Some features of two cultivars of *in vitro* propagated *Rosa hybrida*

Veneta Kapchina-Toteva[1], Dimitrina Stoyanova-Koleva[2] & Elena Iakimova[3]

1 Department of Plant Physiology, Biological Faculty, Sofia University St. Kliment Ohridski, 8, Dragan Tzankov Blvd., 1164 Sofia, Bulgaria, e-mail: veneta@biofac.uni-sofia.bg

2 Department of Botany, Biological Faculty, Sofia University St. Kliment Ohridski, 8, Dragan Tzankov Blvd., 1164 Sofia, Bulgaria, e-mail: koleva@biofac.uni-sofia.bg

3 AgroBioInstitute, 8, Dragan Tzankov Blvd., 1164 Sofia, Bulgaria, e-mail: elena_iakimova@abi.bg

Received: June 27, 2002 ▷ Accepted: July 16, 2002

Abstract. A comparative analysis of two *in vitro* propagated rose cultivars (*Rosa hybrida*) has shown a difference in bud sprouting, shoot number, fresh and dry mass, and chloroplast ultrastructure, in correlation with different degree of apical dominance.

Key words: apical dominance, chloroplast, micropropagation, *Rosa hybrida*

Introduction

Plant micropropagation is often based on the apical or lateral bud formation and their growth into shoots. The effectiveness of *in vitro* propagation is strongly dependent on the type and concentration of plant growth regulators supplemented to the growth medium. Bud sprouting can be inhibited due to dormancy or domination of the actively growing apical bud, but axillary bud outgrowth, considered a process of apical dominance release, can be enhanced in response to exogenous cytokinins (Philips 1975; Bollmark & al. 1995). Apical dominance can be defined as the control exerted by the shoot apex over the sprouting of lateral buds mainly via suppressed branching, as an effect of auxins from the apical meristem (Cline 1994, 1997). They can also be used as a tool for overcoming the apical dominance and, in this context, the effect of BA and kinetin in *in vitro* propagated roses has been extensively studied (Davies 1980; Hasegawa 1980; Skirvin & al. 1984; Lloyd & al. 1988; Campos & Salome 1990; Arnold & al. 1992; van Telgen & al. 1992). Although the roses have been a model system for a number of physiological and morphological studies, to our knowledge, no data are yet available from a comparison between the ultrastructure of chloroplasts of cultivars with different apical dominance. In an earlier study we have observed a difference in chloroplast ultrastructure in *in vitro* cultured *Gypsophila paniculata* L. in response to cytokinins and their antagonists (Kapchina-Toteva & Stoyanova 2003).

The aim of the present work is to compare some morphological and physiological features of two cultivars of *in vitro* propagated *Rosa hybrida* L. that differ in their degree of apical dominance. We have shown that bud sprouting, shoot number, fresh and dry weight, content of pigments and chloroplast ultrastructure are distinct in cultivar *Madelon* expressing a strong apical dominance, and that cultivar *Motrea* represents an easily branching variety.

