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Effects of Dark and TDZ on Callus Formation of Rose Leaf Explants

Fatih A. Canli
Department of Horticulture, SDU Faculty of Agriculture, Central Campus 32260 Isparta, Turkey

Abstract: The objective was to develop a shoot regeneration system for leaf explants of Fairmount 1' 'FM1', a chimeral *R. multiflora* , rose. *Ex vitro* leaves when kept in the dark for three weeks formed abundant semi compact callus. No callus formed in the light. No shoot organogenesis was observed from either dark or light kept leaf explants. Dark grown leaves formed abundant callus. Thus the dark treatment of the leaf is important for callus formation. The reason for the different growth responses in light and dark is unknown. The highest rate of callus formation and highest number of callus colonies were obtained at 1.8, 2.7 and 3.6 μM TDZ. Callus did not develop without TDZ. None of these calli formed shoots. Further studies are needed to determine whether these calli are regenerable.

Key words: Rose, regeneration, callus, TDZ, dark

INTRODUCTION

There is limited information on adventitious shoots regeneration and somatic embryo formation from rose tissues. A few reports have been published (Tweddle *et al.,* 1984; Lloyd *et al.,* 1988; De Wit *et al.,* 1990; Burger *et al.,* 1990; Noriega and Sondahl, 1991; Firoozabady, 1994; Rosu *et al.*, 1995; Hsia and Korban, 1996); but most protocols have been unreliable, useful only for specific genotypes, or occur at such low frequencies to make their protocols of limited value for most roses.

Lloyd *et al.* (1988) obtained adventitious shoots of Rosa *persica* x *xanthina*, from a callus derived from newly forming shoots which had developed after being transferred to medium containing BA (3.0 mg l^{-1}) and α-Naphthalenacetic acid (NAA) (0.1-0.3 m l^{-1}). They reported that their organogenic cells contained few starch grains. Perhaps this observation could help researchers distinguish cells with organogenic potential from recalcitrant cells.

Another important protocol was developed by De Wit *et al.* (1990) for a cut rose cultivar. In this study, low frequencies of somatic embryos were obtained from callus derived from leaf explants of various *R. hybrida* (cvs Domingo and Vicky Brown). Rout *et al.* (1991) reported that they succeeded in obtaining somatic embryos from callus derived from immature leaf and stem segments of *R. hybrida* cv Landora. Noriega and Sondahl (1991) obtained somatic embryos, which gave whole plants, from callus of *R. hybrida* cv Royalty initiated from filament explants. Arena *et al.* (1993) reported direct adventitious shoot regeneration from leaf and root explants as well as somatic embryogenesis from callus derived from various plant (anther, petal, receptacle, leaves) of *R. hybrida* cv Meirutal.

Matthews *et al.* (1991) reported obtaining plantlets from callus of *R. persica* x *xanthina* which was initiated from the isolated protoplasts discussed. Firoozabady *et al.* (1994) reported isolating transgenic rose plants from embryogenic callus of 'Royalty'. However, regeneration of adventitious shoots or somatic embryos are very rare occurrences in roses (Skirvin *et al.*, 1995 and Hsia, 1995).

Rosu *et al.* (1995) reported a regeneration protocol for a chimeral thornless type of *R. multiflora*. They reported that shoots harvested from MS proliferation medium, supplemented with gibberellic acid (GA_3, 0.5 to 1.0 mg l^{-1}) and silver nitrate (3.4 mg l^{-1}), formed nodular callus and occasional putative adventitious shoots when subcultured on the same media supplemented with different levels of thidiazuron (TDZ). The best callus and regeneration occurred on medium with 1 μM TDZ, which produced putative adventitious shoots after a few subcultures.

Finally, Hsia and Korban (1996) reported regeneration of shoot (3.3%) and somatic embryos (6.6%) from the callus derived from stem explants of *R.hybrida* and *R. chinensis minima* on a medium containing N-phenyl-N'1,2,3-Thidiazuron (Thidiazuron) (TDZ) (23 μM) and Gibberellic acid $(GA)_3$(3 μM). The overall purpose of this study was to develop a regeneration protocol for 'Fairmount 1' rose 'FM1', a chimeral *R. multiflora*.

MATERIALS AND METHODS

Plant material, experimental medium, establishment of cultures and culture room conditions: 'Fairmount 1' FM1, a chimeral *R. multiflora* , was found by Dr. Philip Dziuk on his farm in Fairmount, IL. He found the thornless shoot growing from the base of a thorny plant ('FM1 thorny').

