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STUDIES REGARDING CULTURE MEDIUM INFLUENCE ON IN VITRO REGENERATION FROM WHEAT IMATURE EMBRYOS

STUDII PRIVIND INFLUENŢA MEDIULUI DE CULTURĂ ASUPRA REGENERĂRII *IN VITRO* DIN EMBRIONI IMATURI LA GRÂU

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Surnamed „embryos' saving method", embryos culture is an in vitro technique used for over half of the century for saving the distant hybridization products, that would have degenerate in other conditions. Immature embryos culture is used for initiation of in vitro cultures imposed by the impossibility of using other explants for some of the plant species. Wheat is one of the crops that immature embryos culture technique is suitable for. This methods principle is based on aseptic embryos excision and their inoculation to an adequate culture medium. In vitro culture results depend in a greater manner of the basic culture medium and the hormonal balance used. Immature embryos isolated from two Romanian wheat cultivars – Dropia and Lovrin 41 – were inoculated for callus production on two types of basic media added with 2,4 D. The selected calluses were transferred on MS basic medium and several parameters were registered. Both cultivars emphasized a good callusing capacity, in a different percentage depending on the culture media used, such as 71,09 – 94,45%.. big differences between the cultivars regarding embriogenic callus frequency, shooting callus frequency and regenerated plants percentage were registered

Key words: immature embryos, culture medium, callus, regenerated plants.

Introduction

The success of a breeding program depends of the existent natural variability and of its extinction possibilities either by classical procedures either by unconventional methods, such as the biotechnological methods. Immature embryos culture is one of the unconventional methods used in this study.

Excision of the immature embryos can be made in different ways depending on the plant specie. Generally, an incision to the micropilar end of the young ovary is made, that permits embryos extraction by pressing the opposite end. This operation must be done very carefully to protect the embryo that might be hurt by pressing.

Wheat studies realized by Larking *et al*. (1984) proved that wheat callus obtained by immature embryos culture is the most efficient system for this species regeneration and was adopted as the standard method.

Materials and Methods

The immature embryos donor plants were grown in the experimental fields of USAMVB Timişoara. Spikes were yielded when the immature embryos were in the optimum developmental stage (0.8-1 mm). Immature embryos were sampled from the caryopses, which were before sterilized by immersion in mercuric chloride 0.1% and rinsed several times in distilled sterile water.

The explants belonging to both cultivars were cultured in Petri dishes on MS and B_5. Five spikes for each cultivar were inoculated on the culture media surface and the culture mode was one spike per Petri dish.

Hormonal balance was constituted of an auxine presence in the medium, such as 2.4 D, because wheat regeneration mode involves indirect somatic embryogenesis (table 1).

The calluses resulted over few weeks were subcultured on the fresh culture media, same chemical composition, every four weeks in order to ensure better growing conditions.

Table 1

Hormonal balances used for aseptic wheat cultures initiation

Culture system	Culture media	Hormonal balances			
		Lovrin 41		Dropia	
		V_1	V_2	V_1	V_2
Immature embryos culture	MS	2.5 mg/l 2.4D	1 mg/l 2.4D	2.5 mg/l 2.4D	1 mg/l 2.4D
	B_5	2 mg/l 2.4 D	0.5 mg/l 2.4D	2 mg/l 2.4D	0.5 mg/l 2.4D

Culture incubation, both for callus generation and for its growing, was realized in the growing room in the darkness at 24^{o}C.

The calluses were passed to MS medium supplemented with 1mg/l kinetin (KIN) for regeneration. Cultures incubation was done at 24^{o}C and a photoperiodic regime of 16 hours light. Regenerated plants were transferred on MS added with 0.2mg/l naphthalene acetic acid (NAA) for rooting. Rooted plantlets were acclimatized in soil and grown in the green house till maturity.

Results and Discussions

In vitro cultures of immature embryos depends on several factors such as the culture medium chemical composition, the genotype studied, the position of the embryos in the medium - that can be with the scutellum exposed (with the axial part downwards) in order to induce callus formation, or with the scutellum in contact with the culture medium surface (with the axial part onwards) for callus formation form the epiblast.

In this study two cultivars that are very well answering to this type of culture system were used. Two of the most known culture media were used for embryos culture namely Murashige-Skoog and Gamborg. Embryos position on the culture surface was with the scutellum downwards in contact with the medium because the regenerative callus masse is higher in this situation.

For culture initiation, the immature embryos from both cultivars were cultured on both basic culture media used in order to compare their efficiency. An auxin was added to the basic culture media in order to induce callus generation. The number of embryos inoculated was 285 for the cultivar Dropia and 291 for the cultivar Lovrin 41 respectively.

The parameters registered in these experiments were: embriogenic calluses (CE), shooting calluses (CL), the number of regenerated plants reported to shooting calluses (PR/CL) and the number of regenerated plants from the number of embryos inoculated (PR/EI).

Both cultivars used manifested callusing capacity, but in a different manner and depending on the culture media, being between 71.09% and 94.45%. High differences between the two cultivars were registered regarding the frequency of embriogenic calluses, shooting calluses frequency and the number of regenerated plants.

Analyzing the obtained results, can be observed that, for the cultivar Dropia the best results regarding the callusing capacity, the frequency of embriogenic calluses and shooting calluses frequency were registered on the basic medium MS while the number of regenerated plants reported to the number of shooting calluses was superior on B_5 culture medium (Fig 1). The highest number of plants regenerated in total for Dropia was obtained on the medium MS.

