J Plant Growth Regul (2000) 19:232–237
DOI: 10.1007/s003440000013

J P G R
Journal of Plant Growth Regulation

CONTRIBUTED ARTICLES

Role of Phenylurea Cytokinin CPPU in Apical Dominance Release in *In Vitro* Cultured *Rosa hybrida* L.

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ABSTRACT

The effect of purine (BA) and phenylurea (CPPU) cytokinins on apical dominance release in *in vitro* cultured *Rosa hybrida* L., cv. Madelon and Motrea was evaluated. Cv. Madelon shows stronger natural apical growth and fewer branches than cv. Motrea *in vivo* and *in vitro.* We examined the effects under three conditions, without the addition of the auxin IBA, in the presence of IBA, and in material pretreated with a pulse of IBA. Results were scored weekly for 4 weeks. BA and CPPU stimulated axillary bud break, and higher numbers of open buds were recorded in the presence of CPPU. When CPPU cytokinin was added to culture medium, physiologic features such as bud sprouting and shoot fresh and dry weight were enhanced. CPPU was also highly efficient for overcoming IBA inhibition of bud outgrowth. Different cultivar responses were observed.

Key words: Apical dominance; BA; Bud sprouting; *In vitro*; CPPU; Rose

INTRODUCTION

Apical dominance can be defined as the control exerted by the shoot apex over the outgrowth of the lateral buds caused mainly by inhibition by auxins from the apical meristem (Cline 1994, 1997). Hence, type and concentration of plant growth regulators affect the capacity of *in vitro* propagation because they play a major role in cell division, differentiation, and morphogenesis in plant tissue cultures. Axillary bud outgrowth, considered a process of apical dominance release, can be enhanced in response to exogenous cytokinins (Bollmark and others 1995; Philips 1975). In *in vitro* propagated roses, the effect of BA (N^6 benzyladenine) and kinetin on apical dominance has been extensively studied (Arnold and others 1992; Campos and Salome 1990; Davies 1980; Hasegawa 1980; Lloyd and others 1988; Skirvin and others 1984; van Telgen and others 1992), but data are not yet available concerning the application of phenylurea cytokinins (CPPU; N-(2-

Received 27 April 1999; accepted 23 February 2000
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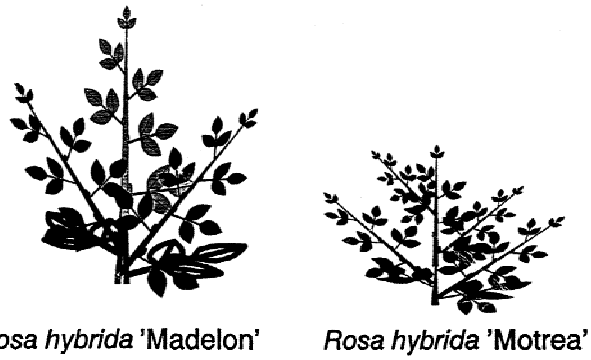

Figure 1. In vitro cultured plants of *Rosa hybrida* L.

chloro-4-pyridyl)-N-phenylurea). Phenylurea cytokinins demonstrated higher activities and a better effect in earlier test systems (Ivamura and others 1980; Karanov and others 1992; Okamoto and others 1978; Shudo 1994; Takahasi and others 1978). When *in vitro* apple and grapevine culture medium was supplemented with thidiazuron and CPPU, enhanced outgrowth of axillary buds was observed (Fellman and others 1987; Gribaudo and Fronda 1991; Niewkera and others 1986).

This study was undertaken to investigate the effect of purine (BA) and phenylurea cytokinins (CPPU) on apical dominance release in *in vitro* cultured *Rosa hybrida* L. We demonstrate that when CPPU is added to culture medium the physiologic features such as bud sprouting and shoot fresh and dry weight are considerably enhanced. The effect of indolyl-3-butyric acid (IBA) and cytokinins on bud break was also characterized. Our results indicate significant differences in cultivar responses.

