THE INFLUENCE OF CYTOQUININE IN THE IN VITRO MORPHOGENESIS AT THE BIRD'S FOOT TREFOIL (LOTUS CORNICULATUS L.)

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Abstract

We have followed the in vitro behaviour of some explants (apex,knot, floral bud) -taken from bird's foot trefoil on culture environments with benzyladenine (BA) and izo-pentynol-adenine(2iP) in different concentrations with an auxine adding (ANA) for the basic environment Murashige-Skoog (MurashigeT., F.Skoog, 1962). The apex generated completely formed plantlets that had total rooting in all the experimental variants (T_0 – T_4) especially on the T_2 and on T_4 environments. The knots, although they led to the formation of new plantlets are numerically inferior in comparison with the apex. A tendency of callose has been noticed at the basis of the new plantlets once the 2iP dose has been increased. Although the floral bud regenerates in a lower percentage it still forms the highest number of new plantlets with a very well emphasized radicle system. The results obtained show the necessity to use moderate doses of cytoquinine and of auxine in the basic environment.

Key words: *in vitro* morphogenesis, bird's foot trefoil, regeneration, cytoquinine, auxine.

INTRODUCTION

Some valuable genotypes' *in vitro* ability of regeneration constitutes a current major preoccupation in the improvement of plants (Varga P. and collaborators., 1998).

The bird's foot trefoil, as well as other fodder vegetables has the possibility, through the *in vitro* cultures, to lead to the creation of a valuable biologic material with increased possibilities of mass vegetative multiplication. The *in vitro* regeneration and the organogenesis form an essential condition to the fulfillment of this aim which is definitely influenced by endogenous and exogenous factors. The nature of the used phytohormones, their concentration and the hormone balance have an important role in the organogenesis processes (Cachiță Cosma Dorina, 1987; Savatti M., Maria Zăpărțan, 1991; Zăpărțan Maria and collaborators., 1989).

The current work has in view to explain the *in vitro* reaction way of the bird's foot trefoil explants under the influence of two cytoquinine elements: the benzyladenine (BA) and the 2 izopentyl-adenine (2iP) related to the regeneration ability and to the organogenesis of this species.

MATERIAL AND METHOD

The meristems have been harvested from the Alina type of bird's foot trefoil plants, type multiplied in greenhouses and completely developed from apex, knot and floral bud. After a 30 minute disinfection of the meristems with 5% calcium hypochlorite and Tween 20 and after the plants have been repetedly rinsed with sterile water they have been placed on a MS (Murashige-Skoog) culture environment in order to regenerate, this environment having also been completed with different stimulative substances according to table 1.

Table 1

Basic environments used in the bird's foot trefoil explants' culture

Elements mg/l	For regeneration	For multiplication
Macro elements	MS	MS
Micro elements	MS	MS
FeEDTA	MS	MS
Meso-inositum	100 mg	100 mg
Thiamine	0.5	1.0
Pyrodixin	0.5	1.0
Nicotinic acid	0.5	1.0
Sugars	30 g/l	30 g/l
Agar	7 g/l	7 g/l
Aden sulphate ($AdSO_4$)	40 mg/l	80 mg/l
pH	5.7	5.7

MS = basic environment Murashige-Skoog, 1962.

The culture environment shown in table 1 has been completed with a hormone balance experimented for the bird's foot trefoil explants (table 2).

Table 2

The hormone balance experimented in the case of the bird's foot trefoil meristems

Variant	Basic environment	Cytoquinine	Concentration mg/l	Auxine	Concentration mg/l	$AdSO_4$ mg/l	Bonus
T_0	MS	-	-	-	-	40	xx
T_1	MS	BA	1.0	ANA	0.5	40	xxxx
T_2	MS	BA	2.0	ANA	1.0	80	xxxxx
T_3	MS	2iP	1.0	ANA	0.5	40	xxxx
T_4	MS	2iP	2.0	ANA	1.0	80	xxxxx

The environments have been abbreviated from T_0 to T_4, T_0 being the witness variant formed by the hormoneless MS basic environment and the other variants with a balanced hormone scales each cytoquinine being completed by a quantity of auxine (ANA) acid-naphthyl-acetic in doses of 0.5 – 1.0 mg/l. A quantity of 40 – 80 mg/l doses of $AdSO_4$ hormone have

been added to the basic environment (MS) and to the above mentioned variants ($T_0 – T_4$).

The light regime from the growing room has been of 16 hours out of 24. The light was provided through strip lighting with an intensity of 2.600 1x. The average temperature has been of 25 – 27ºC. The observations have been done at about six weeks after the inoculation.

