REGENERATION AND IN VITRO MULTIPLICATION OF LOTUS CORNICULATUS L. SPECIES

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Abstract

The work consists of a large experiment initiated in vitro for the Lotus Corniculatus L. species starting from different detached explants from platns existing in a natural life environment (plains). We have followed the regeneration and multiplication capacity of these explants cultivated in vitro on a Murashige – Skoog environment made up of ten variants. In parallel we have used the witness evidence on which the results proved to be modest and we have used huge doses of phytohormones, 50 mg/l of BA and 2 mg/l of Zeatine on which a mass of knotty rhysogene callus had formed, this being an important evolution for the survey of the somatic embryogenesis phenomenon. The floral knot had shown a superior evolution and it regenerated in 100% on V_6, this variant proving to be the best out of the ten variants for all the explants.

Key words: *Lotus corniculatus* L, explants, regeneration, rhysogenesis, callus.

INTRODUCTION

The *Lotus corniculatus* L. species contains many ecotypes that have their origin in the Mediterranean area the same as the majority of the species from the fodder vegetable's group (Flora României - 1957; Dragomir, 1992, 1993). As the alfalfa and as the clover the bird's foot trefoil covers the whole coast area of the Mediterranean Sea (Varga P. et al., 1998; Borsos Sz., Olga, 1976). There are a lot of types and forms of Lotus Corniculatus with a high genetic variability and with a well established place in the wild vegetal species' genetic fund, many authors considering this species extremely important and having a qualitative and productive value as high as other evergreen fodder (Shaney B.B., 1975; Phillips G. C., G.B. Collins, 1984).

In our country there are a lot of gene types with a very important role in the improvement works in order to obtain new plant cultures with superior qualities. In the Romanian space and area the *Lotus Corniculatus* L species is cultivated either alone or together with other evergreen vegetable species (Dragomir N., 1997; M. Savatti et al., 2004). Starting with the development of biotechnologies and of fodder vegetable species a lot of studies have been done in order to establish which is the biological value and the *in vitro* reaction of some improved cultivated plants (Savatti M. et al. 2006). There were also studies related to the behaviour of the *in vitro* species that followed the regeneration capacity (Zăpârţan Maria, M. Savatti,

2004) or related to obtaining mutations after applying some chemical mutagenic substances (Zăpârțan Maria et al., 2006). The current work also follows the in vitro reaction of the different bird's foot tresoil explants on a great number of environments with varied hormone balance in order to establish the best environment formulas of *in vitro* culture for this species.

MATERIALS AND METHODS

The vegetal material had been obtained from the fodder vegetable collection of the Veterinary Medicine and Agricultural University from Cluj-Napoca, the Alina type being improved in this institution. The tissue cultivated in vitro was formed of the following explants: floral bud and knots detached from mature plants collected from the plain, plants which after having been sterilized were inoculated on the culture environment. The sterilized environment used for the culture of these plants was a basic MS environment (after Murashige T., A**.** Skoog**,** 1962), supplied with different hormones (Cachiță D., 2007; Raicu P., O. Dumitru, 1990). The explants have their origin on the plains and fields and their size expressed in millimeters was of approximately 0.4 – 0.5 mm.

Table 1

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

Variants	**Main culture environment**	**A u x i ne s**		**Cytoquinine**		**R e g e n e r a t i o n (bonus)**
		IAA	**IBA**	**BA**	**Z**	
M	MS1/2	-	-	-	-	x
V_1	MS	0.5	-	0.5	-	xx
V_2	MS	-	0.5	-	0.5	xxx
V_3	MS	0.5	-	-	0.5	xxx
V_4	MS	-	0.5	0.5	-	xx
V_5	MS	0.5	-	1.0	-	xxx
V_6	MS	-	0.5	-	-	xxxxxx
V_7	MS	0.5	-	-	1.0	xxxx
V_8	MS	-	0.5	1.0	-	xxxx
V_9	MS	-	0.5	5.0	-	Callus induction, rhysogenesis and embryogenesis
V_{10}	MS	-	0.5	-	2.0	xxxx

(M = control; IAA = indol acetic-acid; IBA = indol butyric – acid; BA = benzylaminopurine ; Z = Zeatine)

The culture environments are presented in table 1 which contains the type of phytohormone (cytoquinine or auxine) and the hormone balance (phytohormone concentration) for the ten variants.

