±14,49	
	I	56,2±5,81	20,0±5,16	
H 1112	I	35,7±7,39	6,4±3,57	Multiple with necrosis of callus
H 1110	I	55,2±9,23	0	
H. cordobense H 1702	EM	83,3±5,38	5,3±5,14	Single
H. chilense I 559966	I	87,7±3,79	16,4±3,13	Multiple with necrosis of callus
		section *Critesion*		
H. jubatum:				
K 1	EM	98,6±1,40	38,9±8,13	Multiple
	I	100	100	Multiple
Sh 2	I	93,7±3,06	96,8±1,82	Multiple
Sh 5	I	92,5±13,09	89,7±3,69	Multiple
Ya 1	I	100	0	
H. procerum:				
I 491049	EM	64,7±6,71	45,5±3,69	Multiple
	CR	6,5±4,43	30,0±14,49	Multiple

	I	95,8±1,84	100	Multiple
W 356	I	71,9±4,09	47,6±4,92	Multiple
H. lechlery H 1496	EM	93,8±4,26	2,8±2,75	Multiple with dominance of one
	I	79,0±5,18	57,8±7,36	shoot
H. arizonicum H 2313	I	42,8±9,35	33,3±19,24	Multiple
		section *Stenostachys*		
H. marinum (2×) H 299	I	45,8±10,17	40,0±12,65	Single
H. marinum (4×):				
I 0118920	EM	82,3±3,35	33,9±6,33	Multiple
	CR	78,1±5,17	–	
	I	13,9±2,82	0	
I 0118921	I	42,6±3,59	4,8±3,30	Multiple
W 493	I	28,2±3,93	28,2±7,21	Multiple
H. depressum:				
W 309	EM	100	23,3±6,57	Multiple with necrosis of callus
	I	100	7,6±2,11	
И 538814	I	100	61,1±11,49	Multiple with necrosis of callus
H. parodii H 1269	EM	66,7±6,12	16,7±7,61	Multiple
	I	57,8±5,21	1,6±1,57	Multiple
H. patagonicum H 1468	EM	92,5±3,73	21,4±5,48	Multiple
	I	47,2±8,27	0	
H. californicum I 559970	EM	80,0±8,94	52,9±12,48	Multiple
	CR	90,0±4,74	–	
	I	42,1±53,07	0	
H. secalinum W 418	I	81,0±5,04	0	

Note. See Table 2. Dashes – formation of non-viable calli which were not used in the experiment.

All studied samples of the section *Critesion* were found to form a compact nodulous callus. The only exception - the sample *H. jubatum* Ya 1 (loose watery callus not capable to regeneration). Other samples demonstrated multiple regeneration, and the dominance of a single shoot was observed in the sample *H. lechlery* H 1496. In the section *Critesion*, type of regeneration was common for all types of explant as well (Table 3), while regeneration of calli obtained from inflorescence was more efficient than from other types of explants. The samples *H. jubatum* K 1 and *H. procerum* I 491,049 manifested regeneration of 100% calli originated from inflorescences, whereas in variants with immature embryos and mature caryopses this indicator varied within 30,0-45,5% (differences in all cases are reliable at $p = 0,001$). In *H. lechlery* H 1496, efficiency of regeneration was slightly lower than in *H. jubatum* K 1 and *H. procerum* I 491049, though this value in the variant with inflorescences significantly exceeded ($p = 0,001$) the corresponding indicator observed in this sample at the use of immature embryos.

Species of the section *Stenostachys*, as a rule, developed a dense nodulous callus (II type) from explants of different types regardless the efficiency of callus formation, with a trend toward lower efficiency of regeneration in calli obtained from young inflorescences than when using embryos (Table 3). Thus, in the variant with immature embryos of *H. depressum* W 309, this value was significantly higher than in the variant with its young inflorescences ($\chi^2 = 5,183$, $p < 0,05$). Within a sample, one type of regeneration was observed in calli derived from different explants (Table 3). Most of the studied forms performed a multiple regeneration (*H. marinum* 4 × - I 0118920, I 0118921, W 493, *H. parodii* H 1269, *H. patagonicum* H 1468, *H. californicum* I 559970). Both studied samples of *H. depressum* showed multiple regeneration accompanied with necrosis of callus. Single regeneration was performed only by the diploid *H. marinum* (Table 3).