Materials and Methods

Plant Material and Growth Conditions

Experiments used shoot cultures of *Rosa hybrida* L., cv. Madelon and cv. Motrea. Both cultivars express different degrees of apical dominance, and *in vitro* (Van Telgen and others 1992) cv. Madelon shows strong apical growth and fewer outgrowing shoots than cv. Motrea (Figure 1). Rose plants cultured on a standard MS medium supplemented with 1.5 mg L^{-1} BA, 4.5% (w/v) sucrose, and 7 g L^{-1} agar were subcultured every 5 weeks according to Van Telgen and others (1992). The explants isolated from these cultures were grown on the same medium containing a reduced amount of BA (1.0 mg L^{-1}). For shoot elongation, liquid basal MS medium (2 mL per plant) was added 1 week later. Axillary buds from the third and fourth position, including a small part of elongated shoots (single nodes), were used as an explant source (30 explants per treatment). Growth conditions were 20°C and 16 h of light (60 µmol × m^{-2} × s^{-1} photosynthetic photon flux density, Philips TLD-33).

Evaluation of Plant Growth Regulator Effect

The single nodes with buds already present in the explant were transferred to standard medium supplemented with 1.6, 2.2, or 4.4 µM of the synthetic cytokinins of purine-type BA and phenylurea-type CPPU and/or 1.0 µM IBA. Pretreatment with 1.0 µM IBA was performed by culturing single nodes for 72 h and subsequent transfer onto media containing BA or CPPU at different concentrations. Bud break was determined as a percentage of open buds 1, 2, 3, and 4 weeks after the transfer. The number of shoots from the open axillary buds, shoot length, and fresh and dry weight were determined in 4 weeks of culture. For statistical significance the data were processed and assessed by LSD at a 5% level of probability and SEM, which was the standard error of M_n; $n \leq 10$.

Results

Bud Outgrowth

In both rose cultivars Madelon and Motrea, the application of 1.6 µM CPPU stimulated bud opening better than BA. This effect was clear in the first week of culture when the sprouted buds were nearly twice that of those treated similarly with BA (Figures 2 and 3). A higher percentage of open buds was also evident at 1.6 µM CPPU after 2, 3, and 4 weeks of subculture. Cv. Motrea showed an obvious differential response to the tested concentrations during the first week (enhanced bud break with increasing BA concentrations and diminished outgrowth with increasing CPPU concentrations). Statistical analysis of LSD between cultivars indicated a difference in cultivar response to cytokinin treatments [LSD ($p \leq$ 0.05) 5.69]. The results revealed that for cv. Madelon BA was the more effective cytokinin, especially at 1.6 µM, whereas for cv. Motrea a similar response was observed at 2.2 µM CPPU.

When CPPU was applied simultaneously with 1.0 µM IBA, about 50% of the axillary buds opened in the second week compared with BA where only 4.4 µM caused slight acceleration of bud outgrowth of cv. Madelon. CPPU response was preserved after 3 and 4 weeks at each of the three concentrations, while similar behavior for BA was observed at 4.4 µM (Figure 2). The percentage of open buds of cv.

