THE IMPLICATIONS OF TIDIAZURON (TDZ) IN THE INDUCTION OF CALLUS AND PLANT REGENERATION IN THE *DIANTHUS SPICULIFOLIUS* SCHUR. VARIETY

Laslo Vasile*, Zăpârţan Maria*, Agud Eliza*, Vicaş Simona*

* University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048, Oradea, Romania: laslovasile@yahoo.com; mariazapartan@yahoo.com

Abstract

The study followed the implication of tidiazuron (TDZ) in the induction of embryogenic callus formation and plant regeneration from this callus. Three different concentrations of TDZ (0.5, 1.0 and 2.0 mg/l) were introduced in the basic Murashige-Skoog environment, each combined with an auxine (ANA), in a single 0.5 mg/l concentration. If on the witness sample (MS), the regeneration of a completely conformed plant occurs, on the variant with a 1 mg/l dose of TDZ, formation of a callus mass of circa 4 cm ø occurs, green, slightly firable, which after 40 days generates 2-3 small but completely conformed plantlets. In the higher concentration, of 2.0 mg/l TDZ, the nodal explant generated the same mass of olive green callus, friable, with a high number of embryoids on its surface. The fresh and dry weights, as well as the water content of the tissue highlighted the relationship between the callus water content and its friability. The uniformity of results regarding the obtainment of embryogenic callus and of plants from callus made possible the conlusion that TDZ has implications on the formation of embryogenic callus (V_3) and on the regeneration of plants from callus (V_2). Thus, tidiazuron in the culture medium, în moderate concentration of 1 mg/l, behaves like a cytochinine, with real implication on the regeneration of plants from callus.

Key words: *Dianthus spiculifolius* Schur. tidiazuron (TDZ), naftil acetic acid, embryogenic callus, embryoids, regeneration, fresh weight, dry weight, water quantity.

INTRODUCTION

Studies regarding the *in vitro* evolution of callus in some plant species have lately led to the elucidation of some issues regarding somatic embryogenesis. Thus, organ genesis can now be initiated from embryogenic callus (Ammieato, 1987); remarkable results were also obtained by the efficient induction of *in vitro* regeneration via callus (Cardona, Duncan, 1997). In a wider range of species of varying natures, a higher plant regeneration frequency from embryogenic callus was also observed (Luo et al., 1999), as well as the induction of embryogenic callus in some ornamental plants (familial Liliaceae, specia Agapanthus), with the tissue's response to the selective action of some agents (Suzuki, 2000). The callus of varieties like *Dianthus* has been studies from the perspective of growth regulators involved in *in vitro* organ genesis, as well as the genotype (Chamani et al., 2007; Kallak, 1997), thus establishing the most reactive variety and the optimal hormonal balance. The Dianthus caryophyllus L species also

supported the induction of somatic embryo-genesis via callus (Karami, 2008), highlighting the role of cytochinines in the process.

Dianthus spiculifolius Schur. is an endemic specie in Romania, vulnerable and going extinct. In order to extend its presence in cultures, it was successfully reproduced *in vitro*, like other endangered species, analyzed from a genotype standpoint, environment composition and *ex vitro* adaptation ability (Zăpârțan, 2000). For the *in vitro* culture of this specie, the role of natural extracts on explants was tested, in order to replace phytohormones (Butiuc-Keul, Deliu, 2000) and also to observe the behavior of different types of explants on mediums with varied hormonal balances.

MATERIALS AND METHODS

The implications of tidiazuron (TDZ) were observed in the formation of callus in the *Dianthus spiculifolius* Schur. Variety and in the plant regeneration via callus, using *nodal tissues*, detached from plants obtained *in vitro*. The basis of our experiment lies in the results concerning tidiazuron (a potential cytochinine) in the culture of species which have a slow reaction or are reluctant to *in vitro* culturing (Huerreman, Preece, 1993). In other studies, the introduction of callus and plant regeneration in the Musa sp. variety, triploid form, was attempted, by adding tidiazuron to the Murashige-Skoog medium (Srangsam, Kanchanapoom, 2003). The substance was also experimented with, in association with an auxine, in order to obtain direct embryo-genesis from the leaf explant, in the Oncidium variety (Tsung et al., 2001). The reaction of the callus of some species to repeated transfers on mediums with enhanced hormonal balances (modified) was also observed with great interest (Hurgoiu, Cachiță, 2000).