RESULTS AND DISCUSSION

The aim of the work which is to follow the in vitro regeneration capacity of the bird's foot trefoil explants had been evaluated after 40 days

from the in vitro culture and contained the following parameters: the regeneration percent (number and length of the plants), the capacity to differentiate the radicle system (number and length) according to the variant composition and to the explant type, being known that 50 explants had been inoculated for each variant. The results obtained related to the multiplication and to the regeneration are presented in table 2 and 3 respectively. The MS environment culture supplied with 0.5 mg/l IBA + 1.0 mg/l Z (V_6) is the best variant. Good results had also been obtained on the following variants V_7, V_8 and V_{10}. The callus that has been induced on the V_9 variant (0.5 mg/l AIB + 5 mg/l BA), is an important tissue which can induce the somatic embryogenesis, being known that high concentrations of cytoquinine stimulate this process (Savatti M., et al., 2006).

Table 2

The in vitro evolution of the bird's foot trefoil floral bud

Variant	**No. Plant**	**Length of plants (cm)**	**No.roots**	**Length of roots (cm)**	**% Regeneration**	**% Rhysogenesis**
M	1	2.5	2	2.0	5	6
V_1	22	1.2	5	1.9	15	20
V_2	18	0.9	8	1.6	13	82
V_3	19	0.5	6	1.8	24	23
V_4	17	0.7	11	1.5	20	85
V_5	40	0.3	5	1.2	40	21
V_6	58	0.8	7	0.4	100	29
V_7	56	0.7	5	0.8	85	32
V_8	46	0.7	9	1.0	83	40
V_9	-	-	2	0.2	Callus	13
V_{10}	49	0.4	5	0.5	40	35

The evolution of the explant from the bud is presented in table 3 from which one can notice that on the V_6 environment we have obtained the 100% regeneration percent (see figure 1) and a number of approximately 58 neoplantula/explant. On the V_{10} variant (MS + 0.5 mg/lAIB + 2.0 mg/Z) a great number of neoplantula /explant had also been obtained (approximately 50) and on the environments that contained AIB the formation of the radicle system is stimulated (approximately 9-11 roots/explant). On the AIB environments the rooting percent is between 82 – 85% (see figure 2).

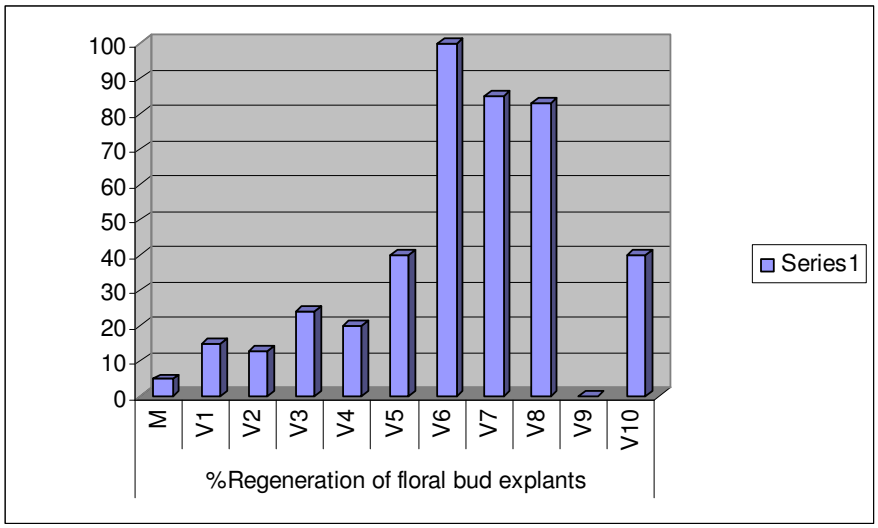


Fig. 1 Regeneration percent of the bird's foot tresoil floral bud on the experimented environments