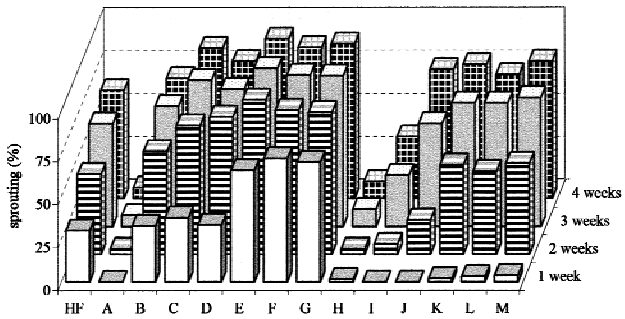

Figure 2. Effect of BA and CPPU and IBA on sprouting of axillary buds in *in vitro* cultured *Rosa hybrida* L., cv. Madelon. Significance of the effect of cytokinin and time is determined at LSD ($p \leq 0.05$) 7.51. Significance of the effect of IBA, cytokinin and time is determined at LSD ($p \leq 0.05$) 12.04. HF, hormone free; *A,* 1.0 µM IBA; *B,* 1.6 µM BA; *C,* 2.2 µM BA; *D,* 4.4 µM BA; *E,* 1.6 µM CPPU; *F,* 2.2 µM CPPU; *G,* 4.4 µM CPPU; *H,* 1.0 µM IBA + 1.6 µM BA; *I,* 1.0 µM IBA + 2.2 µM BA; *J,* 1.0 µM IBA + 4.4 µM BA; *K,* 1.0 µM IBA + 1.6 µM CPPU; *L,* 1.0 µM IBA + 2.2 µM CPPU; *M,* 1.0 µM IBA + 4.4 µM CPPU.

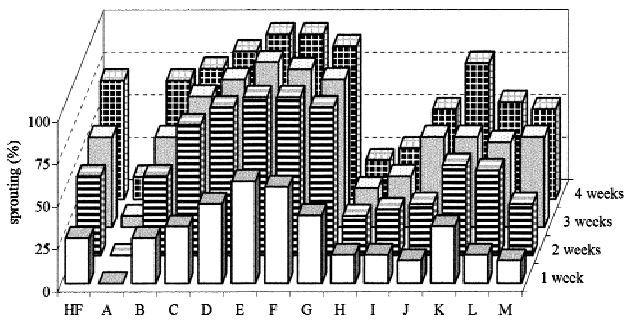

Figure 3. Effect of BA and CPPU and IBA on sprouting of axillary buds in *in vitro* cultured *Rosa hybrida* L., cv. Motrea. Significance of the effect of cytokinin and time is determined at LSD ($p \leq 0.05$) 8.91. Significance of the effect of IBA, cytokinin and time is determined at LSD ($p \leq 0.05$) 7.81. *HF,* hormone free; *A,* 1.0 µM IBA; *B,* 1.6 µM BA; *C,* 2.2 µM BA; *D,* 4.4 µM BA; *E,* 1.6 µM CPPU; *F,* 2.2 µM CPPU; *G,* 4.4 µM CPPU; *H,* 1.0 µM IBA + 1.6 µM BA; *I,* 1.0 µM IBA + 2.2 µM BA; *J,* 1.0 µM IBA + 4.4 µM BA; *K,* 1.0 µM IBA + 1.6 µM CPPU; *L,* 1.0 µM IBA + 2.2 µM CPPU; *M,* 1.0 µM IBA + 4.4 µM CPPU.

Motrea compared with IBA control was greater in the first week in response to both cytokinins, especially treatment with 1.6 µM CPPU (Figure 3). Cultivar effect to IBA + cytokinin treatment was assessed at LSD ($p \leq 0.05$) 7.00. In 1 week of culture, BA and CPPU caused opening of more buds in cv. Motrea. In the subsequent period, BA was more effective in cv. Motrea and CPPU was more effective in cv. Madelon.

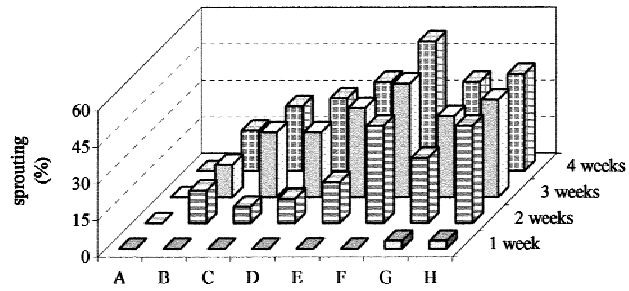

Figure 4. Effect of BA and CPPU on sprouting of axillary buds in *in vitro* cultured *Rosa hybrida* L., cv. Modelon after 72 h pre-incubation with 1.0 µM IBA. *A,* 1.0 µM IBA; *B–H,* 72-h pretreatment with 1.0 µM IBA and then: *B,* hormone free; *C,* 1.6 µM BA; *D,* 2.2 µM BA; *E,* 4.4 µM BA; *F,* 1.6 µM CPPU; *G,* 2.2 µM CPPU; *H,* 4.4 µM CPPU. Significance of effect of IBA pretreatment, cytokinin, and time is determined at LSD ($p \leq 0.05$) 6.2.