The culture medium for inoculation of the *Dianthus spiculifolis* Schur. Nodal tissue was made up from the basic medium after Murashige – Skoog-1962, to which an auxine in a constant concentration was added (0.5mg/l ANA) and tidiazuron in three different concentrations (0.5, 1.0 and 2.0 mg/l). The mediums are presented in Table 1, in which the experimented working formulas are also lsited. After inoculation, the explants were kept in growing chamber conditions, and observations were made after circa 40 days.

Table 1

Culture environment of the nodal explant of *Dianthus spiculifolius* Schur.

Var.	Basic medium	ANA mg/l	TDZ	Explant type/ variety *Dianthus spiculifolius* Schur.
Mt.	MS	-	-	Basal node
V_1	MS	0.5	0.5	Basal node
V_2	MS	0.5	1.0	Basal node
V_3	MS	0.5	2.0	Basal node

MS = medium after Murashige – Skoog; ANA = α naftil acetic acid; TDZ = tidiazuron

RESULTS AND DISCUSSION

40 days after the incubation of nodules on tidiazuron mediums, the regenerative ability of the node was observed along with the callus differentiation, the size of the callus mass, the color, consistency, the formation of embryoids, the fresh and dry weight of the callus, as well as water contents of the tissue, along with other particular aspects appearing at the level of differentiated callus mass (necrosis). The evolution of the nodes is listed in Table 2.

Table 2

The evolution of nodal explants of *Dianthus spiculifolius* on TDZ enhanced mediums (after circa 40 days)

Var.	Regeneration	Ø callus mass (cm)	Color	Consistency	Particular aspects
Mt.	1 plant of circa 2cm with -5 thin roots				-
V_1	callus	2,8 ± 0, 2	Dark green (Fig. 2)	Hard	Callus harness (Fig. 2 and 10)
V_2	Callus	4,0 ± 0,1	Greenish (Fig. 4)	Slightly friable	Regeneration of 2-3 plantlets of circa 0.5cm with 3-4 leaves. (Fig. 4, 5, 7, 9)
V_3	callus	4,3 ± 0,1	Olive green (fig. 6)	Friable	High number of embryoids, embryonic cones (fig. 1, 3, 4, 6, 8,)

On the witness sample, on Murashige-Skoog (Mt) medium only, plant regeneration was observed of around 2 cm, with afferent root system (thin, fragile roots, a mean number of about 5 roots/plant); it is known that the variety does not encounter problems regarding *in vitro* regeneration (Zâpărţan, 1995). However, on TDZ variants, callus differentiation was observed, with friability directly proportional to the concentration of tidiazuron in the medium. On the variant with a small dose of TDZ (0.5 mg/l) and ANA (V_1), a callus mass was differentiated, with a diameter of circa 2,8 cm, dark green, solid, with high hardness. On V_2, with 0.5mg/l ANA + 1mg/l TDZ, the differentiated callus mass reaches circa 4 cm in diameter, it is greenish and slightly friable. From this callus mass, 2-3 plantlets regenerate of circa 0.5 cm, with 3-4 afferent leaves. On V_3 (MS+ 0.5mg/l ANA + 2.0mg/l TDZ) the callus mass is slightly larger (ø > 4.0 cm), olive green, friable, with a high number of differentiated embryoids on the callus surface.

