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OBTAINING AND CYTOMORPHOLOGICAL CHARACTERIZATION OF LONG-TERM EMBRYOGENIC AND REGENERABLE WHEAT CALLUS TISSUES

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Two types of long-term highly embryogenic and regenerable calli has been obtained in tissue culture of several wheat genotypes: compact (CE) – from seven and friable (FE) – from three investigated cultivars. Both types of tissues maintained their embryogenic potential over 10-12 passages. Regeneration capacity of CE calli was very high (100 %) and took place spontaneously on the maintenance media with 1,0 mg/l 2,4-D. Regeneration capacity of FE calli was significantly lower (0-2 %) and took place only on differentiation media with BAP, kinetin and organic substances. It has been revealed, that the distinctions in regeneration ability of FE and CE calli were related to the peculiarities of their cytomorphological structure. Reproducible genotype-independent system for long-term regeneration in wheat tissue culture has been developed on the base of this investigation.

INTRODUCTION

Elaboration and widely application of cell biotechnology for the improvement of cereal crops is limited by two major reasons. First, in vitro culture of cereals shows strong genotype dependence and production of the appropriate culture type is generally limited to few cultivars or cell lines [1, 2]. Second, the majority of cereals cultures lose their morphogenic capacity after several subcultures [3]. Therefore, the one of the main goals of plant biotechnology is to develop genotype independent systems of long-term embryogenesis and regeneration in vitro for further application in biotechnology for the genetics improvement of important agricultural crops. The aim of this investigation was the obtaining and cytomorphological characterization of long-term embryogenic and regenerable callus tissues from different wheat genotypes and search of ways to overcome genotype limitation of processes of embryogenic potential maintenance.

MATERIAL AND METHODS

Immature embryos (1,0-1,4 mm long) of eight *Triticum aestivum* genotypes (Otan, Celinnaya 3S,

Akmola 2, Kazakhstanskaya 10, Kazakhstanskaya 15, Kazakhstanskaya 25, Bobwhite) were cultured on MS [4] medium with 2,4-D (1,0 and 5,0 mg/l). Morphogenetically perspective globular calli have been selected after one month and subcultured for the obtaining of long-term embryogenic calli by two ways: a) incubation on the MS medium supplemented with different level of 2,4-D (1,0 mg/l and 5,0 mg/l), 30 g maltose, 1000 mg/l casein hydrolyzate and 500 mg/l l-proline; b) incubation on the MS medium with high level of 2,4-D (5,0 mg/l) and KH_2PO_4 (1700 mg/l) and following subculture on the MS medium with 5,0 mg/l 2,4-D and normal concentration of KH_2PO_4 (170 mg/l) [5]. All cultures were incubated at 26±2 C? with a 16-h photoperiod and subcultured every 30 days. Regenerated shoots were transferred to rooting media with 0,5 mg/l NAA. Regenerated plants were grown to maturity in soil.

Different types of embryogenic callus tissues (28 days of culture, passages №5 and №10) were fixed for hystologycal study in Chemberlen fixing solution (ethanol-formalin-acetic acid) [6]. Paraffin sections (10-12 mkm) were stained with gematoxiline, Schiff's reagent and alcian blue according to Kamelina O.P. [6].

RESULTS AND DISCUSSION

Previous investigation of morphological heterogeneity and metamorphosis of wheat calli revealed that globular tissues are most morphologically stable callus type and was found as perspective for morphogenesis processes and universal for different genotypes [7]. In the present work we have used globular calli for the experiments on the obtaining of long-term embryogenic and regenerable wheat callus tissues. Long-term compact embryogenic (CE) calli were induced from globular tissues after incubation on the MS medium with different level of 2,4-D (1,0 mg/l and 5,0 mg/l) supplemented with 30 g maltose, 1000 mg/l casein hydrolyzate and 500 mg/l l-proline. Friable embryogenic (FE) tissues were derived from globular calli after subculture on MS medium with high level of 2,4-D (5,0 mg/l) and KH_2PO_4 (1700 mg/l) and following subculture on the MS medium with 5,0 mg/l 2,4-D and normal concentration of KH_2PO_4 (170 mg/l) [4].

