# STUDIES CONCERNING THE *IN VITRO* CALLUSOGENESIS AT ALFALFA (*Medicago sativa*) GENOTYPES

# STUDII PRIVIND CALUSOGENEZA *IN VITRO* LA DIFERITE GENOTIPURI DE LUCERNĂ (*Medicago sativa*)

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***Abstract:*** *We use in this study three alfalfa genotypes cultivated in Romania (Topaz, Coral, Super). Our analyzed is reference to their ability to regenerate callus from different type of explants. Preliminary studies indicate that alfalfa in vitro response is strongly genotype dependent.*

***Rezumat:*** *În cercetările noastre am utilizat trei genotipuri cultivate în România (Topaz, Coral, Super). Studiile se referă la abilitatea de formare a calusului în funcție de tipul de explant utilizat. Studii preliminarii arată că in vitro lucerna răspunde diferit în funcție de genotip.*

***Cuvinte cheie:*** *cultura in vitro, calusogeneză, lucernă*
***Key words:*** *in vitro culture, callusogenesis, alfalfa.*

## INTRODUCTION

*Medicago sativa* is a leguminous plant species that originated in Asia and Iran. This plant species has been grown for a variety of purposes such as soil improvement, animal feed, medicinal uses and suitable foliage. It represents one of the oldest forage crops. Alfalfa is also a good nitrogen soil fixer, by symbiosis with bacteria of the genus *Rhizobium.* Alfalfa is also one of the most frequently studied crops from the point of view of tissue culture derived embryo production (CHEN, et al.). *In vitro* selection technology combined with spontaneous or active mutagenesis has been effective in altering or isolating genetic variability for desirable characters. It is also considered the species with the most advanced synthetic seed system, although its use for commercial propagation purposes is still under evaluation (PICCIONI et al., 1997). Moreover, significant progress has been made using genetic engineering methods for improving its nutritional value (SCHROEDER et al., 1991), to enhance tolerance to abiotic stress (MCKERSEI et al., 1993), and to use alfalfa as a source of value-added products. The present paper describes callusogenesis from three varieties of *M. sativa* L.

## MATERIAL AND METHODS

Seeds of three alfalfa genotypes Topaz,, Coral, Super, cultivated in Romania, were disinfected by immersion in a $70^0$ EtOH for 10 sec. followed by 0,1% $HgCl_2$ solution for 3 min, followed by 5 rinses in sterilized water. Seeds were placed on half-strength MS basal medium (MURASHIGE & SKOOG, 1962) to obtain aseptic material. We tested 10 individuals from each considered genotypes.

The explants (roots, petiole and leaflet), three weeks after culturing, were used to induction of callus. MS media with 2 mg/l 2,4 D and 0,5 mg/l kinetin was used. A high amount of callus has been generated for all of the individuals.

## RESULTS AND DISCUSSION

In our experiments frequency of callus production varied with type of explants. Petioles, roots and leaflets explants showed higher responses of callus production, being registered also differences between genotypes. (table 1)

*Table 1*

Experimental results concerning the callus process of the studied genotypes

| Genotypes | Explants | Callus process % | | |
|---|---|---|---|---|
| | | Period I (7 days) | Period II (14 days) | Period III (21 days) |
| Topaz (I) | Petioles | 80 | 10 | 10 |
| | Leaflets | 10 | 65 | 25 |
| | Roots | 90 | 5 | 5 |
| Coral (II) | Petioles | 70 | 15 | 10 |
| | Leaflets | 25 | 50 | 25 |
| | Roots | 85 | 10 | 5 |
| Super (III) | Petioles | 65 | 25 | 10 |
| | Leaflets | 35 | 50 | 25 |
| | Roots | 70 | 20 | 5 |

The induced callus percentage was higher for root explants with values ranging between a minimum of 70 for genotype Super and a maximum of 90 for genotype Topaz for the first observation decade. For the rest of observation periods, callusogenesis was reduced for all studied genotypes (fig 1)

Fig. 1. Callusogenesis at different genotypes of alfalfa from petiol explants

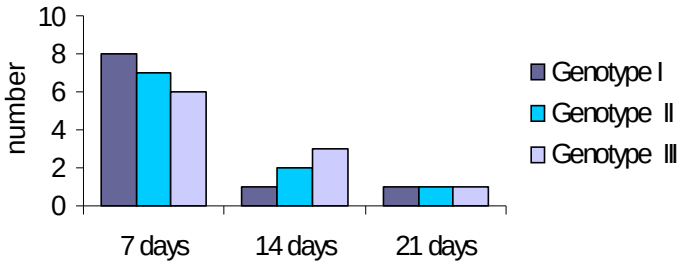
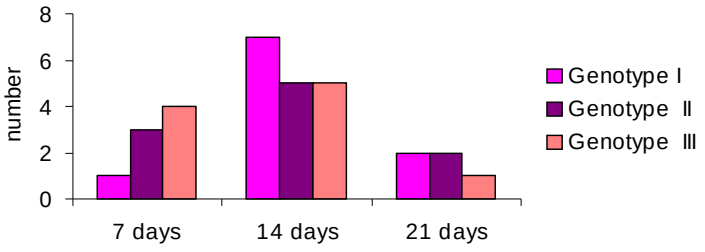


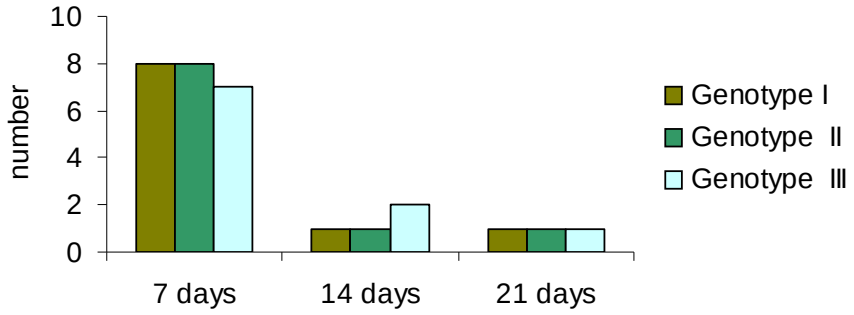

Fig. 2. Callusogenesis at different genotypes of alfalfa from leaflets explants



The use of leaflet explants demonstrated that callusogenesis is more delayed in the first decade for all studied genotypes. High rates were registered for the subsequent observation periods ranging between 65 for genotype Topaz and 50 for genotype Coral and Topaz (II period) and with 25% for all genotypes at the third period (Fig 2).

The use of root explants in case of all three genotypes taken into study proved effective inducing callus formation in the first decade ( with percentage between 70 - 90) and more reduced for the following decades (5 – 20 %depending on genotype) (fig. 3). In case of genotype Super and compared with the rest of studied genotypes, it has been emphasized very good callusogenesis.

Fig. 3. Callusogenesis at different genotypes of alfalfa from roots explants




**CONCLUSIONS**

Experimental results obtained, allow us to point few conclusions:

1. A high amount of callus has been generated for all of the individuals.
2. The roots are the best tissue for callus regeneration.
3. The leaflet explants generated a smaller amount of callus than another studied genotypes.

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