This clone grows very vigorous and erect. This clone was established in tissue culture.

We used procedures developed by Skirvin and Chu (1979) described earlier to establish the plant *in vitro*. Shoots were proliferated on Skirvin and Chu's (1979) modification of the Murashige and Skoog (1962) which contains two growth regulators, 6-benzylaminopurine (BA, 2 mg/liter [8.8 μM]) and naphthaleneacetic acid (NAA, 0.1 mg l^{-1} [0.54 μM]). We called this media standard MS (SMS). The cultures were maintained in a culture room with 16 h days (cool white fluorescent light) between 24 and 26°C. The standard Murashige and Skoog (SMS) high mineral salts (appendix A) were supplemented with, Staba vitamins (1969, appendix B), myo-inositol (100 mg l^{-1}), Na_2EDTA (37.25 mg $3l^{-1}$), $FeSO_4.7H_2O$ (27.85 mg l^{-1}), sucrose (30 g l^{-1}) and agar (7.5 g l^{-1}). The pH of the media were adjusted to 5.4-5.6 prior to autoclaving. The photosynthetically active radiation (PAR) at the level of the agar surface was 131 $\mu Mm^{-2}s^{-1}$ as measured with a Li-cor, Inc. integrating quantum/radiometer/photometer, model LI-188B).

Leaf regeneration experiment: In first experiment, the objective was to determine the effect of different TDZ concentrations on callus formation and adventitious shoot development from leaf explants of one-year-old greenhouse grown 'FM1'. Shoot tips (10 cm) of 'FM1' rose were harvested from greenhouse plants. Leaves were sterilized in 10% commercial bleach (0.525 NaOCL) two times (ten min. treatment^{-1}) and rinsed with sterile destilled H_2O (SDW) for 5 minutes at the each sterilization. Single leaflets were isolated from compound leaves, given two to three perpendicular to the midrib, by slicing them about half way through and placed with the abaxial side downward onto medium supplemented with NAA (0.54 μM) and eight levels of TDZ (0, 0.23, 0.45, 0.9, 1.4, 1.8, 2.7 and 3.6 μM). Petri dishes were placed in a completely randomized experimental design in a culture room as described earlier. Data regarding callus development were collected seven weeks later.

In the second experiment, leaves were prepared, handled and explanted on media supplemented with NAA (0.54 μM) and eight levels of TDZ (0, 0.23, 0.45, 0.9, 1.4, 1.8, 2.7 and 3.6 μM) as described in Experiment 1, but the petri dishes covered with aluminum foil and placed in dark for three weeks. Data were collected seven weeks after starting the experiment.

Data were analyzed using ANOVA; means were subjected to LSD tests at the 5% level using the SAS.

Table 1: Effect of TDZ on regeneration and callus formation of 'Fairmount 1' ('FM1') rose leaves in the dark *in vitro* (n=30)

Treatments TDZ (μM)	No. explants with adven. shoots	No. explants with callus	Callus formation (%)	Mean no. callus colonies
0.0	0	0	0.0d	0.00c
0.23	0	1	3.3d	0.03c
0.45	0	1	3.3d	0.03c
0.9	0	9	30.0c	0.90c
1.4	0	17	58.6b	2.60c
1.8	0	25	85.0a	47.66a
2.7	0	30	100.0a	8.10bc
3.6	0	29	100.0a	20.1ab
Significance:		*	*	

*, NS significant or not significant by ANOVA at 5%. Mean separation by LSD (Least Significant Difference); numbers within columns followed by different letters are significantly different

RESULTS AND DISCUSSION

In first experiment, no adventitious shoot formation was observed. No obvious callus development observed in the light. In second experiment, dark brown leaves formed abundant callus. The highest rate of callus formation and highest number of callus colonies were obtained at 2.7 and 3.6 μM TDZ (Table 1).

When two experiments were considered together, TDZ was not effective in forming callus from *in vitro* leaf explants in light. Leaves kept in light did not show any obvious callus growth in any of the TDZ levels. In contrast, relatively high concentrations of TDZ (1.4, 1.8, 2.7 and 3.6 μM TDZ) were effective in inducing callus formation in dark (Table 1). There were significant differences between TDZ concentrations on formation of callus in light. Callus did not developed without TDZ, however, 100% of the leaf explants formed callus at 2.7 and 3.6 μM TDZ in dark. Mean number of callus colonies was highest at 1.8 6 μM TDZ and lowest at 0.0 μM TDZ. Abundant callus formed in the dark on *ex vitro* leaves, but not in the light. The reason for the different growth responses in light and dark is unknown.