Both the compact and friable embryogenic calli maintained their morphogenic capacity over many subcultures (over 10-12 passages). Type I calli has been obtained from all seven investigated cultivars, type II calli has initiated from three of seven tested genotypes (Otan, Akmola 2, Celinnaya 3S). Frequency of compact embryogenic calli formation varied for different genotypes and 2,4-D concentrations in the media (tab. 1). For example, high frequency of CE calli initiation on the media with both concentration of 2,4-D has been observed for cultivars Otan and Bobwhite (46,2 – 46,7% at 1,0 mg/l 2,4-D; 84,1 – 87,5% at 5,0 mg/l 2,4-D), low frequency – for cultivars Kazakhstanskaya 15 and Kazakhstanskaya 25 (19,7 – 22,5% at 1,0 mg/l 2,4-D; 44,0 – 58,8% at 5,0 mg/l 2,4-D). Frequency of CE calli formation on the media with 1,0 mg/l 2,4-D for all seven genotypes was higher (from 44,0 % up to 87,5 %), than on the media with 5,0 mg/l 2,4-D (from 19,7 % up to 46,7 %). Frequency of FE calli induction was very low and approximately equal for different genotypes (0 – 1,0%).

The table 1. **Frequency of compact embryogenic calli initiation from the globular type of wheat calli (*Triticum aestivum*)**

Genotypes	% frequency of compact embryogenic calli	
	1,0 mg/l 2,4-D	5,0 mg/l 2,4-D
Otan	84,1 ± 2,3	46,7 ± 1,9
Bobwhite	87,5 ± 1,6	46,2 ± 2,2
Celinnaya 3S	62,9 ±1,2	43,3 ± 0,8
Kazakhstanskaya 10	53,1 ± 1,8	23,6 ±2,0
Kazakhstanskaya 15	44,0 ± 1,5	22,5 ± 0,5
Kazakhstanskaya 25	58,8 ± 0,9	19,7 ± 1,4
Akmola 2	57,5 ± 1,6	28,0 ± 0,8

Despite of some distinctions in the frequency of CE calli formation embryogenic and regenerative potential of this tissue type were maintained during the long-term subculture (over 10-12 passages) at all investigated wheat genotypes. Regeneration capacity of CE calli was very high (100 %) and plant regeneration from the embryoids took place spontaneously on the maintenance media containing 1,0 mg/l 2,4-D. No any replacement on the regeneration media was necessary for the shoot formation in CE calli in the presence of 1,0 mg/l 2,4-D. Embryo differentiation and plant regeneration in the media with 5,0 mg/l 2,4-D were blocked and occurred only after the transfer on the media with 1,0 mg/l 2,4-D. Regenerated shoots have been grown on MS media with 0,5 mg/l NAA for the rooting and landed in a ground. All obtained plants were fertile and gave seed progeny.

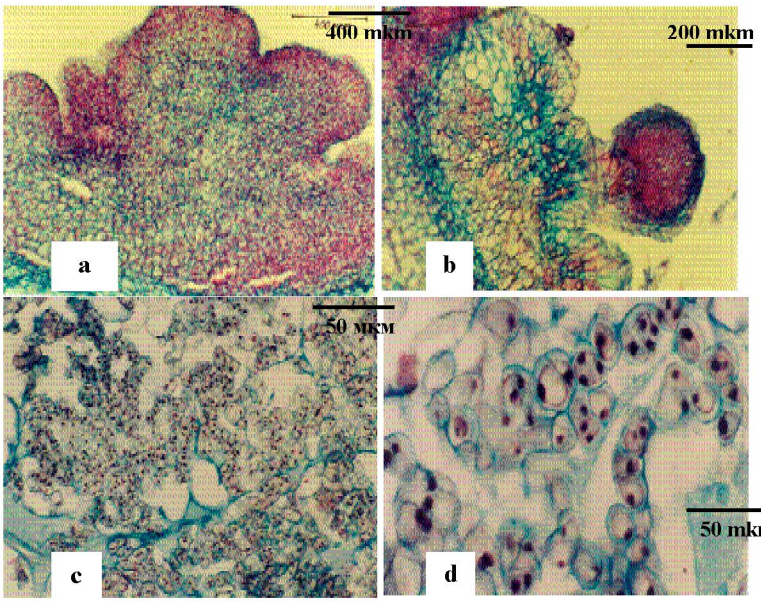

Figure 1. – (a) – General view of compact embryogenic (CE) calli;
(b) – Embryogenic complex and globular embryoid arising from CE calli (Type I);
(c, d) – Totipotent cells and 2-, 3-, 4-celled proembryoids of FE calli (Type II) situated in the dense net of mucilage

Morphogenic potential of FE calli was significantly different from CE tissues. FE calli produced embryoids on the maintenance medium with 5,0 mg/l 2,4-D during the 3-5 years, but the plant regeneration capacity of embryoids was difficult and took place very rare (0-2 %). Plant regeneration of FE calli took place only after subculture from the maintenance medium to the medium for embryoids differentiation supplemented with 0,5 mg/l BAP, 0,5 mg/l kinetin, 1000 mg/l casein hydrolyzate and 500 mg/l l-proline.

