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IMPROVED PLANT REGENERATION FROM MATURE EMBRYO DERIVED CALLUS OF WHEAT (*TRITICUM AESTIVUM* L.)

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Compact, yellowish and smooth callus of five wheat cultivars was obtained by culturing mature (ripe) embryos on MS (M u r a s h i g e and S k o o g, 1962) medium with 2.0 mg/l 2,4-D. Callus developed from the embryo axis and not from scutellum which turned brown. Differentiation took place on MS medium uppon decrease of 2.4-D concentration in the medium to 0.2 mg/l. Regeneration of whole plantlets from this callus was low ranging from 0 to 7% of explants irrespectivelly of the orientation of the embryo on the medium. However, the exscision of scutellum from the embryo significantly increased the percentage of explants (embryos) which regenerated plantlets. Depending on genotype within range from 9 to 22%. We believe that this increase of the regenerative ability of mature embryos is an important first step which will finaly enable mature embryos to be used as starting material in various *in vitro* techniques aimed at breeding new wheat cultivars and genetic engineering.

Key words: mature embryo culture, scutellum, somatic embryogenesis, *Triticum aestivum* L., wheat.

Ključne reči: kultura zrelih embriona, skutelum, somatska embriogeneza, *Triticum aestivum* L., pšenica.

INTRODUCTION

Wheat is one of the oldest and most important food crops in the world. It is a cereal grass of the Gramineae (Poaceae) family, genus *Triticum*.

During the past 20 years, numerous attempts have been made to obtain wheat tissue cultures which possess the capacity for efficient plant regeneration as a basic prerequisite for crop improvement programs. However, only a small number of plants have been obtained from cultures initiated from mature embryo and differentiated tissues (Shimada et al., 1969; Dudits et al., 1975; Bhojwani and Hayward, 1977; Chin and Scott, 1977). It has been showed that only calli derived from immature embryo scutellum manifest high-frequency regeneration of plants (Shimada, 1978; Shimada and Yamada, 1979; Gosch-Wackerle et al., 1979; Ozias-Akins and Vasil, 1982; Sears and Deckard, 1982; Lazar et al., 1983). Since the most responsive explant source – immature embryo is available only in a very short period of the year, then a procedure based on the use of mature embryos would be wellcome.

MATERIALS AND METHODS

Seeds of five cultivars, *Triticum aestivum* L.: Jugoslavija, Lepenica, Sana, San Pastore and Bankuty were surface sterilised sequentially with 95% ethanol (3 min), 20% commercial bleach (4-5% sodium hypochlorite) (20 min) and 0.2% mercuric chloride (15 min) and thoroughly washed with sterile destilled water. Mature embryos were excised under a binocular microscope. In treatment A, mature embryos of all five cultivars were placed on medium with axial side up (scutellum in contact with medium), in treatment B with the axial side down (scutellum exposed) and in C scutellum was removed and discarded.

The induction medium consisted of MS inorganic salts, 30 g/l sucrose, 100 mg/l inositol, 2.0 mg/l glycine, 0.5 mg/l B6, 0.5 mg/l nicotinic acid, 0.4 mg/l B1 and 2.0 mg/l 2,4-D. The medium was adjusted to pH 5.8 with NaOH, solidified with 0.64% agar, and autoclaved at 114°C for 20 min. Tissue cultures were cultured in plastic sterile Petri dishes (15 x 90 mm), each dish contained 40 ml medium and 8 explants or calli.

Temperature in the growth room was 25 ± 2°C, photoperiod 16/8 hours light to darkness and irradiance 33.5-46.5 μmol $m^{-2}s^{-1}$ provided by 65W 4500°K white fluorescent lamps.

After 28 d of incubation explants forming callus were transferred to differentiation medium supplemented with 0.5 mg/l 2,4-D. After next 28 d explants were transferred again to the medium where the hormone was reduced to 0.2 mg/l.

RESULTS AND DISCUSSION

Mature embryos of *Triticum aestivum* cultured on MS supplemented with 2.0 mg/l 2,4-D showed the initiation of callus within a week and at the end of 4 week, slowly proliferating, compact, smooth surfaced and yellowish calli were obtained. In all three experimental treatments (A, B, C) callus formation was associated with embryo axis, whilst scutellum, if present, turned brown and deteriorated within 1-2 weeks. According to histological investigations performed by Ozias-Akins and Vasil (1983a) in mature embryo callus arises from tissues within and near the procambium of the axis,

whilst in immature embryo callus is formed from parenchyma cells of the scutellum. Their results are in accordance with our findings that in mature embryos scutellum is not required for callus proliferation.

The frequency of callus induction in all five cultivars was similar and generally high, ranged from 89% to 100% (Tab. 1). Such a small variation indicate that the genotype plays no important role in the frequency of callus induction. This result is consistent with the report of O'Hara and Street (1978) and is in contrast with that of Lazar et al. (1983).