RESULTS AND DISCUSSIONS

After the explants have been re-cultivated on the environments mentioned in table 2 a series of macroscopic parameters have been followed related to their development and growing, to the number of regenerated plants, to their conformation, to the regeneration percentage, to the presence of the radicle system, to the callose tendency, etc (table 3).

Table 3

The *in vitro* morphogenesis of the bird's foot trefoil explants cultivated on environments with BA, 2iP and ANA

Variant	Explant	Regeneration percentage %	Number of plants	Size (cm)	Number of roots	Length of the root (cm)	Other types of tissues
T_0	Apex	80	1	1.5	1-2	2.0	-
T_1		83	2	1.1	3	2.5	-
T_2		52	5	1.2	4	1.5	-
T_3		90	2	1.0	3	2.5	-
T_4		94	3	1.0	6	2.0	-
T_0	Knot	68	2	1.1	2	1.5	-
T_1		78	6	1.0	4	1.0	-
T_2		80	6	1.0	-	-	callose (50%)
T_3		84	8	1.0	4	1.5	-
T_4		88	6	0.8	-	-	callose(30%)
T_0	Floral bud	49	2	0.8	2	1.0	-
T_1		60	7	1.5	5	1.0	-
T_2		54	12	0.8	2	0.5	callose
T_3		63	10	1.5	6	0.5	-
T_4		58	10	0.5	2	0.5	callose

From the data expressed in table 3 one can notice an obvious difference of the explants' reaction in what the regeneration aspect is concerned, oscilating between 49 – 94%.

In the apex case the results obtained show that this tissue has got a good regeneration ability (80 – 90%). Comparing the T_0 variant, without growing hormones with those variants in which growing hormones have

been introduced, the regeneration tendency is obvious: the T_4 variant overcoming the witness variant (T_0) with 14% and the T_2 variant overcame the witness one with 12%. The obtained plantlets are completely standard even in what the radicle system is concerned especially in the presence of 2iP and ANA, an auxine that is known to be strongly implied in rhizogenesis (Cachiță Cosma Dorina, 1987). On the BA environments the apex has also got a good regeneration ability but it is weaker than in the case of 2iP environments.

It has been noticed that the use of the knot meristems present a pretty great regeneration ability even on the environments without hormones. In the case of this explant, on the cytoquinine environments the highest number of regenerated plants have been obtained. At a concentration of 0.5 – 1.0 mg/l 2iP a regeneration percentage of 84 – 88% can be noticed. The radicle system, though stimulated by the presence of ANA 0.5mg/l, in case the cytoquinine's concentration increases one can notice that there is a lower tendency of the rootlets to form at about 30 – 50% of the knot explants generating a callose muff.

On one hand, the *in vitro* evolution of the bird's foot trefoil floral bud presents a regeneration in a lower percentage in comparison with the explants harvested from the apex or from the knot, that one being of 49 – 63%. On the other hand on the environments with higher cytoquinine concentrations (BA and 2iP) the highest number of new plantlets are formed (10 – 12 newplantlets per plant) surrendered by a callose tissue that negatively influences the development of the rootlets.

CONCLUSIONS

Summarizing the data obtained in our experiment related to the *in vitro* behaviour of some bird's foot trefoil eplants (apex, knot, floral bud) on MS culture environments completed with two cytoquinines (BA and 2iP) in different concentrations and with an ANA auxine adding we can mention the following:

1. The meristems harvested from the apex regenerate completely formed plants, with multiplication and rooted in all the variants (T_0 – T_4), and they have proved to be the most suitable for the *in vitro* vegetative multiplication of the bird's foot trefoil;

2. In what the knot meristems were concerned they were able, on almost all the variants to produce new plantlets but per cent they were inferior in comparison with the apex. When there are high concentrations of cytoquinine in the basic environment this substance leads to callose thus inhibiting the rhizogenesis;

3. The floral bud shows a lower percentage of regeneration, 49 – 63% but it forms the highest number of new plantlets (10 – 12) with an

appropriate radicle system. In case the doses of cytoquinine are increased then there is a tendency of the callose to form;

4. The results obtained make us recommend the use of moderate doses of cytoquinine in the (MS) basic environments which favorize the regeneration of explants and we recommend small doses of cytoquinine that favorize the multiplication of explants. The use of a 40 mg/l adenine sulphate ($AdSO_4$) dose favorizes the rhizogenesis process in the case of the bird's foot trefoil new plantlets.

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