REGENERATIVE CAPACITY *IN VITRO* OF SOME EXPLANT TYPES OF DIFFERENT AGES IN BIRD'S FOOT TREFOIL

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Abstract

The experiments of the study were carried out using a bird's foot trefoil culture at the beginning of its flowering phase. The shoots chosen for explant harvest were of the same size, belonged to the same biotype, but to plants of different ages (I, II, III).

The two explant types used, apexes and nodes, were inoculated soon, approximately 24 hours, after being harvested from the donor plants (of different ages). The apexes were trimmed to 0.1-0.2 cm (basically a meristem), and the nodes cut very short, without internodal portions. Before inoculation, the plant material was sterilized for 30 minutes in 5% calcium hypochlorite solution with Tween 20 added, after which the solution was removed by repeated rising with distilled water.

The explants were inoculated in culture media consisting of macro- and micro-elements Fe-EDTA, Thiamine HCl – 2 mg/l, Pyridoxine HCl – 0.4 mg/l, meso-inositol – 252 mg/l, sucrose 22 g/l and agar 7 g/l. This basal culture medium (BM) was supplemented with two combinations of growth hormones, IAA – 0.002 mg/l + BA – 0.5 mg/l (medium abbreviated L1) and IBA – 1 mg/l + BA – 0.5 mg/l (medium abbreviated L2) respectively. The medium Ph was 6.8. After inoculating the explants in the two culture media, the vials with the inocula were kept at a temperature of 20-24˚C, for a photo period of 16 hours, at a luminous intensity of 2000 lux.

Key words: apexes, bird's foot trefoil, explants, *in vitro,* nodes

INTRODUCTION

In vitro cultures are used in plant breeding programs in order to select and multiply productive genotypes, resistant to climatic stress, diseases and pests. In the case of forage legumes, interesting results were obtained by selecting biotypes with increased capacity to fix atmospheric nitrogen, with bigger and more numerous root nodules (Zăpârţan Maria et al., 1989).

In the case of perennial forage legumes such as alfalfa, the factors involved in regeneration are considered to be the chemical structure, the quality and quantity of the growth regulators used and the low level of nitrogen in the regeneration medium (Zăpârţan Maria et al., 1989; Köteles N., et al., 2014).

MATERIAL AND METHOD

The experiments were carried out using a bird's foot trefoil culture at the beginning of its flowering phase. The shoots chosen for explant harvest were of the same size, belonged to the same biotype, but to plants of different ages (I, II, III).

The two explant types used, apexes and nodes, were inoculated soon, approximately 24 hours, after being harvested from the donor plants (of different ages). The apexes were trimmed to 0.1-0.2 cm (basically a meristem), and the nodes cut very short, without internodal portions. Before inoculation, the plant material was sterilized for 30 minutes in 5% calcium hypochlorite solution with Tween 20 added, after which the solution was removed by repeated rising with distilled water (Köteles N., et al., 2014).

The explants were inoculated in culture media consisting of macro- and micro-elements (Evans J. R., 1983) Fe-EDTA (McCay P.B. et al., 1984), Thiamine HCl – 2 mg/l, Pyridoxine HCl – 0.4 mg/l, meso-inositol – 252 mg/l, sucrose 22 g/l and agar 7 g/l. This basal culture medium (BM) was supplemented with two combinations of growth hormones, IAA – 0.002 mg/l + BA – 0.5 mg/l (medium abbreviated L1) and IBA – 1 mg/l + BA – 0.5 mg/l (medium abbreviated L2) respectively.

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RESULTS AND DISSCUSIONS

The inoculated bird's foot trefoil nodes and apexes were observed with regard to their behaviour *in vitro* concerning regeneration, multiplication and rooting, based on the hormonal balance in the culture medium and the age of the donor plant. The observations were recorded 6-8 weeks after inoculation. The results obtained in the case of culture medium (L1) are shown in Table 1.