Tab. 1. – Frequency of callus induction, embryogenic callus formation and total number of regenerated plants from five Triticum aestivum cvs.: Jugoslavija, Lepenica, Sana, San Pastore and Bankuty after 8 weeks on MS medium with decreased 2,4-D (A – mature embryos with scutellum in contact with medium, B – mature embryos with scutellum that is not in contact with medium and C – scutellum-less mature embryos)

Treatment	Cultivar	Embryos	Calli		Embryogenic calli		Regenerated plants
		No	No	(%)	No	(%)	No
A	Jugoslavija	45	40	(89)	0	(0)	0
	Lepenica	45	42	(93)	1	(2)	2
	Sana	45	40	(89)	2	(5)	3
	San Pastore	45	43	(95)	1	(2)	4
	Bankuty	45	41	(91)	3	(7)	23
B	Jugoslavija	45	43	(95)	1	(2)	5
	Lepenica	45	45	(100)	1	(2)	8
	Sana	45	43	(95)	1	(2)	4
	San Pastore	45	44	(97)	1	(2)	2
	Bankuty	41	41	(100)	2	(5)	7
C	Jugoslavija	45	43	(95)	4	(9)	48
	Lepenica	60	57	(95)	10	(17)	108
	Sana	60	57	(95)	9	(16)	99
	San Pastore	60	59	(98)	13	(22)	73
	Bankuty	45	41	(91)	7	(17)	53

The obtain plant regeneration calli were transferred to media with decreased concentration of auxin. After 3-4 weeks of incubation some calli extensively produced only roots and others exhibited localised nodular area from which evantualy somatic embryos developed. Thus in wheat plant regeneration was coupled with somatic embryogenesis.

Appart from normal somatic embryos, often multiple shoots formed as a result of precocius germination of the primary embryo (Fig. 1 and 2). This is a common situation in wheat previously described by Ozias-Akins and Vasil (1982, 1983b).

In this case first the scutellum of the primary somatic embryo enlarges (leaffy scutellum structure) and then in its base numerous shoots appear. According to Ozias-Akins and Vasil (1982) multiple shoot formation from somatic embryos result from the absence of apical dominance.

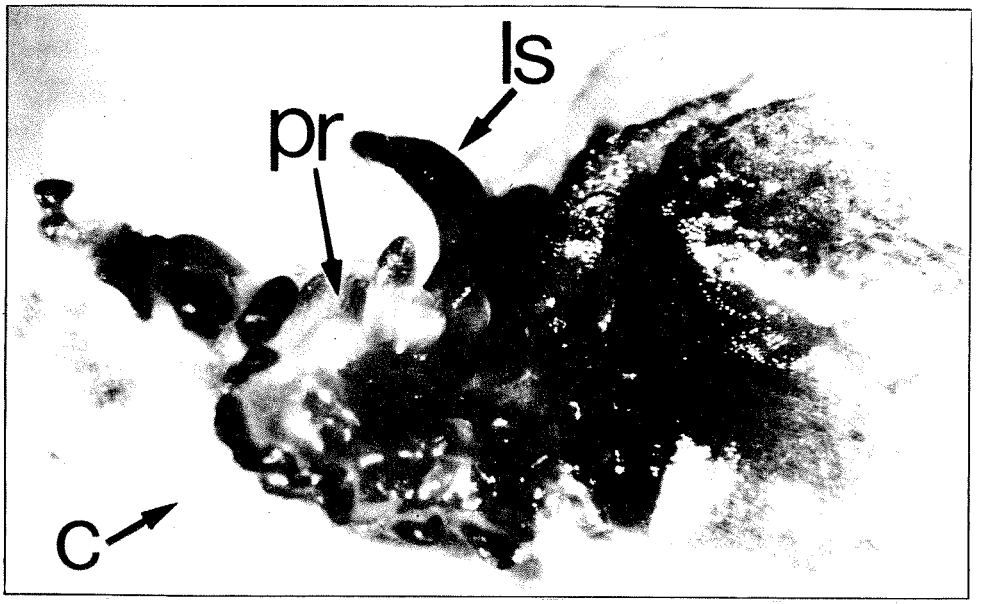

Fig. 1. – Callus derived from mature embryo with numerous shoot primordia after 4-8 weeks on regeneration medium (*pr* – shoot primordia, *ls* – leafy scutellum, *c* – callus)

The results of three treatments for callus formation and plant regeneration are presented in Table 1. The induction frequency of embryogenic callus of all 5 cultivars was significantly higher (≈ 10 times) when scutellum-less embryos were used (treatment C) instead of whole embryos (treatments A and B) as primary explants. In the third group genotypic variation could be observed since embryogenic calli production ranged from 9% (Jugoslavija) to 22% (San Pastore). Regenerative potential as indicated by the frequency of embryogenic callus formation obtained in this way was significantly increased in comparison to previously reported results of 0,4-3,2% (Lazar et al., 1983), 0-4% (Shimada and Yamada, 1979) and 7% (Chin and Scott, 1977).