Regarding the cultivar Lovrin 41, the results shows that, comparing the two basic culture media used, superior callusing capacity (88.24%) were registered on the basic culture medium MS. Murashige & Skoog culture medium proved to be more suitable for embriogenic callus inducing as the best embriogenic calluses frequency results were obtained on this culture medium.

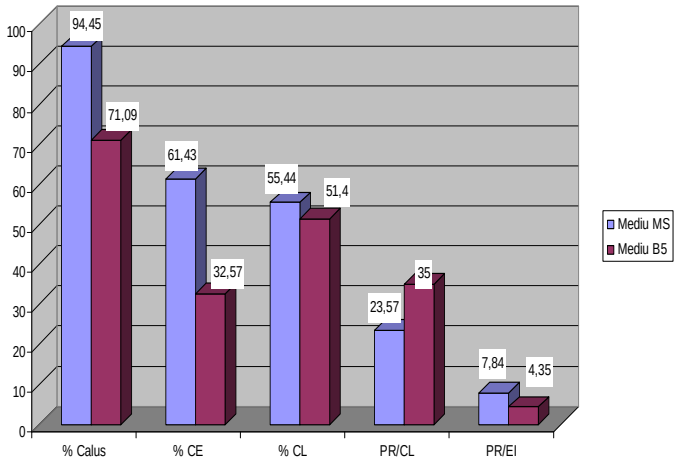

Fig.1 Graphic representation of experimental results obtained from *in vitro* immature embryos on MS and B_5 media, for the wheat cultivar Dropia

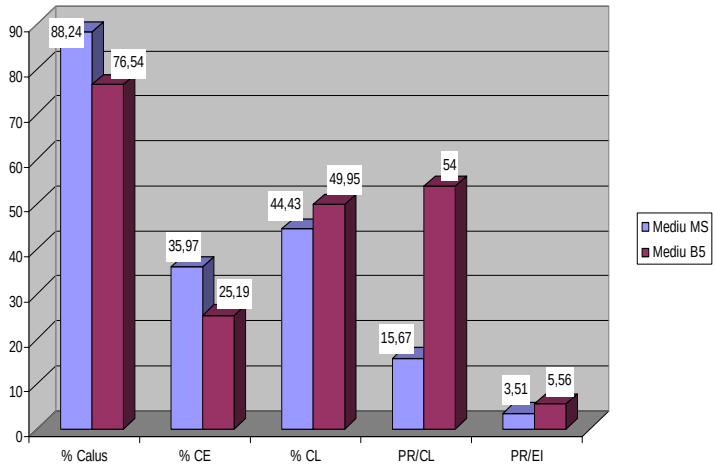

Fig.2 Graphic representation of experimental results obtained from *in vitro* immature embryos on MS and B_5 media, for the wheat cultivar Lovrin 41

Results obtained for Lovrin 41 on B_5 basic culture medium were superior to those obtained on MS for the parameters shooting calluses frequency and the number of plants regenerated from this type of calluses. But also the highest number of plants regenerated per total was registered on the medium B_5 for the cultivar Lovrin 41 (Fig 2).

Comparing both cultivars answer on both basic culture media used, on can observe that in general the best results were registered for the basic culture medium

MS. The results in the case of cultivar Dropia were superior for all the parameters registered comparing with the cultivar Lovrin 41.

Conclusions

1. The highest and the lowest callusing capacity were registered for the cultivar Dropia, 94.45% on the basic culture medium MS and 71.09% on the B_5 basic culture medium respectively.
2. The culture medium proved to be the decisive factor that influenced callusing capacity for the cultivar Dropia.
3. Both cultivars expresed the best results regarding callusing capacity on the basic culture medium MS added with 2.5 mg/l 2.4D.
4. Regarding embriogenic calluses frequency the best results were registered for the cultivar Dropia on MS basic culture medium – 61.43%, comparing with the cultivar Lovrin 41 that emphasized the lowest embriogenic calluses number on the basic culture medium B_5 – 25.19%.
5. The highest number of plants regenerated from the embriogenic calluses was registered for the cultivar Dropia on the basic culture medium MS – 11 while the lowest numbers were registered for the cultivar Lovrin 41 (5 plants) on the same basic culture medium.
6. The percentage of the plants regenerated from calluses with shoots varies between 15% to 54%, both values were registered for the cultivar Lovrin 41, on the cultures media MS and B_5 respectively.

Bibliography

1.**Badea Marcela Elena, Papa D., Lupusanschi I., Verzea M**. (1991) - *Study of regenerative potential and chromosomal variation in a long term culture of Triticum aestivum*. Probl. Genet. Teor. Apl. 3-4: 123-131.

2.**Larkin P.J**.(1984) - *In vitro cultures and cereal breeding*, In: Dordrecht, Boston, Lancaster, pp 273-286.

3.**Maddock S.E, Lancaster V.A., Risiott R., Franklin J**.(1983) - *Plant regeneration from cultured immature embryos and inflorescens of 25 cultivars of wheat (Triticum aestivum)*; J.Exp.Bot., 34: 915-926.

4.**Mohmand A.S., Nabors M.W**.(1990) - *Somaclonal variant plants of wheat derived from imature embryo explants of three genotypes*, Plant Cell Reports, vol 9, pp 558-560.

5.**Vrînceanu V., Judras Monica** (1980) - *Aplicații ale culturii de celule și țesuturi în ameliorarea plantelor*. Probleme de genetică teoretică și aplicativă, I.C.C.P.T. Fundulea.