THE REACTION OF THE *LOTUS CORNICULATUS L.* MERISTEM CULTIVATED *IN VITRO* ON LINSMAIER-SKOOG (LS) BASAL MEDIUM *CORNICULATUS L.*)

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Abstract

It was followed the reaction of the meristematic tissue detached from the young plants, from spring (March), of Lotus corniculatul L, which after disinfection were cultivated on the Linsmaier-Skoog, 1965 (LS) basal medium with the variants: M_o *= LS (control);* G_1 *= LS + ANA – 0.5mg/l + K - 1.0mg/l;* G_2 *= LS + ANA - 0.5mg/l + 2iP-1.0 mg/l;* G_3 *= LS + ANA - 0.5mg/l + K - 2.0mg/l;* G_4 *= LS + ANA - 0.5 mg/l + 2iP - 2.0 mg/l;* G_5 *= LS + ANA - 0.5mg/l +5 g/l GP;* G_6 *= LS + ANA – 0.5mg/l + 80 mg/l* $AdSO_4$*. After 60 days of in vitro incubation of the meristem, on the recalled variants, observations were made concerning the regeneration, multiplication and differentiation percentage of the radicular system. Among the experimented variants the best proved to be the presence of 2iP, in a concentration of 2mg/l (*G_4*) on which the meristem has regenerated and rooted in a percentage of 90% and has multiplied in a percentage of 95%. The variant with maize germ extract of 5g/l GP (*G_5*) reaches values which are better than the ones obtained on the variants with kinetin (*G_1 *and* G_3*) and namely 85% regeneration and rooting, and over 75% multiplication. Also, adenine sulfate,* G_6 *(with 80mg/l* $AdSO_4$*) has proved more efficient than the addition of kinetin (see Table 2 and fig. 1 and 2). We recommend the use of the meristematic explant of Lotus corniculatus L (bird's-foot trefoil) for the in vitro regeneration and multiplication and also the mediums with maize germ extract (GP-5g/l) and adenine sulfate,* G_6 *(*$AdSO_4$*-80mg/l), proving to be much more economical at the in vitro culture of the meristem, then the mediums with phitohormones.*

Key words: *Lotus corniculatus* L, meristem, Linsmaier – Skoog (LS) medium, regeneration, multiplication, radicular system.

INTRODUCTION

The ecotypes of bird's-foot trefoil (*Lotus corniculatus* L) have their origin in the Mediterranean region like most perennial forage legumes (Flora României, 1957; Dragomir, 1992). Like other forage legumes, bird's-foot trefoil occupies the entire coast range of the alpine regions (Varga et al., 1998; Borsos, 1976), the types and forms of *Lotus corniculatus* L. with a high genetic variability have a well established place in the genofond of the wild plant species, being considered very important species for the amelioration of this group of plants (Shaney, 1975), which made possible the establishment, over time, of the qualitative and productive value which proved as high as the one of other perennial species (Phillips, Collins, 1984). In Romania there are also many forms and ecotypes with an important role in the amelioration for obtaining new biotypes or cultivars with superior qualities (Dragomir, 1993). In our country, *Lotus corniculatus* L specie is cultivated either single or together with other perennial legumes

species (Dragomir, 1997). The interest of the ameliorators in the field of forage plants was directed towards following the in vitro behavior of the new genres or obtained lines.

Modern biotechnologies also comprise aspects related to the *in vitro* reaction of different plant tissues (Raicu, Dumitru, 1990), also the method presents interest for many species with economic value (Cachiță, 2007), which can multiply by automatic and robotic technologies. Extensive studies have pointed out the importance of some species of economic interest and the implications of plant biotechnologies in the success of the cultures of these species (Cachiță et al., 2004; Cachiță, Ardelean, 2009).

The legume forage species have been researched *in vitro* for establishing what is the biological value and also the reaction of the different types of explants and of the different species of ameliorated legumes in the country (Zăpârțan et al., 1991). At the *Lotus corniculatus* L. specie, a certain system of *in vitro* regeneration was followed (Zăpârțan, Savatti, 2004; Savatti et al., 2006), being studied also in terms of obtaining mutations after applying some chemical mutagenic agents (Zăpârțan et al., 2006), research based on the results concerning the technique of inducing mutations to another specie of forage legumes (Savatti et al., 2006). Our preoccupations continued with studies concerning the *in vitro* culture of *Lotus corniculatus* L specie insisting on the aspects related to the value of the germplasm, following the stimulation of *in vitro* germination (Köteles, Pereș, 2011), and also aspects of stimulating the regenerative, multiplication capacity and callus induction at bird's-foot trefoil (Köteles, 2011).