Regenerative efficiency of the embryogenic callus of the cultivaras was very low in treatments A and B (with exception in cultivar Bankuty). This was not the case with calli derived from mature embryos without scutellum where we observed originated single plantlets and multiple shoot formation in evidently higher number. In cultivar Lepenica, 108 was a total number of regenerated plantlets and in cultivars Lepenica and Sana 34 plantlets were obtained from a single callus.

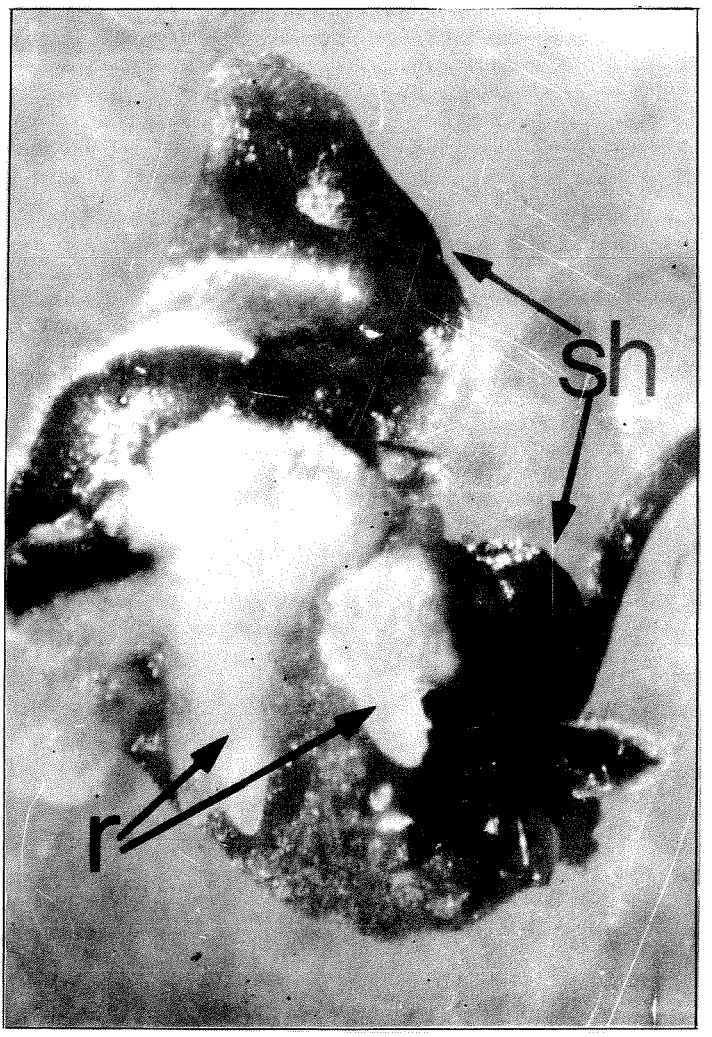

Fig. 2. – Regeneration of plantlets in callus derived from mature embryos (*r* – root, *sh* – shoot)

Results of this study show that we have improved the regeneration system for *Triticum aestivum* which enables a higher frequency of plant regeneration from callus obtained from mature embryos. With this findings and the main advantage of mature embryo explants – the availability thought the whole year, we open up the possibility of utilising this technique for studies related to wheat breeding program.

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Rezime

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POSPEŠIVANJE REGENERACIJE BILJAKA U KALUSU POREKLOM OD ZRELIH EMBRIONA PŠENICE *(TRITICUM AESTIVUM* L.)

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Kultivisanjem zrelih embriona 5 različitih kultivara pšenice na MS (Murashige i Skoog, 1962) medijumu sa 2.0 mg/l 2,4-D dobili smo žućkast, gladak kalus kompaktne konzistencije. Kalus se obrazovao od osnove embriona a ne od skuteluma koji je zadobijao braon boju i propadao. Diferenciranje je usledilo nakon smanjenja koncentracije 2,4-D u medijumu na 0.5 mg/l, a potom na 0.2 mg/l. Nezavisno od načina orijentacije embriona u odnosu na površinu podloge (skutelum okrenut ka i od površine) samo 0-7% kalusa regeneriše cele biljke dok je taj procenat značajno veći u grupi gde je skutelum na samom početku odstranjen i odbačen sa primarnog eksplantata (embriona) i u zavisnosti od genotipa iznosi 9-22%. Smatramo da ovo povećanje regenerativne sposobnosti zrelih embriona kultivisanih bez skuteluma otvara interesantan put korišćenja zrelih embriona u različitim *in vitro* tehnikama koje se koriste u genetičkom inžinjeringu i oplemenjivanju biljaka.