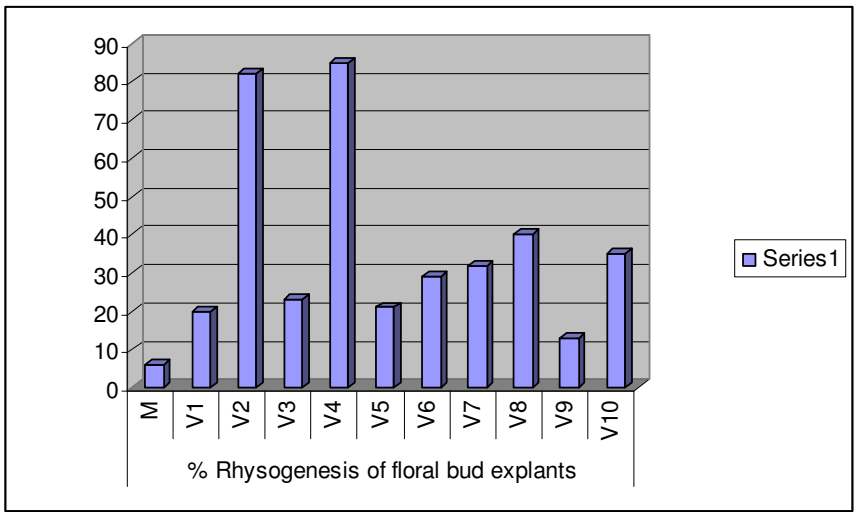


Fig. 2 Rooting percent of the neoplantula obtained from floral bud

The evolution of the knot explant is presented in table 3. The highest number of neoplantula/explant had also been obtained on V_6 that is an average of 32 neoplantula. The knot explant reacts better to moderate phytohormone concentrations thus, on V_7 and on V_8 when adding 1 mg/l Zeatine a number of 30 neoplantula had been obtained.

Table 3

The in vitro evolution of the bird's foot tresoil knot explant

Variants	No. of Plants	Length of plants (cm)	No. of roots	Length of roots (cm)	% Regeneration	% Rhysogenesis
M	2	2.1	2	2.0	2	4
V_1	4	1.8	3	1.8	8	28
V_2	5	1.8	9	2.0	19	88
V_3	6	1.6	3	1.7	60	25
V_4	5	2.0	9	1.5	37	86
V_5	10	0.8	4	1.0	31	27
V_6	32	1.5	6	0.5	**80**	41
V_7	30	1.1	4	0.7	**81**	39
V_8	30	1.5	4	0.7	**81**	28
V_9	-	-	2	0.1	Callus	8
V_{10}	8	0.9	2	0.3	31	25

The regeneration percent of the knot explant reaches maximum values of 80% on the 1 mg/l cytoquinine environments (BA or Z) and with one auxinic (V_6, V_7 şi V_8); on the small doses of cytoquinine the percent is lower (see figure 3). The rooting process of the neoplants that come from the knot are directly dependent on the presence of the butyric indolil acid (AIB), in combination with the small dose of BA or Z, this can reach 86 – 88% (see figure 4).

