

Effect of culture medium and cultivars on callus formation and plants regeneration from anthers and immature embryos culture of wheat (*Triticum aestivum* L.)

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Abstract Experiments were conducted to investigate callus induction and plant regeneration in two types of explants from two wheat cultivars Dropia and Lovrin 41. Explants used were anthers and immature embryos that give the best results when are cultured in vitro. Plants initiation and regeneration from cells and tissues cultured in vitro constitute a first stage but also an essential condition in using biotechnologies in order to develop different programs for cultured species breeding. Thus, callus initiation technique can be used in wheat breeding programs by inducing somaclonal variation generated by in vitro culture (1).

Key words

callus, anthers culture, immature embryos culture, culture medium

Somaclonal variation is an important source of genetic variability used in many situations to develop new agronomical, economical and industrial important genotypes. The best way to induce somaclonal variation is to generate plants from callus culture. In order to induce callus formation we used two culture systems that differ by the explant type used: anthers for experimental androgenesis and immature embryos for callus culture. Callus induction and also plants regeneration involved different culture media and the results showed which of them the best for the genotypes we used is. It is known that all the parameters in a in vitro culture system are genotype dependent thus it is very important to find the proper protocol for this.

Biological Material and Methods

The biological material was represented by two Romanian western wheat cultivars Dropia and Lovrin 41. Anthers and immature embryos from both cultivars constituted the initiation explants in order to generate callus induction using the two different culture systems. Callus initiation culture media starting from anthers were N₆ and P₂, and MS and B₅ when the initiation explants were represented by immature embryos, respectively.

Hormonal balance was different for each of the culture systems depending on the explant needs and

culture media used. Thus, auxin/cytokinin combination was used to generate callus from anthers, generally accepted being the fact that for cereals both hormones types are necessarily for callus induction from anthers. Only an auxin (2.4 D) when the explants were represented by immature embryos was used. Both anthers and immature embryos were carefully excised and immediately cultures on the media surface. The cultures were incubated in the growth room, in the darkness and 24°C, for several weeks, till callus formation was observed.

Results and Discussions

Results concerning callus induction and plants regeneration from anthers culture

In our experimental conditions, both genotypes manifested a relatively high callus generation capacity of about 81-95%.

It can be easily observed that callus generation capacity answer differs depending on the culture media, but also on the genotype. Thus for, a superior callus generation capacity was presented by the genotype Dropia on both culture media. Comparing the culture media used in this purpose the highest callus generation capacity was obtained for both genotypes on P₂ culture medium than on N₆ (Fig.1).

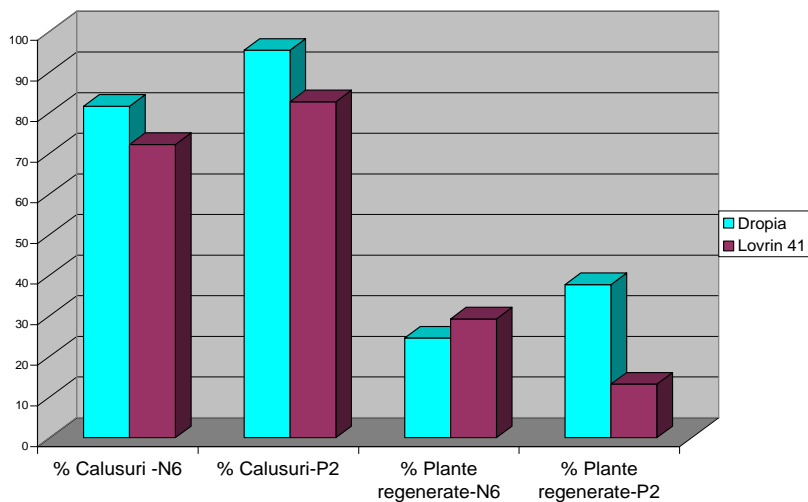


Fig.1 Results concerning callus and regenerated plants percentage from wheat anthers culture

Regenerated plants percentage is highly influenced by genotype and culture media used. Thus, figure 1 shows clearly this difference that the best results were obtained on P₂ culture medium for genotype Dropia whereas for Lovrin 41 the best results were obtained on N₆ culture medium.

Results concerning callus induction and plants regeneration from immature embryos culture

Callus is a particular formation constituted by a cells mass with a histological uniform structure.

Larkin and its collaborators (1984), obtained for the first time wheat callus starting from immature embryos, and proved that this system is the most efficient in cereals plants regeneration, thus for, this procedure was accepted as a standard method for wheat regeneration from *in vitro* culture (3).

In the same manner, as anthers culture, the immature embryos culture depends on different factors as culture media composition, genotype, and also embryos position on the media surface. The embryos were placed with their scutelum in direct contact with the culture medium, to induce callus formation from epiblast.

The graphic above shows that both genotypes present a high callus generation capacity of about 70%. It can be also observed that MS culture medium permits a superior callus generation capacity for both genotypes comparing with B₅ culture medium.

Analyzing comparatively both genotypes answers to *in vitro* culture, the superior results have been obtained by the cultivar Dropia for callus generation capacity, calli with shoots and regenerated plants percentages on MS culture media while Lovrin 41 had a better callus generation capacity and a higher regenerated plants percentage on B₅ culture media.

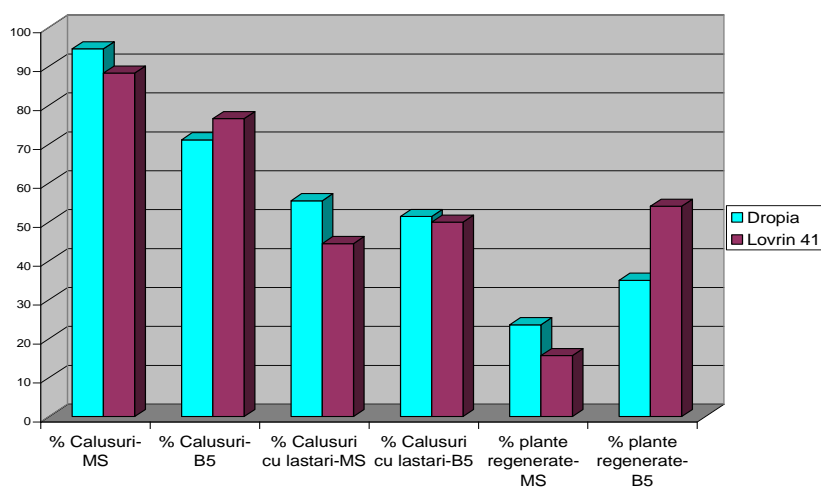


Fig.2 Results concerning callus and regenerated plants percentage from wheat immature embryos culture

These results can be explained by different chemical composition of culture media and the difference in nutritive substances between the cultivars studied. There are also differences between both genotypes for both culture systems explained by the different needs that each culture system require.

Conclusions

1. Both culture systems permit obtaining good results for wheat callus generation capacity and plants regeneration from callus.
2. Both callus generation capacity and plants regeneration rate are influenced by the culture system and genotype.
3. The highest callus generation capacity was obtained of the cultivar Dropia from anthers on P₂ culture medium.
4. The cultivar Lovrin 41 gave the highest regenerated plants percentage on B₅ culture media using immature embryos culture system.

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