Table 3

TDZ effect on the quantity of fresh substance – dry substance, and water content resulted from the callus tissue (after circa 45 – 50 days)

Var.	Fresh weight (g)	Dry weight (g)	Water content (g)	Creditworthness
Mt.	-	-	-	-
V_1	**4,6** ± 0,4	**0,2877** ± 0,0001	1,0 1 ± 0,0009	xxxxx
V_2	3,5 ± 0,5	0,1797 ± 0,0008	1,93 ± 0,0007	xxxx
V_3	4,0 ± 0,7	0,1317 ± 0,0005	**2,82** ± 0,0007	xxxxx

Given sufficient callus samples for each variant, determination of *callus mass weight (fresh, dry and water content)* in the tissue was performed. Table 3 presents the mean of the values of these parameters and the creditworthness of these variants, as well as Fig. 1 graphically presenting the mean values of these parameters for each variant.

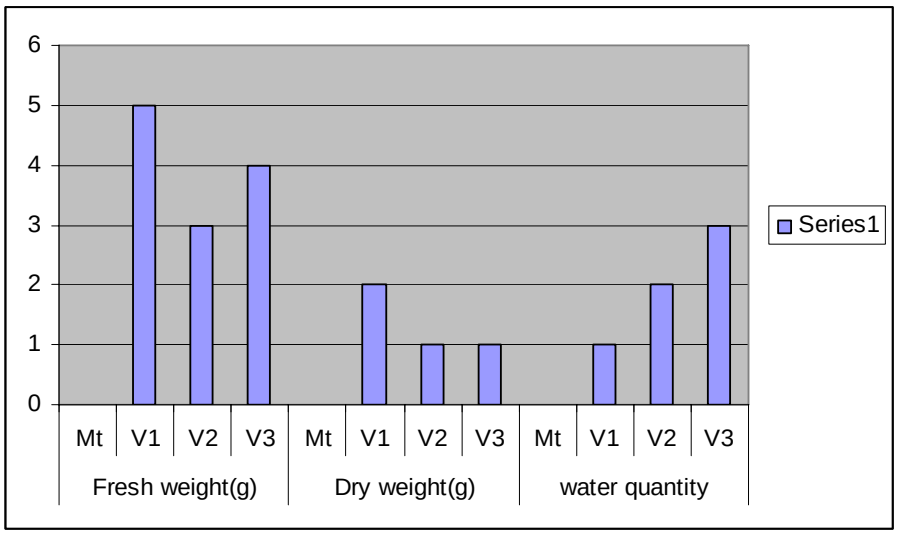

Fig. 1. Values of the callus mass after 40 days (fresh weight, dry weight and water content)

CONCLUSIONS

1. On the base medium MS (witness), the regeneration of a completely conformed plantlet can be observed, with good acclimation ability.
2. On the variant with a small dose of TDZ (V_1), the node generates callus (ø of circa 2.8 cm), of green color and high hardness, with small water content (after determining the amount of dry substance).
3. At 1 mg/l TDZ concentration (V_2), the generated callus mass is double (ø of approximately 4 cm), greenish callus, slightly friable,

with regenerative ability, regenerating circa 2-3 small plantlets with afferent leaves.

4. The high TDZ concentration, 2 mg/l (V_3), generated approximately the same mass of callus, olive green, with embryoids, but with higher friability.
5. The differences in fresh weight of the callus mass were relatively small: 0,4 in V_1, 0,5 in V_2 and 0,7 in V_3. Small differences of 0,0001 up to 0,008 were observed in dry weight.
6. The water content is given by the callus friability; the more friable it is, the more water it contains. V_3 has the highest water content, V_2 has less water than V_3, and V_1 has the lowest water content.
7. Due to the evenness of samples on each variant, the results are conclusive and valuable, both for the differentiation of neo-plantlets from callus, and for the possibility of obtaining a cellular suspension.