Lloyd *et al.* (1988) reported direct shoot regeneration from the petiole and midribs of leaflets of *R. persica* x *xanthina*, *R. laevigata* and *R. wichuraina*. Shoots were formed directly without interference by callus. When transferred to multiplication medium, only shoots of *R. persica* x *xanthina* survived.

Jemmali *et al.* (1994) reported that they had obtained spontaneous shoot regeneration from leaf stipules of strawberry (*Fragaria* spp.). They stated that adventitious shoots were formed on stipules without any intermediate callus. They also stated that stipular buds could be accurately distinguished from axillary buds. Axillary buds were more vigorous while stipular buds stayed attached to the stipule after even three subcultures, so no confusion was possible.

In current study, relatively high levels of TDZ induced formation of calli in light, none of these calli formed plants. Further studies are needed to determine whether these calli are regenerable. We observed no shoot regeneration in the present study. This might be attributed to a different genotype used in present experiments and also to the fact that frequencies of regeneration in earlier reports were very low. Further experiments should be conducted to assess the regenerability of the leaf explants.

REFERENCES

Arena, L., C. Pellegrino and S. Gudin, 1993. A comparison of the somaclonal variation level of Rosa hybrida L. c.v. Meirutral plants regenerated from callus or direct induction from different vegetative and embryonic tissues. Euphytica, 71: 3-90.

Burger, D.W., L. Liu, K.W. Zary and C.I. Lee, 1990. Organogenesis and plant regeneration from immature embryos of *R. hybrida* L. Plant Cell Tiss. Org. Cult., 21: 147-152.

De Wit, J.C., H.F. Esnadam, J.J. Honkanen and U. Tuommen, 1990. Somatic embryogenesis and regeneration of flowering plants in rose. Plant Cell Rep., 9: 456-458.

Firoozabady, E., Y. Moy, N. Courtney-Gutterson. and K. Robinson, 1994. Regeneration of transgenic rose (*Rosa hybrida*) plants from embryogeneic tissue. Bio-Technol., 12: 611-13.

Hsia, C. and S. Korban, 1996. Organogenesis and somatic embryogenesis in callus cultures of *Rosa hybrida* and *Rosa chinensis minima*. Plant cell Tiss. and Org. Culture, 44:1-6.

Hsia, C., 1995. Regeneration and genetic transformation of rose and evergreen azalea. Ph.D. Thesis. University of Illinois at Urbana-Champaign.

Jemmali, A., P. Boxus, D. Dekegel, G. Heule, 1994. Occurrence of spontaneous shoot regeneration on leaf stipules in relation to hyperflowering response in micropropagated strawberry plantlets. *In vitro* cell dev. Biol. Plant, 30: 192-195.

Lloyd, D., A.V. Roberts and K.C. Short, 1988. The induction *In vitro* adventitious shoots in *Rosa*. Euphytica, 37: 31-36.

Matthews, D., J. Mottley, I. Horan. and A.V. Roberts, 1991. A protoplast to plant system in roses. Plant Cell Tiss. Org. Cult., 24: 173-80.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physio. Planta, 15: 473-97.

Noriega, C. and M.R. Sondahl, 1991. Somatic embryogenesis in hybrid tea roses. Bio Technol., 9: 991-93.

Rosu, A., R.M. Skirvin, A. Bein, M.A. Norton and M. Kushad, 1995. The development of putative adventitious shoots from a chimeral thornless rose (*Rosa multiflora* Thunb. ex J. Murr.) *in vitro*. J. Hort. Sci., 70: 901-907.

Rout, G.R., B.K. Debata and P. Das, 1991. Somatic embryogenesis in callus culture of *Rosa hybrida* L. cv. Landora. Plant Cell Tiss. Org. Cult., 27: 65-9.

Skirvin, R.M. and M.C. Chu., 1979. *In vitro* culture of 'Forever Yours' rose. Hort. Sci., 14: 608-610.

Tweddle, D., A.V. Roberts and K.C. Short., 1984. *In vitro* culture of roses: In Novak, F.J., L. Havel and J. Dolezel (Eds.) Plant Tissue and Cell Culture Application to Crop Improvement. Czechoslovak Academy of Sciences, Prague, pp: 529-530.