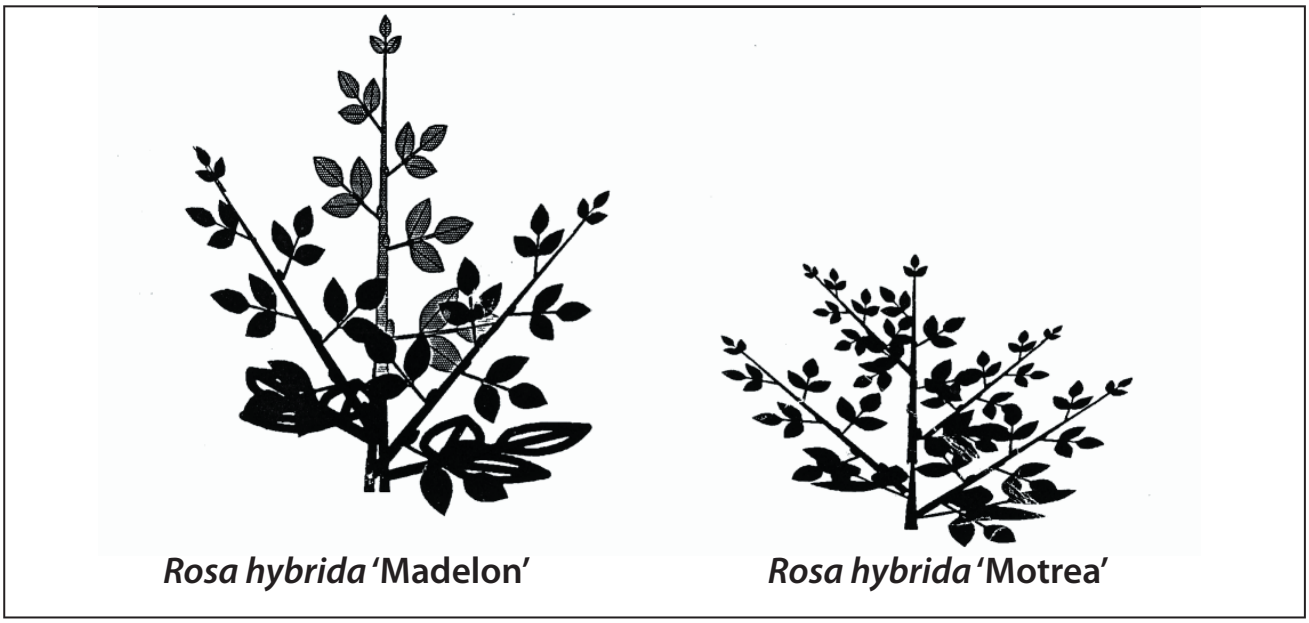


Fig. 1. *In vitro* cultured plants of cultivars *Rosa hybrida* L.

Materials and methods

The experiments were carried out with shoot cultures of *Rosa hybrida*: cultivar *Madelon* and cultivar *Motrea*. Both cultivars are known to express a different degree of apical dominance (Van Telgen & al. 1992). Cultivar *Madelon* shows strong apical growth and a lesser number of outgrowing shoots than cultivar *Motrea* (Fig. 1). Rose plants were subcultured on a standard MS medium supplemented with 1.0 mg.l^{-1} BA, 4.5 % (w/v) sucrose, and 7 g.l^{-1} agar. Axillary buds from 3rd and 4th position with a small part of elongated shoots (single nodes) were used as an explant source. Growth conditions were 20°C and 16 hours of light (60 µmol.m^{-2}.s^{-1} photosynthetic photon flux density, Philips TLD-33).

Budbreak was determined as a percentage of open buds ($n = 30$). The physiological performance was evaluated by the number of shoots from the open axillary buds, shoot length, and fresh and dry mass after four weeks of culture.

Plastid pigment content was determined spectrophotometrically in acetone extract, following the method of Arnon (1949) and expressed in mg pigment per gram fresh mass.

The samples for electron microscopy were taken from the middle part of the lamina of the 3rd leaf, fixed in 3 % (w/v) glutaraldehyde in phosphate buffer (pH 7.4) for 12h at 4 °C, and postfixed in 2 % (w/v) $KMnO_4$ for 4 h at room temperature. After dehydration the material was embedded in Durcupan (Fluka, Swizerland) and cut with Tesla (Prague, Czech Republic) ultramicrotome. Observations were carried out with JEOL 1200 EX (Japan) electron microscope.