The present paper analysis the *in vitro* reaction of the bird's-foot trefoil meristem on the Linsmaier, Skoog (LS) basal medium, with a variable hormonal balance, in order to establish the regenerative and multiplication capacity of this tissue, and also the best hormonal mixture (the ideal variant) for the *in vitro* culture of the specie.

MATERIALS AND METHODS

Bird's-foot trefoil meristem was detached form field plants in March from young sprouts before the first scythe. The plant material was sterilized by following the subsequent steps: the rinse in alcohol 80% for 2 minutes; then the maintenance of the material in sodium hypochlorite solution 10% for ten minutes, followed by repeated rinses (about four rinses) with sterile water. After the detachment of the apical *meristematic tissue* of about 0.2 – 0.4 cm it was passed on the medium variants specified in table 1. In establishing the hormonal balance in this experiment we kept in mind the results obtained by us by the use of some phitohormones in a balanced mixture with citokinin and auxin on a MS basal medium (according to Murashige, Skoog, 1982), hence establishing the best hormonal balance

(Köteles, Pereş, 2012). The basal medium now experimented by us is LS (according to Linsmaier, Skoog, 1965), to which it was established the hormonal combination specified in table 1.

Table 1

Culture mediums used for the *in vitro* culture of the *Lotus corniculatus* L meristems

Var.	Basal culture medium	ANA (mg/l)	K (mg/l)	2iP (mg/l)	Admixture GP (g/l)	Admixture $AdSO_4$ (mg/l)	Regeneration (evaluation)
M_o	LS1/2	-	-	-	-	-	x
G_1	LS	0.5	1.0	-	-	-	xx
G_2	LS	0.5	-	1.0	-	-	xxxx
G_3	LS	0.5	2.0	-	-	-	xxx
G_4	LS	0.5	-	2.0	-	-	**xxxxx**
G_5	LS	0.5	-	-	5	-	xxxxx
G_6	LS	0.5			-	80	xxxx

(LS = medium according to Lnsmaier-Skoog, 1965: M_o = LS1/2 = control; ANA = naphtyl acetic-acid; K = kinetin: 2iP = 2-izopentyl-adenine; GP = maize germ extract; $AdS0_4$ = adenine sulfate)

RESULTS AND DISCUSSION

The observations were made after 60 days of *in vitro* culture and they concerned: *the regenerative capacity* of the bird's-foot trefoil meristem cultivated on the experimented variants; *the percentage of rooted neoplantlets* and the evolution of the regenerated explants under the aspect of *the number of neoplantlets and of their length*, and also *the value of the radicular system* after this period, the average of the results of the recalled parameters being presented in table 2.

Table 2

The *in vitro* evolution of the bird's-foot trefoil meristem (after 60 days)

Var.	No. of Plant	Length of plants (cm)	No. of roots	Length of roots (cm)	Regeneration %	Multiplication %	Rhizogenesis %
M_o	1	**2.8**	3	2.0	5	1	5
G_1	6	1.0	5	1.6	62	60	62
G_2	12	1.0	8	1.6	70	65	70
G_3	14	0.6	6	1.5	75	70	75
G_4	**25**	0.7	**12**	1.6	**90**	**95**	90
G_5	10	0.5	5	1.0	85	75	85
G_6	8	0.6	7	0.5	80	70	80

Following table 2 we can see the evolution of the bird's-foot trefoil meristem depending on the Linsamier-Skoog basal medium, on the hormonal components form the medium and on the admixture, in this case

maize germ extract (GP) and adenine sulfate ($AdSO_4$). The evolution of *the number of regenerated plants and roots* on the seven mediums is very different, the largest number of about 25 neoplantlets/meristem is obtained on G_4 medium, with the highest concentration of 2iP (LS + ANA -0.5 mg/l + 2iP – 2.0 mg/l), on this medium the number of roots also reaches the greatest average (about 12 roots/neoplantlet). In terms of the height of the bird's-foot trefoil neoplantlets we can see that on the control medium M_o (MS1/2) the plants are the highest, being known from the research literature that on simple mediums, without hormones and with halved salts, the distance between the nodes is greater (internodes are longer), so the stems are higher (Zăpârțan M. et. al., 1991), also the roots are longer. We can state that the variant with 2iP in the highest concentration (2 mg/l) proved to be the best, followed then by the variant with maize germs G_5 (5 mg/l GP), the values of the parameters on the other variants being lower but close.

