INDUCTION AND ACCELERATION OF THE IN VITRO PROLIFERATION OF THE AXILLARY BUDS (THE MULTIPLE AXILLARY SPROUTING) OF EIGHT GENOTYPES OF BIRD'S FOOT TREFOIL (*LOTUS CORNICULATUS L.*)

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Abstract

For the in vitro multiplication of the bird's foot trefoil explants we have used mini seedlings more exactly nodal segments with at least one axillary bud. Two types of medium have been tested which have been supplimented with auxines as well as with cytokinins. The medium used for the induction of axillary bud proliferation was Murashige-Skoog (1962) supplimented with different concentrations of hormones, realizing two variants of medium culture.. The basic MS medium has been supplimented with 30 g sucrose/l and 7 g agar/l for all the variants of hormonal concentrations.The pH of the medium had been adjusted to 5.8 before the agar was added. The in vitro evaluation of the nodal segment culture has been performed six week after the incubation and consisted in determining the following parameters: the number of formed sprouts and the length of the sprouts. The in vitro obtained plantlets have been divided and periodically redropped on the same type of medium. The inoculation of the explants had been done in jars. In each jar 4-5 explants have been inoculated. In what the number of sprouts is concerned as well as in what the length of the sprouts is concerned, through micropropagation, it is to be noticed that the Alina type stands out. In comparison with the other genotypes the Alina genotype shows a very special sprout formation tendency, following the propagation.

Key words: mini seedlings, *Lotus corniculatus L., in vitro*, bird's foot trefoil genotypes, sprouts, axillary bud.

INTRODUCTION

The in vitro regeneration and organogenesis at certain species of plants form an essential condition for those plants to multiply in a vegetative way.

The improvement programs for the perennial forage plants allow the *in vitro* culture application in order to select and multiply some productive genotypes, resistant to stress (Savatti et al., 2003).

The *in vitro* cultures with an important role in the improvement of the plants allow the selection and multiplication of some productive lines, resistant to biotic and abiotic factors. The *in vitro* culture has also got the aim to preserve some newly formed genotypes, to supervise their *in vitro* reaction, under their regeneration and multiplication and it also follows their capacity to obtain embriogenous gags from different explants according to the hormonal balance from the culture medium (Halmagyi, Butiuc-Keul, 2007).

MATERIAL AND METHOD

For the *in vitro* multiplication of the bird's foot trefoil explants we have used mini seedlings, more specifically nodal segments with at least one axillary bud. In order to obtain the highest number possible of clones it has been suggested to test two medium variants which have been each supplimented both with auxines as well as with cytokinins.

The medium used for the induction of axillary bud proliferation was Murashige-Skoog (1962) supplemented with different concentrations of hormones, realizing two variants of culture medium:

- V1 - MS (Murashige-Skoog) + 1.00 mg/l AIA (indolyl acetic acid) + 0.50 mg/l BAP (benzylaminopurine);
- V2 - MS + 1.00 mg/l AIA (indolyl acetic acid) + 1.50 mg/l BAP (benzylaminopurine).

The basic MS medium was supplemented with 30 g sucrose/l and 7 g agar/l for all the variants of hormonal concentrations. The medium's pH was adjusted to 5.8 before adding the agar. The hormones have been dissolved in ethyl alcohol or in sodium hydroxide. The used auxine (indolyl acetic acid- AIA) was dissolved in a small quantity of ethyl alcohol 96º, after which it was diluted with two times distilled water until obtaining a concentration solution of 10%. The cytokinins (benzylaminopurine - BAP), was also dissolved in a small quantity of 1 N of NaOH solution and then it was diluted with two times distilled water until obtaining the stock final solution with a concentration of 10%.

RESULTS AND DISCUSSION

The *in vitro* evaluation of the nodal segment culture was done six weeks after the incubation and consisted in determining the following parameters: the number of the formed sprouts and the length of the sprouts.

Table 1

The number of shoots/sprouts produced by micropropagation at the Bird's-foot trefoil

Genotype	*Culture medium*	
	MS-1.0 mg/l AIA + 0.5 mg/l BAP	MS – 1.0 mg/l AIA + 1.5 mg/l BAP
Suceava1	3.5	5.2
Suceava2	3.3	3.8
Suceava3	2.6	6.5
Suceava4	3.5	7.3
Suceava5	4.7	6.8
Nico	2.7	3.3
Alina	5.5	12.5
Danitim	2.6	3.4

The *in vitro* obtained plantlets have also been divided and periodically redropped (at about eight weeks) on the same type of medium. The

inoculation of the explants was done in jars. In each jar 4-5 explants have been inoculated. Six weeks after the inoculation the number of the sprouts obtained through micropropagation, at the eight studied genotypes of Lotus Corniculatus L. bird's foot trefoil is presented in table 1.

In case of some increased doses of BAP (benzylaminopurine) we can notice a proliferation of the number of sprouts in the case of Alina genotype, on the level of the soil, that is a number of 12.5 sprouts and for the Suceava4 and Suceava5 local populations there are 7.3 and 6.8 sprouts respectively. The length of the sprouts (cm) obtained through micropropagation, six weeks after the inoculation, is presented in the following table.

Table 2

Length (cm) of shoots/sprouts obtained through micropropagation at the Bird's-foot trefoil

Genotype	*Culture medium*	
	MS-1.0 mg/l AIA + 0.5 mg/l BAP	MS – 1.0 mg/l AIA + 1.5 mg/l BAP
Suceava1	4.5	6.0
Suceava2	5.2	6.3
Suceava3	3.7	5.5
Suceava4	4.6	8.2
Suceava5	4.5	8.0
Nico	3.2	4.3
Alina	4.3	8.8
Danitim	3.6	6.2

Fig. 1. Micropropagation of Alina genotype

The Alina type stands out once more in this case too, through the length of the sprouts (see Figure 1). At a dose of 1.5 mg/l BAP (benzylaminopurine) the sprouts develop until they reach a length of 8.8 cm. Suceava4 and Suceava5 genotypes have got a sprout length of 8.2 and 8.0 cm respectively.

CONCLUSIONS

From the point of vies of the number of sprouts as well as from the point of view of the sprouts' length, through micropropagation, the Alina

genotype stands out obviously. In comparison with the other genotypes, the Alina genotype shows a special tendency of sprout forming after the micropropagation process.

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