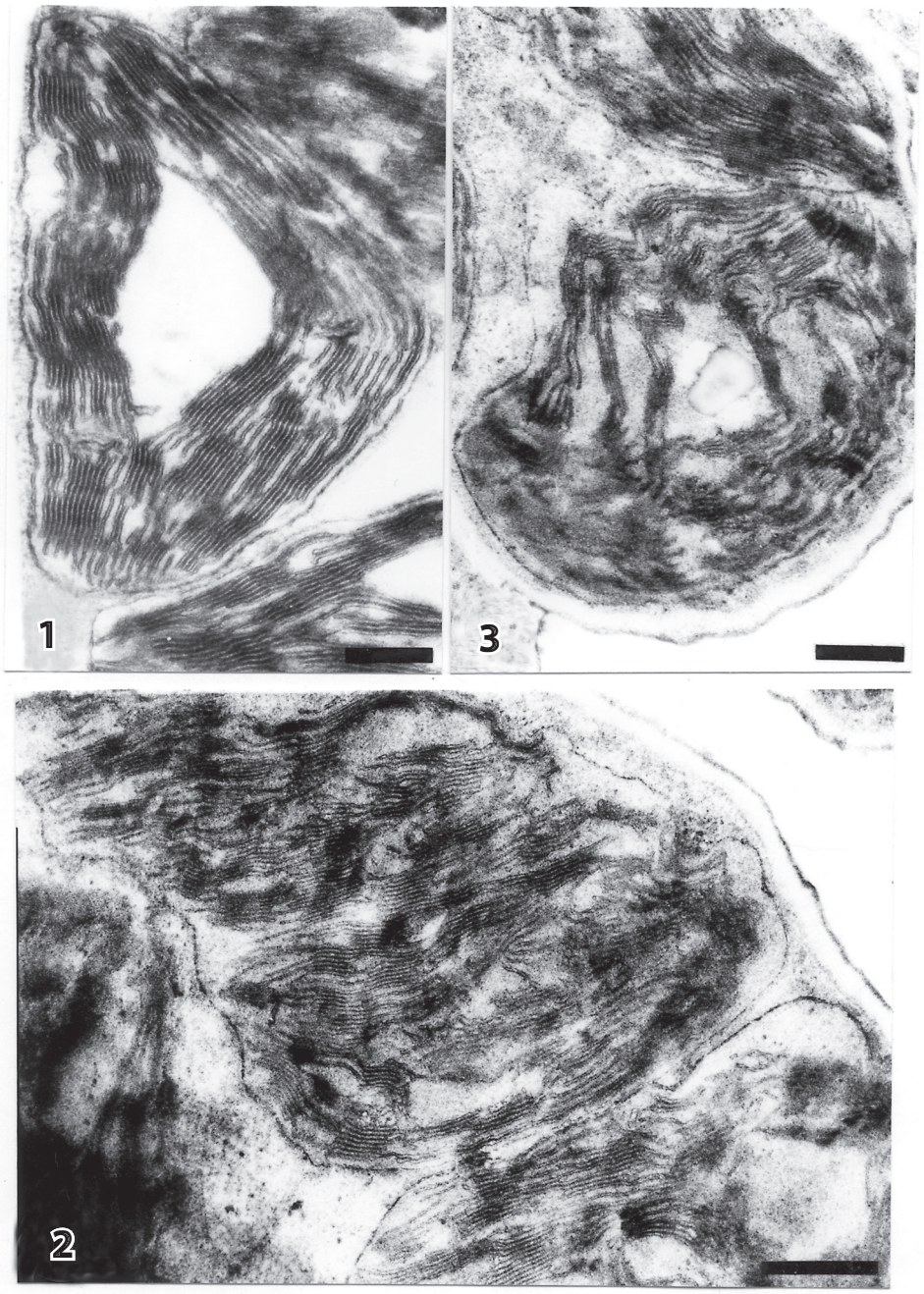
For statistical significance the data were processed and assessed by LSD at a 5 % level of probability.

Results and discussion

In both rose cultivars, *Madelon* and *Motrea*, more than 50 % of buds opened after four weeks of culture. A higher percentage of open buds was established in cultivar *Motrea* and this corresponded to a lesser extent of apical dominance. LSD analysis of both cultivars indicated an enhanced budbreak of cultivar *Motrea* and statistical difference of cultivar response [LSD ($p \leq 0.05$) 8.67]. Cultivar *Motrea* developed three times more shoots per single node.

The correlation of higher fresh weight with lower dry weight is one of the features characterizing vitrification caused by cytokinins in the medium during *in vitro* propagation of carnations and other plant species (Pasqualetto & al. 1986; Leshem & al. 1988). Changes in dry weight provide information whether the increase of fresh weight, if any, is due to biomass accumulation, or to an increase of water content, called vitrification (Gaspar 1991). The percentage of dry and fresh mass in cultivar *Madelon* was significantly higher than in cultivar *Motrea*, but during four weeks of culture no sign of vitrification was observed in either of the studied cultivars. The dry mass per single shoot have been calculated (Table 1). Although the

Plate I



Figs 1-3. *Rosa hybrida* - cross section of the leaf:
1. cultivar *Madelon*; **2.** cultivar *Motrea*; **3.** cultivar *Motrea*

fresh and dry mass of cultivar *Madelon* was higher than that of cultivar *Motrea*, the accelerated number of healthy and well-developed shoots of cultivar *Motrea* has shown a better propagation rate *in vitro*. This testifies to the fact that cultivars with lower degree of apical dominance are more appropriate for mass micropropagation.