Table1

Evolution of the *in vitro* regeneration process in bird's foot trefoil based on culture medium (L1) and age of mother plant

Nature of the explant	Culture medium	*Age of mother plant*	% Viability	New plantlets		Rooting		Obs.
				No. of new plant	Height (cm)	No. of roots	Root length (cm)	
Node	L1	1 2 3	93 96 94	2 3 3	4.1 4.0 4.3	- - -	- - -	Callus 0.1-0.3 cm
Apex	L1	1 2 3	63 65 84	6	2.8	-	-	Without root Callus

The results obtained in the case of culture medium (L2) are shown in Table 2. The data obtained show that the bird's foot trefoil has a good regenerative and multiplication capacity *in vitro*, strongly influenced by the hormonal balance of the culture medium (rhizogenesis in particular), regardless of the age of the explant. In the case of nodal explants, the neoformation of plantlets and multiplication *in vitro* on the culture medium L1, shows are high percentage of viability, above 90%, regardless the age of the donor plant. From each node 2-3 neo-plantlets are formed, of 3.8-4.3 cm without

root system, but with a callus sleeve of 0.1-0.3 cm around the explant. After three months of *in vitro* culture roots could not be observed, which shows that the hormonal balance of this medium disadvantaged rhizogenesis.

The evolution of the node based biological material on culture medium L2 shows a good *in vitro* viability, of 87-89%, a bit higher for the explants harvested from the 3-year-old donor plants. The number of neo-plantlets generated is lower, two plants/node, of 3.8-3.9 cm in height, but with rich root systems, thick roots, approximately 5-6 roots/explant, of 3.6-3.8 cm in length. The newly formed roots are thick, whitish, with thicker parts on them (similar to nodules).

Table2

Evolution of the *in vitro* regeneration process in bird's foot trefoil based on culture medium (L2) and age of mother plant

Nature of the explant	Culture medium	*Age of mother plant*	% Viability	New plantlets		Rooting		Obs.
				No. of new plant	Height (cm)	No. of roots	Root length (cm)	
Node	L2	1	87	2	3.8	6	3.6	Callus 0.1-0.3 cm
		2	87	2	3.8	6	3.6	
		3	89	2	3.9	5	3.8	
Apex	L2	1	60	5	3.4	4	2.7	Without root Callus
		2	61	3	2.8	3	2.5	
		3	68	4	3.0	3	2.0	

An essential and at the same time particular characteristic of the *in vitro* node evolution is the regeneration of one plant, rarely two, but in full conformity with their type, that is, leaves characteristic to the species. The bird's foot trefoil apex proved to have the highest regenerative and multiplication capacity *in vitro*, though the viability percentage is lower than in the case of nodes. This situation is probably the result of the trauma experienced by the meristem after trimming. On medium L1, the viability percentage varies with the age of the plant, increasing with age.

The tissue excised from the plants in their first year shows a viability of over 60%, from those in their second year 65%, and in the case of the third year 84%. The number of plantlets/apex is approximately 5-6, of 2.2-2.8 cm in height. Just like in the case of nodes, the apex explants also do not form roots on this medium, only a small green callus sleeve at their base.

On medium L2, the apex performs lower percentages of viability, but with significant differences against the age of the donor plant, with values increasing as the donor plant ages. On this medium a strong process of multiplication can be seen, with 5-7 neo-plantlets/apex, of 2.5-3.0 cm. At the base a small number of roots are formed, 3-4, with 2.0-2.7 cm in length. They are whitish, thin and with no thicker parts. It can be seen that the evolution of apexes is better than that of nodes in respect of multiplication, but the viability percentage is lower. The hormonal supplement consisting of IBA – 1 mg/l + BA – 0.5 mg/l is better balanced, causing both multiplication and the formation of an appropriate root system.

CONCLUSIONS

From those presented above it can be concluded that there is a strong correlation between the culture medium and the contribution of the growth regulators and the organogenesis process and root formation, influenced more or less by the age of explants. This last aspect is present only in the case of apex, which proves to have an influences on the *in vitro* regenerative process, being stronger for explants excised from older plants (3 years) than for those of one year.

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