The efficiency of regeneration from different types of explants was established to be dependant on current stage of development (optimal or non-optimal) on which they were isolated to be a source for primary callus (23, 25). It is common that size of an explant is used as a guide in such cases; therefore, the efficiency of regeneration can vary from experiment to experiment. However, qualitative characteristics of regeneration are not associated with any particular type of the explant.

Thus, the genus *Hordeum* was found to express three types of regeneration: single – in species the section *Hordeum* and in the samples of *H. cordobense* and *H. californicum*, multiple - in the section *Critesion* and majority of studied species from the section *Stenostachys*, multiple with necrosis of callus - in *H. depressum* and *H. chilense*. Type of regeneration is the specific feature of a sample developed independently on type of explant and the callus inducing medium.

REFERENCES

1. Nevskii S.A., Materials to the Knowledge about Wild Barley Species in Relation to the Origin of *Hordeum vulgare* and *Hordeum distichum* L. (Experience of Study of the Genus *Hordeum* L.), in *Flora i sistematika vysshikh rastenii*, Moscow - Leningrad, 1941, issue 5, pp. 64-255.
2. Bothmer R., Jakcobsen N., Baden C., Jorgensen R.B. and Linde-Laursen I., An Ecogeographical Study of the Genus *Hordeum*, IPGR, Rome, 1991.
3. Luhrs R. and Lörz H., Plant Regeneration In Vitro from Embryogenic Cultures of Spring and Winter Type Barley (*Hordeum vulgare* L.) Varieties, *Theor. Appl. Genet*., 1987, vol. 75, pp. 16-25.
4. Ziauddin A., Simon E. and Kasha K.J., Improved Plant Regeneration from Shed Microspore Culture in Barley (*Hordeum vulgare* L.) Cv. Igri, *Plant Cell Reports*, 1990, vol. 90, pp. 69-72.
5. Rikiishi K., Matsuura T., Maekawa M. and Takeda K., Light Control of Shoot Regeneration in Callus Cultures Derived from Barley (*Hordeum vulgare* L.) Immature Embryos, *Breeding Sci.,* 2008, vol. 58, pp. 129-135.
6. Powell W. and Dunwell J.M., In Vitro Genetics of Barley (*Hordeum vulgare* L.): Response of Immature Embryos to 2,4-Dichlorophenoxyacetic Acid, *Heredity*, 1987, no. 8, pp. 75-80.
7. Isaeva N.A., Shumnyi V.K. and Pershina L.A., The Study of Callusogenesis Characteristics of Different Barley Species, *Izv. Sibirskogo otdeleniya AN SSSR, Ser. Biologicheskie nauki*, 1980, vol. 1, no. 5, pp. 70-74.
8. Rotem-Abarbanell D. and Breimann A., Plant Regeneration from Immature and Mature Embryo Derived Calli of *Hordeum marinum*, *Plant Cell Tissue and Organ Cult.,* 1989, vol. 16, pp. 273-276.
9. Breiman A., Plant Regeneration from *Hotdeum spontaneum* and *Hordeum bulbosum* Immature Embryo Derived Calli, *Plant Cell Rep.,* 1985, no. 2, pp. 70-73.
10. Orton T.J., A Quantitative Analysis of Growth and Regeneration from Tissue Cultures of *Hordeum vulgare*, *H. jubatum* and Their Interspecific Hybrid, *Environ. Exp. Botany*, 1979, no. l, pp. 319-335.
11. Chernov V.E. and Pendinen G.I., Callusogenesis and Regeneration in Immature Embryo Culture of the Genus *Hordeum*, *Nauch.-tekhn. bul. VIR*, 1990, vol. 204, pp. 20-23.
12. Chernov V.E. and Pendinen G.I., Possibilities for the Use of *Hordeum* Wild Species in Ornamental Purposes and Peculiarities of Their Micropropagation, *Sci. Works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture*, 2001, vol. 20, no. 4/2, pp. 124-131.
13. Ellis R.P., Forster B.P., Robinson D., Handley L.L., Gordon D.C., Russel J.R. and Powell W, Wild Barley: a Source of Genes for Crop Improvement in the 21st Century?, *J. Exp. Botany*, 2000, vol. 51, no. 342, pp. 9-17.
14. Kartel' N.A. and Maneshina T.V., Callus Formation in Barley Plants with Different Genotypes (*Hordeum vulgare* L.), *Tsitologiya i genetika,* 1977, no. 11, pp. 486-490.
15. Pendinen G.I., Peculiarities of Micropropagation In Vitro of Barley-Wheat and Wheat-Barley Hybrids, *Sb. nauch. tr. po prikl. bot., gen. i sel*., Leningrad, 1989, vol. 128, pp. 39-45.
16. Nakamura C., Keller W. and Fedak G., In Vitro Propagation and Chromosome Doubling of *Triticum crassum* × *Hordeum vulgare* Hybrid, *Theor. Appl. Genet*.,