Pretreatment of the single nodes for 72 h with 1.0 µM IBA reversibly inhibited bud outgrowth. A limited number of buds opened in the first week after the transfer from the medium with IBA onto the medium with BA or CPPU (Figures 4 and 5). After 2 weeks the highest percentage of sprouted buds was 33% for cv. Motrea and 40% for cv. Madelon at various concentrations of CPPU. At the same duration of BA treatment 26.7% of the buds opened in cv. Motrea and 16.7% in cv. Madelon. After 4 weeks of subculture, the impact of 1.6 µM CPPU was better expressed in both cultivars compared with BA and the other two CPPU concentrations. The statistical analysis showed that in the case of IBA pretreatment CPPU led to a significant increase in the number of spouted buds, mainly for cv. Madelon; LSD ($p \leq 0.05$) 3.92.

Number of Shoots, Shoot Length, Shoot Fresh and Dry Weight

The number of shoots per single node is presented in Figure 6. Culture on CPPU containing medium caused an increase in shoot numbers, an effect more apparent in CPPU treatment than for BA treatment in both cultivars. No considerable differences in stem lengths were observed in response to the two types of cytokinins, although a slight reduction was noticed with increasing CPPU or BA concentration (data not presented).

The fresh and dry weight of shoots was accelerated during culture on medium with either CPPU or BA (Figures 7 and 8). Higher fresh weight was measured in response to CPPU. The percentage of dry weight of cv. Madelon was significantly higher than cv. Motrea and increased both at exposure to BA

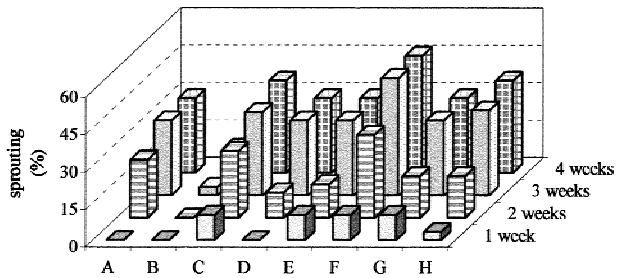

Figure 5. Effect of BA and CPPU on sprouting of axillary buds in *in vitro* cultured *Rosa hybrida* L., cv. Modelon after 72 h pre-incubation with 1.0 µM IBA. *A,* 1.0 µM IBA; *B–H,* 72 h pre-treatment with 1.0 µM IBA and then: *B,* hormone free; *C,*1.6 µM BA; *D,* 2.2 µM BA; *E,* 4.4 µM BA; *F,* 1.6 µM CPPU; *G,* 2.2 µM CPPU; *H,* 4.4 µM CPPU. Significance of effect of IBA pretreatment, cytokinin, and time is determined at LSD ($p \leq 0.05$) 5.09.

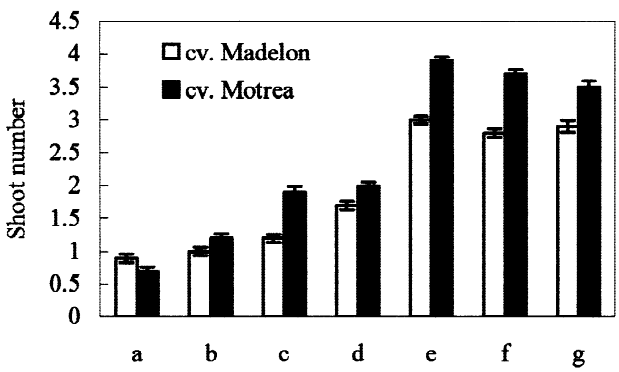

Figure 6. Effect of BA and CPPU on shoot numbers from single nodes of *in vitro* cultured *Rosa hybrida* L. Error bars represent SEM. *a,* hormone free; *b,* 1.6 µM BA; *c,* 2.2 µM BA; *d,* 4.4 µM BA; *e,* 1.6 µM CPPU; *f,* 2.2 µM CPPU; *g,* 4.4 µM CPPU.

and CPPU. For cv. Motrea the differences of BA and CPPU effect were negligible.