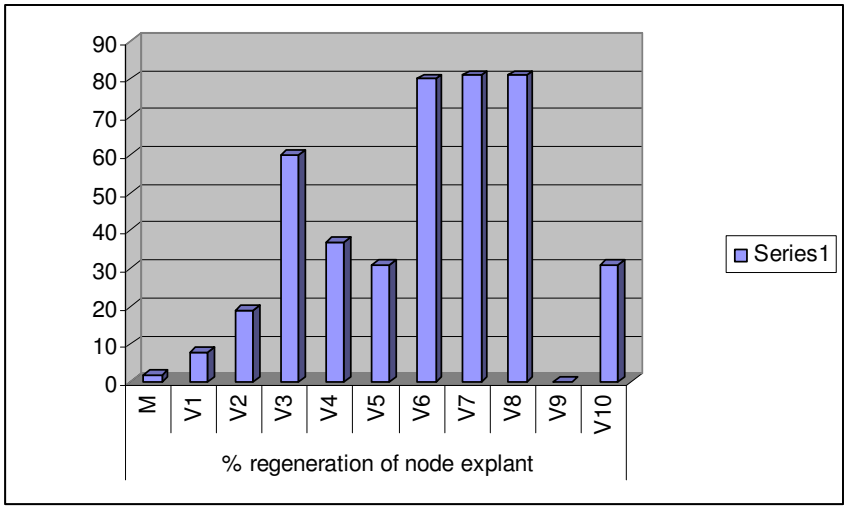


Fig. 3 The in vitro regeneration percent of the bird's foot tresoil knot

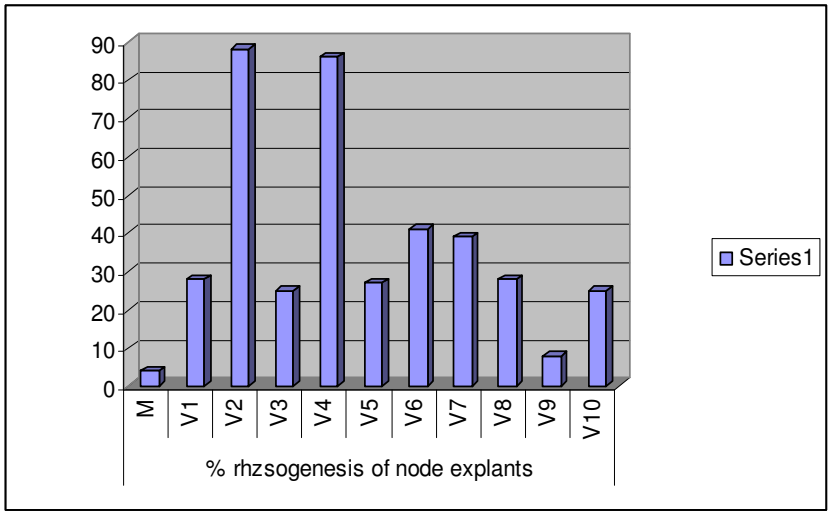


Fig. 4 The rooting percent of the new plantula from the knot

CONCLUSIONS

The explants from floral bud and from bird's foot trefoil knot collected from the field showed a good regeneration and *in vitro* multiplication according to the nature and concentration of phytohrmones.

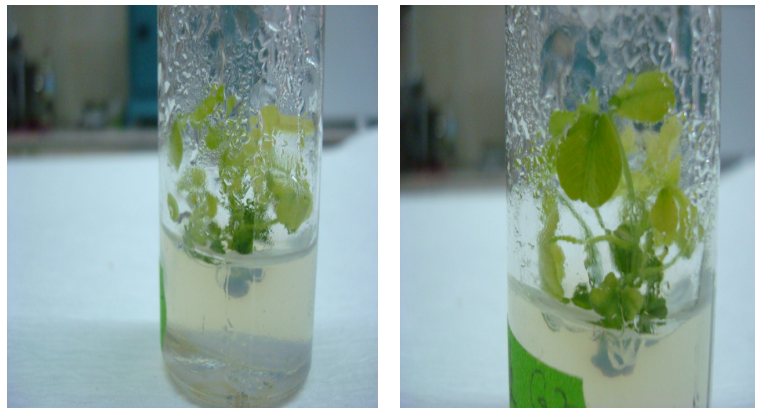
The *in vitro* regeneration percent of the bud floral explant reached the maximum percent of 100% on V_6 (MS + AIB - 0.5 mg/l + 1 mg/l Z), this environment formula proving to be the best.

The evolution of the knot explant is inferior to the budstill the regeneration process on the environment with the dose of 1 mg/l cytoquinine (Z or BA), the percent reaches 80%, and the number of multiplied plants at approximately 30 neoplantula/explant.

The Murashige – Skoog environment supplied with 0.5 mg/l AIB + 1.0 mg/l Zeatine is the best variant for the regeneration and for the *in vitro* multiplication of the bud and of the bird's foot trefoil knot.

On the V_9 environment with a high dose of cytoquinine - 5 mg/l BA the callose rhizogenous was formed and the different small knots.

For the *in vitro* regeneration of totally standard bird's foot trefoil we recommend floral or bud explant or knot cultivated on the MS environment supplied with 0.5 mg/l AIB plus 1.0 mg/l zeatine (V_6).



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4

Photo . 1,2,3 and 4: bird's foot trifoil neoplantula on an environment without phytohormones

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