Fig. 2. V_3, olive callus
> 4cmø (circa 40 days)ø< 4cm

Fig. 3. V_1, dark green callus

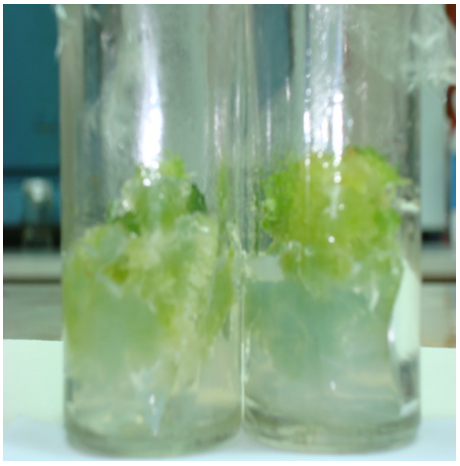

Fig. 4. V_3 (circa 40 days), differentiated

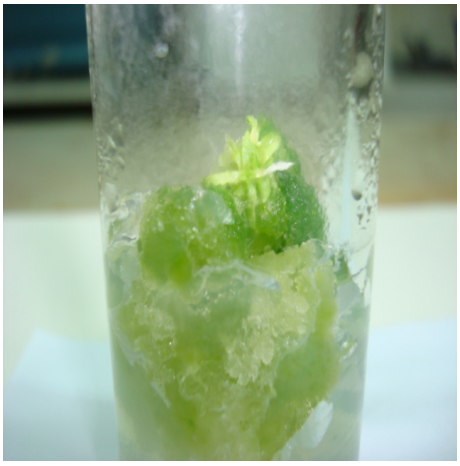

Fig. 5. V_2 (circa 40 days) plant embryoids regeneration

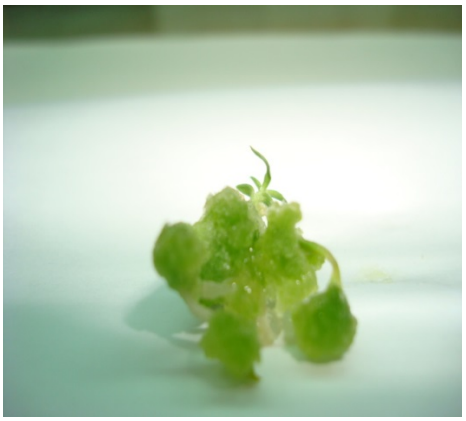

Fig. 6. V_2 (circa 40 days), differentiated

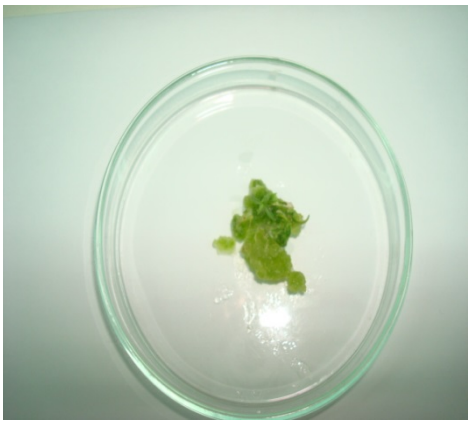

Fig. 7. V_3 (circa 40 days), plantlet embryonic cones

Fig. 8. V_2 regenerative callus, plantlet

Fig. 9. V_3 friable callus, olive color

Fig. 10. V_3 (after circa 40 days)

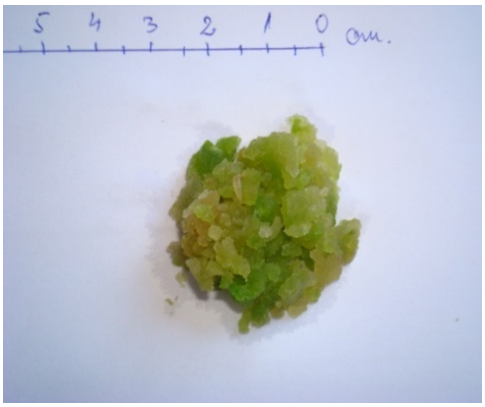

Fig. 11. V_1 (after circa 40 days)

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