Discussion

Apical dominance is an auxin inhibition from apically produced auxin on the outgrowth of axillary buds. Cytokinins are considered an important factor in controlling and breaking the dormancy and apical dominance (Cline 1994, 1997; Martin 1987; Tamas and others 1989). Recently, the distribution of endogenous cytokinins in relation to bud break of *Rosa hybrida* cv. Madelon have been demonstrated by Dieleman and others (1997). These authors measured a high cytokinin concentration in stems and young leaves. A number of physiologic effects of natural and synthetic cytokinins are well documented, but

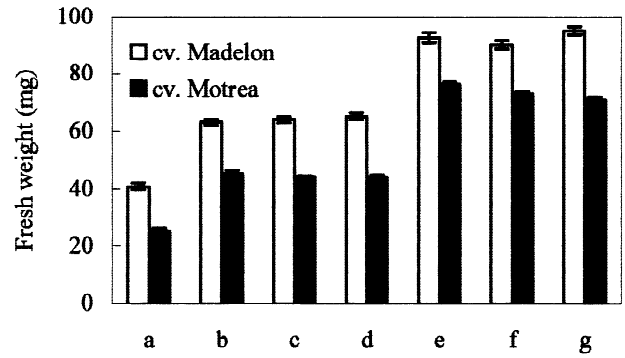

Figure 7. Effect of BA and CPPU on fresh weight of shoots in *in vitro* cultured *Rosa hybrida* L. Error bars represent SEM. *a,* hormone free; *b,* 1.6 µM BA; *c,* 2.2 µM BA; *d,* 4.4 µM BA; *e,* 1.6 µM CPPU; *f,* 2.2 µM CPPU; *g,* 4.4 µM CPPU.

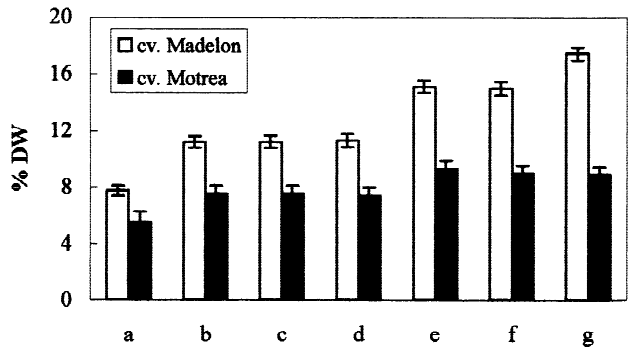

Figure 8. Effect of BA and CPPU on % DW of shoots in *in vitro* cultured *Rosa hybrida* L. Error bars represent SEM. *a,* hormone free; *b,* 1.6 µM BA; *c,* 2.2 µM BA; *d,* 4.4 µM BA; *e,* 1.6 µM CPPU; *f,* 2.2 µM CPPU; *g,* 4.4 µM CPPU.

the mechanism through which these plant growth regulators, and in particular the phenylurea cytokinins, control the processes of growth and development are still not quite clear. Because CPPU exhibits essentially the same physiologic activities at much lower concentrations than BA or zeatin (such as growth, promotion of callus of tobacco, pea, ginseng, and datura and stimulation of shoot formation of tobacco, azalea, petunia, mulberry, and torenia) (Shudo 1994), we hypothesized that this compound could successfully substitute for the widely used BA in *in vitro* culture of roses. Our expectations were confirmed in the current experiments in which the effect of CPPU exceeded the effect of BA, causing stimulation of bud sprouting and an increase of shoot number, fresh, and percent dry weight (Figures 2, 3, 6–8). In general, the application of both types of cytokinins (BA and CPPU) stimulated the bud break, but a greater number of open buds was recorded when the medium was supplemented with

CPPU. The effect was more pronounced in cv. Madelon, a cultivar showing stronger natural apical growth and fewer outgrowing shoots.