1984, vol. 60, pp. 80-96.
17. Chernov V.E. and Pendinen G.I., In Vitro Micropropagation of Interspecific Barley Hybrids, *Sb. nauch. tr. po prikl. bot., gen. i sel*., St.Petersburg, 1992, vol. 148, pp. 53-57.
18. Isaeva N.A., Pershina L.A. and Shumnyi V.K., Organogenesis in Callus Tissues of Barley Different Species and Interspecific Hybrids, *Izv. Sibirskogo otdeleniya AN SSSR, Ser. Biologicheskie nauki*, 1983, no. 5, pp. 76-81.
19. Lupotto E., Callus Induction and Plant Regeneration from Barley Mature Embryos, *Annals of Botany*, 1984, vol. 54, pp. 523-529.
20. Seguin-Swartz G., Kott L. and Kasha K.J., Development of Haploid Cell Lines from Immature Barley, *Hordeum vulgare*, Embryos, *Plant Cell Rep*., 1984, no. 3, pp. 95-97.
21. Chu C.C., Wang C.C., Sun C.S., Hsu C., Jin K.C., Chu C.Y. and Bi F.Y., Establishment of an Efficient Medium for Anther Culture of Rice trough Comparative Experiments of the Nitrogen Sources, *Scientia Sinika*, 1975, vol. 65, pp. 559-568.
22. Murashige T. and Skoog F.A., A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures, *Physiologia Plantarum,* 1962, vol. 15, pp. 473-497.
23. Zaitsev G.N., *Matematicheskaya statistiva v jeksperimental'noi botanike* (Mathematical Statistics in Experimental Botany), Moscow, 1984.
24. Dale P.J. and Deambrogio E.A., Comparison of Callus Induction and Plant Regeneration from Different Explants of *Hordeum vulgare*, *Z. Pflanzenpphysiol*., 1979, vol. 94, pp. 65-77.
25. Goldstein C.S. and Kronstadt W.E., Tissue Culture and Plant Regeneration from Immature Embryo Explants of Barley *Hordeum vulgare*, *Teor. Appl. Genet*., 1986, vol. 71, pp. 631-636.
26. Dunaeva S.E., Kovaleva O.N., Golubeva E.A., Moskalevea G.I. and Kozyreva O.G., Plant Regeneration from Cultured Immature Embryos of Early- and Late-Ripening Barley Cultivars, *Fiziologiya rastenii*, 2000, vol. 47, pp. 58-64.