Microscopically, considerable morphological distinctions between the compact and friable embryogenic calli have been revealed (Figure 1).

Type I callus consists of large groups of densely packed parenchyma and meristematic cells, which produce embryoids in globular stage (Figure 1 a,b). Many vascular bundles are present at the base of the meristematic regions. The origin of embryoids in type I callus is not clearly visible in hystologycal preparations. We suppose that they have both multicellular and unicellular origin.

Type II callus completely lacks any vascular system and consists of friably disposed competent embryogenic cells, two-three celled proembryoids and globular embryoids (Figure 1c). All these cells and structures are isolated each from other and situated in the dense net of extracellular mucilage matrix. Presence of competent cells and two-three celled proembryos surrounded by thickened cell wall (Figure 1d) confirm unicellular origin of embryoids FE calli. The last make this callus type very useful for study of the regulation of somatic embryogenesis process from the one cell to embryoid and whole plant.

Thus, the distinctions in regenerative ability of FE and CE callus tissues on maintenance media could be explained by the difference of their cytomorphological organization. For example, somatic embryoids of CE calli reach the globular stage, differentiate and spontaneously regenerate green plants on the maintenance media with 1,0 мг/л 2,4-D. Embryoids in FE calli remain at the early stage of development - competent cells and two-three celled proembryos, which can not differentiate and produce plants because of embryo development arrest on the maintenance medium with high concentration of 2,4-D (5,0 mg/l).

On the base of the obtaining of compact embryogenic calli the system of stable long-term plant regeneration of has been elaborated. This system has been successfully tested by the use of six wheat hybrid combinations as well. It means that the developed system of long-term regeneration is genotype-independent and it allows overcome distinctions in morphogenic ability between the genotypes and hybrids. Therefore, developed system allows to overcome two difficulties in cell technologies: the dependence of morphogenesis processes in vitro from genotype and the loss of cells and tissues regenerative ability during the long-term subculture. These results confirm hypothesis that differences in cultivar response are physiological in nature and could be overcome by the selection of stable morphogenic tissues and optimization of media

composition [2, 8]. Developed in vitro system could be used for elaboration of cell biotechnologies for the genetic improvement of previously recalcitrant commercially important wheat cultivars.

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Резюме

Бидай ұлпа культурасында екі түрлі ұзақ мерзімді жоғары эмбриогенді және регенерацияға қабілетті каллус ұлпалары алынды: тығыз (ТЭ) – жеті генотиптеріне және борпылдақ (БЭ) – зерттелген жеті генотиптердің үшеуінен. Ол түрлердің екеуі де эмбриогенді қабілетін 10–12 пассажға дейін сақтайды. ТЭ каллустардың регенерациялыэ қабілеті өте жоғары (100 %), және өсу үрдісін сақтайтын 1,0 мг/л 2,4-Д бар қоректік ортада өсімдіктер өздерінен өзі пайда болады. БЭ каллустарда өсімдіктер регенерация үрдісі өте төмен (0-2 %) және дифференциацияға арналған БАП, кинетин және органикалық заттар қосылған қоректік ортаға көшіргеннен кейін ғана пайда болады. БЭ және ТЭ каллустардың әр түрлі регенерациялыэ қабілеттері олардың цитоморфологиялық құрылымының өзгешеліктеріне байланысты екені анықталды. Бұл зерттеудің нәтижесінде бидай ұлпа культурасында генотиптен егеменді ұзақ мерзімді регенерация әдісі жасалынды.

Резюме

Получены два типа длительно культивируемых высокоэмбриогенных и регенерационноспособных каллусов в культуре тканей некоторых генотипов пшеницы: плотный (ПЭ) – у семи, рыхлый (РЭ) – у трех из семи изученных генотипов. Оба типа тканей сохраняют эмбриогенный потенциал в течение более чем 10–12 пассажей. Регенерационная способность ПЭ каллусов очень высокая (100 %), и регенерация растений происходит спонтанно на среде для поддержания роста с 1,0 мг/л 2,4-Д. Способность к регенерации растений у РЭ каллусов очень низкая (0–2 %) и проявляется только после переноса на среду для дифференциации, содержащую БАП, кинетин и органические добавки. Различия в регенерационной способности РЭ и ПЭ каллусов связаны с особенностями их цитоморфологического строения. На основе результатов исследования была разработана воспроизводимая генотип-независимая система длительной регенерации в культуре тканей пшеницы.