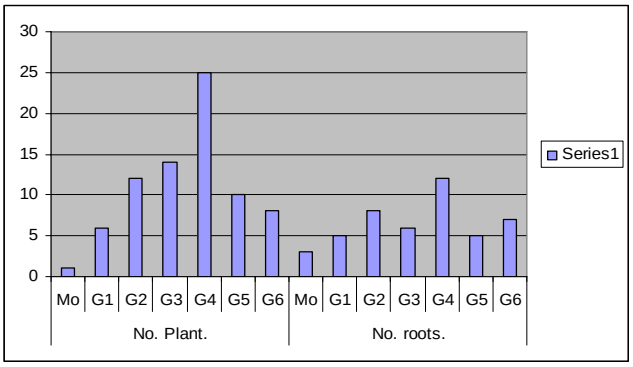

Fig. 1. The evolution of the number of neoplantlets and roots at the bird's-foot trefoil (*Lotus corniculatus* L) meristem cultivated *in vitro*

Figure 1 shows those stated above, highlighting variant G_4 in comparison with the other variants concerning the number of plants and of roots, followed by variant G_3 with 2iP in a lower concentration, this citokinin proving to be more effective than kinetin, effect also reported in the research literature (Cachiță D. et al., 2004). The effect of the maize germ extract GP (5 g/l) and of $AdSO_4$ (80 mg/l), in the concentrations mentioned in Table 1, proves superior to some variants which contain phitohormones.

The percentage of meristematic tissue regenerated, multiplied and with a differentiated radicular system is presented in a comparative manner in the three graphics with in figure 2. *The percentage of in vitro regenerated meristems* on the medium with a high concentration of 2iP, proved to be the best (see Fig. 2 A), determining a regeneration percentage of 90% on G_4 and of 85%, and respectively of 80% on the variants with maize germ (G_5) and adenine sulfate (G_6).