Table 1. Physiological features of two cultivars of *in vitro* propagated *Rosa hybrida*

Cultivar/LSD (5%)	Bud sprouting (%)	Shoot number	Fresh mass (mg)	Dry mass (%)	Chlorophyll (a+b) mg/g.-1 FM	Carotenoids mg/g.-1 FM
cultivar Madelon	53.04	1.14	60.00	11.21	1.252	0.174
cultivar Motrea	68.00	3.06	48.07	7.57	1.941	0.223
LSD (5%)	8.67	1.11	6.89	2.13	0.40	0.03

The conte nt of plastid pigments in cultivar *Madelon* was lower than in cultivar *Motrea* (Table 1). The chloroplasts in mesophyll cells of cultivar *Madelon* had a well-developed inner membrane system occupying almost the entire volume (Pl. I, Fig. 2). The well-developed grana were relatively high (from 10 to 25–30 thylakoids) and linked by a net of short stromal thylakoids. Thylakoid density of the inner membrane system was due to a greater number of wide grana and well-structured stromal thylakoids. Such dimensional structural organization is typical for well-structured chloroplasts. Starch grains of medium size were observed in the stroma.

In cultivar *Motrea* the inner membrane system was also well developed, similarly to that of cultivar *Madelon* (Pl. I, Fig. 3). Despite the high thylakoid density, the chloroplast architecture of cultivar Motrea was distinct from cultivar *Madelon*. The plastids had a wavelike irregular shape. The change of chloroplast shape corresponded to a change in the dimensional orientation of the entire inner membrane system. In most chloroplasts the membrane system was mainly perpendicularly oriented. A difference was also observed in the wavelike shape of thylakoids, expressed to a higher or lesser extent in one chloroplast (Pl. I, Fig. 4). The starch grains were smaller and fewer in number than in cultivar *Madelon*.

The chloroplasts of the two cultivars were similar in volume of their inner membrane system and differed in chloroplast shape and orientation of the inner membrane system. This corroborated the important fact that cultivar *Motrea* had untypical plastid structure. Such observation could be contributed to different apical dominance. Further discussion and study will be necessary so as to shed more light on this assumption.

References

Arnold, N., Binns, M., Barthakur, N. & Cloutier, D. 1992. A study of the effect of growth regulators and time of plantlet harvest on the *in vitro* multiplication rate of hardy and hybrid tea roses. – J. Hort. Sci., **67**(6): 727-735.

Arnon, D. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. – Pl. Physiol., **24**: 1-15.

Bollmark, M., Chen, Hao-Zie., Moritz, T. & Eliasson, L. 1995. Relation between cytokinin level, bud development and apical control in Norway spruce *Picea abies*. – Physiol. Pl. (Copenhagen), **95**: 563-568.

Campos, S. & Salome, S. 1990. Mass propagation of the dwarf rose cultivar *Rosamini*. – Sci. Hort., **43**: 321-330.

Cline, M. 1994. The role of hormones in apical dominance. New approaches to an old problem in plant development. – Physiol. Pl. (Copenhagen), **90**: 230-237.

Cline, M. 1997. Concepts and terminology of apical dominance. – Amer. J. Bot., **84**(8): 1064-1069.

Davies, D. 1980. Rapid propagation of roses *in vitro*. – Sci. Hort., **13**: 385-389.

Gaspar, Th. 1991. Vitrification in micropropagation. – In: **Bajaj, Y. P. S.** (ed.), Biotechnology in Agriculture and Forestry. Vol. **17**, pp. 116-126. High-Tech. and Micropropagation. Springer-Verlag, Berlin.

Hasegawa, P. 1980. Factors affecting shoot and root initiation from cultured rose shoot tips. – J. Amer. Soc. Hort. Sci., **105**: 216-220.

Kapchina-Toteva, V. & Stoyanova, D. 2003. Effect of cytokinins and cytokinin antagonists on *in vitro* cultured *Gypsophila paniculata* L. – Biol. Pl., **46**(3): 337-343.

Leshem, B., Werker, E. & Shalev, D. 1988. The effect of cytokinins on vitrification in melon and carnation. – Ann. Bot. (König & Sims), **62**: 271-276.

Lloyd, D., Roberts, R. & Short, K. 1988. The induction *in vitro* of adventitious shoots in *Rosa*. – Euphytica, **37**: 31-36.

Pasqualetto, P., Zimmerman, R. & Fordham, I. 1986. Geling agent and growth regulator effects on shoot vitrification of Gala apple *in vitro*. – J. Amer. Soc. Hort. Sci., **111**: 976-980.

Phillips, D. 1975. Apical dominance. – Annual Rev. Pl. Physiol., **26**: 341-367.

Skirvin, R., Chu, M. & Walter, J. 1984. Tissue culture of the rose. – Amer. Rose Annual, **69**: 91-97.

Van Telgen, H., Ellagoz, V., Van Mil, A., Paffen, A. & De Klerk, J. 1992. Role of plant hormones in lateral bud growth of Rose and Apple *in vitro*. – Acta Hort., **319**: 137-142.