It is assumed that augmentation of endogenous auxin to the cytokinin ratio suppresses bud outgrowth. The exogenous supply of cytokinins can change this ratio (Hillman 1984). In previous experiments we have established that 1.0 µM IBA inhibited the sprouting of axillary rose buds, but they remained capable of outgrowth under appropriate conditions (unpublished data). Here we have shown that when apical dominance is strongly exhibited, as in the case with cv. Madelon, exposure to CPPU can overcome the inhibitory effect of IBA (Figure 2, *K–M*).

To assess the physiologic activity of cytokinins to stimulate bud sprouting, the single nodes were incubated for 72 h on medium with 1.0 µM IBA. Although it remains to be explored, we have suggested that pretreatment with IBA can change the endogenous auxin/cytokinin ratio thus accelerating the level of auxins and diminishing the level of active endogenous cytokinins. This system provides a good opportunity for more precise evaluation of the exogenous cytokinin effect. Preliminary inhibition with IBA led to fewer sprouted buds during the subculture on medium with 1.6 µM CPPU (50%), whereas when IBA was continuously combined with the same concentration of CPPU, bud sprouting was about 75%. BA treatment (1.6 µM) was less efficient in overcoming IBA inhibition (Figures 2–5).

CPPU-treated medium also enhanced the outgrowth of more shoots per explant (Figure 6), which is evidence for stronger CPPU action compared with BA. These results coincide with the view that exogenous cytokinins can be a successful tool for controlling the outgrowth of axillary buds (Bollmark and others 1995; Philips 1975) and provide new information about the physiologic effects of CPPU cytokinins.

Explant growth depends on cytokinin concentration in the medium, but the impact of exogenous cytokinins involves complicated physiologic processes including uptake, use, and hormonal effects (Auer and others 1992). Low cytokinin concentrations are not appropriate for formation of sufficient numbers of shoots, whereas high concentrations stimulate adventitious shoot formation but cause harmful effects on explant quality leading to vitrification (Dunstan and others 1985). Dry weight changes indicate whether increases in fresh weight, if any, are due to biomass accumulation or to vitrification (Gaspar 1991). Lateral shoot fresh and dry weights from both rose cultivars were augmented in response to BA and CPPU treatments. These weight increases coincided with higher numbers of shoots and a lack of vitrification (Figures 6–8). In our previous study on the same rose cultivars we examined the effect of CPPU on peroxidase activity during the first 7 days of culture. Accelerated peroxidase activity was noted in association with apical dominance release. Plant peroxidases are involved in different physiologic processes and also play a role in cell wall lignification (Asada 1992). We have suggested that among the other physiologic effects cytokinins might affect apical dominance by stimulating guaiacol-peroxidase in *in vitro* cultured roses (Kapchina-Toteva and Yakimova 1997). Increased dry weight and enhanced peroxidase activity are expected to prevent the process of vitrification. As in these experiments no sign of vitrification was observed when CPPU and BA were used, and thus we have assumed the appropriateness of applied cytokinins.

The reported results support the view that phenylurea cytokinins possess higher activities at lower concentrations compared with purine cytokinins (Karanov and others 1992) and offer an opportunity to use *in vitro* propagated roses as a successful model system for studies on physiologic effects of phenylurea cytokinins. Because the economic implications of apical dominance in ornamental and agricultural species in respect to crop yield, stem position, and other considerations are substantial, further investigations can provide a better understanding of the detailed role of substances with phenylurea structure in *in vitro* propagation.

ACKNOWLEDGMENTS

This study was supported by the Bulgarian National Foundation for Scientific Investigations (Project No. B-529) and the Centre for Plant Tissue Culture Research, Lisse, The Netherlands

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