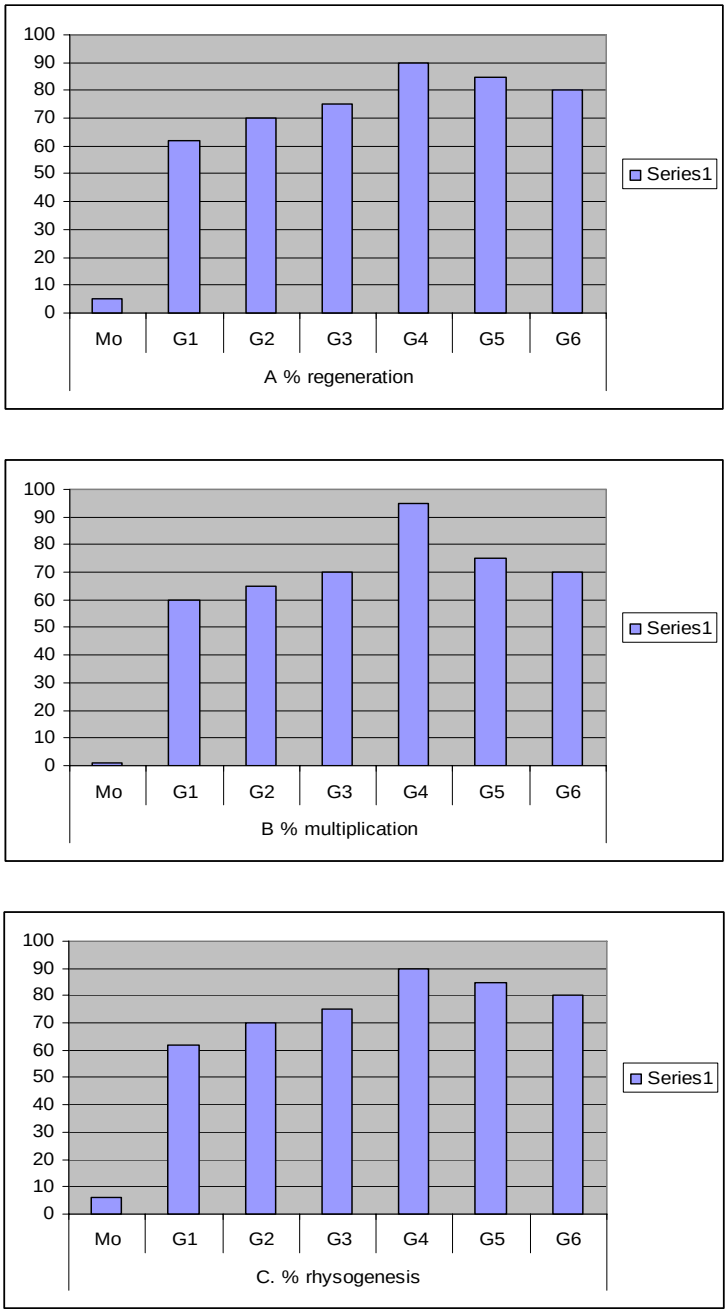

Fig. 2. The regeneration (A), multiplication (B) and rooting (C) percentage of the bird's-foot trefoil (*Lotus corniculatus* L) meristem cultivated *in vitro*

On the other variants the percentage is between 62-75%, depending on the composition of the medium, and on the control sample the percentage is of only 5%.

The multiplication percentage of the bird's-foot trefoil meristem after 60 days of *in vitro* culture is presented in fig. 2B, from which we can see some smaller values than for the regeneration, but a similar evolution. And at this parameter G_4 is being highlighted with the following composition: G_4 = LS + ANA - 0.5 mg/l + 2iP - 2.0 mg/l, on which multiplication exceeds 95%, and on the variants with additional maize germ extract (G_5 = LS + ANA - 0.5 mg/l + 5 g/l GP) the percentage is of 75%, and on the variant with adenine sulfate (G_6 = LS + ANA – 0.5 mg/l + 80 mg/l $AdSO_4$ and G_6) the percentage is of 70%. The beneficial effect of the adenine sulfate at the *in vitro* culture of the meristem of some species of white and red clover is well known (Vicaş G., 2011). For the same period of time, on the control sample there is no evidence of multiplication, remaining a single regenerated plant.

The rooting percentage of the neoplantlets is the highest also on the variant with a high concentration of 2iP (G_4 = LS + ANA – 0.5 mg/l + 2iP – 2.0 mg/l) on which we register a percentage of 90% rooted plantlets, in a number of 12 roots/neoplantlet of about 1.5 cm length. In this case also the additional admixture stimulates favorable the differentiation of the radicular system in a percentage of 85-80%. The experiments which tracked the role of the natural extracts in the *in vitro* culture of some species had as a purpose also the replacement of some phitohormones from the medium, hence proving to be efficient (Agud E., 2011). At the other variants, the percentage is somewhat smaller, but good (62-75%), in comparison with the control sample where the percentage reaches only 6% rooting (see Fig. 2C).

CONCLUSIONS

1. Bird's-foot trefoil meristem reacts *in vitro* depending on the hormonal balance and on the nature of the basal medium. It was found that the evolution of the meristem of this forage legume specie on the Linsmaier – Skoog (LS) medium is better than the one on the basal medium according to Murashige – Skoog (MS);
2. Among the citokinines from the composition of the medium, 2iP proved to be superior to kinetin especially in a concentration of de 2 mg/l (G_4) variant on which it was obtained the largest number of bird's-foot trefoil neoplantlets with an average of the related roots of 12 roots/plantlet;
3. The presence of the maize germ extract in the LS medium can substitute the citokinin necessary in regeneration and multiplication: on G_5 (LS + ANA – 0.5 mg/l + 5 g/l GP), the number of neoplantlets/meristem is over 10, each of them with about 5 small roots;
4. Adenine sulfate ($AdSO_4$) in a concentration of 80mg/l has a beneficial effect similar to a citokinin, on G_6 = LS + ANA – 0.5 mg/l +

$Ad.SO_4$ – 80 mg/l there can be obtained about 8 neoplantlets/meristem, with about 7 roots;

5. We recommend economical mediums for the *in vitro* multiplication of the bird's-foot trefoil, with low concentrations of citokinines or even without them, but with additional admixture of natural extracts: maize germs 5 g/l or adenine sulfate 80 mg/l ($AdSO_4$), with a law concentration of auxin (0.5 mg/l ANA).

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