Micropropagation and Growth Regulation of *Salvia greggii* Variegated©

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Micropropagation and growth regulator treatments have the potential to produce dense, compact *Salvia greggii* variegated commercially. We identified suitable media for shoot proliferation and rooting and studied the effects of paclobutrazol and gibberellic acid on shoot and internode length.

We achieved 100% rooting with explants cultured on MS medium supplemented with 1.0 mg·L^{-1} IBA. Survival of explants on weaning was 93% to 100%. High concentration of paclobutrazol in vitro reduced both the internode length and number of nodes produced per shoot. After weaning, treated shoots were not significantly shorter than those untreated.

For glasshouse-grown plants, paclobutrazol drenches significantly reduced both plant height and internode length and increased bud break but did not affect the number of nodes produced. The maximum loss of variegation was 19% in all treated plants.

INTRODUCTION

Salvia greggii is a biennial plant native to Mexico and Texas and is commonly known as autumn sage (Kawahara et al., 2003). The variegated form has attractive leaves and a long flowering period and is required in large numbers by the nursery trade. Previous studies with *S. fruiticosa* showed that micropropagation methods could be applied (Naser et al., 2004). Micropropagation methods are being developed to multiply and produce large numbers of uniform plants of *S. greggii*, which retain their variegation.

Salvia greggii tends to grow tall with an irregular shape. Growth regulators containing paclobutrazol (e.g., Bonzi) effectively reduce internode extension of various plant taxa (Sugavanum, 1983; Arnold, 1998), which enables production of more compact and densely branched plants.

Paclobutrazol is poorly soluble in water and is absorbed rapidly by plant stems and petioles or through the roots. It inhibits cell division in the subapical meristem of the shoot but has little effect on the production of leaves or on root growth (Gianfagna, 1995). Application reduces stem elongation, and in certain species results in a plant with improved market specification. It has been used to control height growth in the commercial production of poinsettias (Niu et al., 2002), chrysanthemums (Smith et al., 1990), geraniums and bedding plants (Keever et al., 1990).

We had three objectives for this study:

1) To investigate the use of micropropagation techniques and the application of growth regulators for establishing viable shoot cultures in vitro, with the successful production of roots and shoots.

2) To determine if paclobutrazol treated plants propagated in vitro and in the greenhouse exhibit any dwarfing effects after treatment.
3) To develop a commercially viable means to wean plants to market specifications.

MATERIALS AND METHODS

Initiation. We collected shoots from nursery stock plants and sterilised them with 15-min immersion in 7% calcium hypochlorite containing two drops of Tween 80 in 250 ml, followed by three 5-min washes in sterile water. We took nodal explants and cultured them in vitro on MS modified medium (Murashige and Skoog, 1962 macronutrients and micronutrients) with B5 vitamins (Gamborg et al., 1968) to which were added 30 $g \cdot L^{-1}$ glucose, 3.3 $g \cdot L^{-1}$ phytagel, 0.1 $mg \cdot L^{-1}$ BAP, and 0.01 $mg \cdot L^{-1}$ IBA. We adjusted the pH to 5.8 and autoclaved the medium in jars containing 35-ml aliquots for 20 min at 121 °C and 103.4kpa (15 $lb/inch^2$). We maintained all the cultures in a growth room with a 16-h photoperiod at 22 °C under Philips cool white fluorescent tubes (58.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at bench level).

Rooting. We compared the rooting of proliferated shoots on half strength MS and 15 $g \cdot L^{-1}$ sucrose, with IBA added to the medium at 0, 0.01, 0.1, 1.0, or 10.0 $mg \cdot L^{-1}$ IBA. We incubated the cultures for 2 weeks.

Paclobutrazol and Gibberellic Acid Treatment in Vitro. We tested the effects of paclobutrazol and gibberellic acid on shoot and internode length of 2-node explants cultured on MS medium. We compared five different treatments with an untreated control: paclobutrazol at 0.1, 1.0, and 2.0 $mg \cdot L^{-1}$ of medium; giberellic acid at 1.0 $mg \cdot L^{-1}$; and paclobutrazol at 1.0 $mg \cdot L^{-1}$ plus giberellic acid at 1.0 $mg \cdot L^{-1}$. We incubated these cultures for 4 weeks. The medium composition and culture room conditions were the same as in the rooting experiment. Prior to weaning in the greenhouse we removed the explants from the growth room, and measured their internode length, shoot length, and the number of nodes produced. We performed our statistical analysis using a General Linear Model (SAS Institute, Cary, North Carolina)

Weaning. We took plants from the in vitro growth regulator experiment (above) and planted them in trays containing a potting mix of 2 peat potting medium : 1 vermiculite (v/v). We grew them in a greenhouse for 4 weeks at 20 °C under a 16-h photoperiod with supplementary lighting. We recorded internode and shoot length at the end of the period and performed the same statistical analysis as in the growth regulator experiment.

Paclobutrazol Treatment of Weaned Plants. In May we potted micropropagated *Salvia greggii* plants into 2-L pots containing a standard nursery potting mix and cut them back to 7 cm in height. We grew them for 2 weeks to allow roots to establish and bud break to occur before applying the paclobutrazol treatments. We randomly assigned the established plants to three blocks with each block containing six treatment groups (including untreated control) with seven replications per treatment.

We applied paclobutrazol (as Bonzi) as a drench at concentrations of 0, 0.5, 1.0, 2.0, 4.0, and 8.0 mg a.i. per pot (i.e., per 2 L of potting mix). We diluted the chemical to the desired concentrations with distilled water and applied it in 100-ml aliquots per pot in one application. We pruned the plants back to 7 cm in height, 24 h after treatment

and left them unwatered for 3 days to allow maximum uptake. We recorded plant height, internode length, shoot length, and variegation 4 weeks after treatment and performed the same statistical analysis as in the growth regulator experiment.

RESULTS AND DISCUSSION

Salvia greggii variegated established easily in the MS modified medium and produced an average of six shoots per jar per sub-culture period. IBA stimulated rooting (without IBA rooting was 67%; with 1.0 mg·L^{-1} IBA rooting was 100%). Survival of explants on weaning was 93% to 100% with the maximum loss of variegation at 19% (Table 1).

Elevated concentrations of paclobutrazol applied in vitro significantly reduced both the length of the internodes (Fig. 1) and the number of nodes produced (Fig. 2), with giberellic acid having the opposite effect ($p \leq 0.0002$). This agrees with the findings of Smith et al. (1990). The effect had vanished by 6 weeks after weaning, when the treated shoots were not significantly shorter than those untreated (P=0.619) suggesting that the application of paclobutrazol in vitro did not persist through the weaning phase (Table 2).

When applied to weaned plants established in potting media in the greenhouse, paclobutrazol significantly reduced plant height even at the lowest dose (0.5 mg a.i.). At the highest dose (8.0 mg a.i.) treatment resulted in plants 70% shorter than untreated plants (Fig. 3). Internode length at the highest dose was 60% shorter than for untreated plants (Fig. 4). In contrast with the in vitro paclobutrazol treat-

Table 1. Effect of IBA on rooting *Salvia greggii* variegated.

IBA (mg·L)	Rooting (%)	Mean no. roots/plant	Survival (%)	Variegated (%)
0	66.7	2.9	92.3	100
0.01	66.7	2.6	79.3	100
0.1	58.8	3.6	92.3	100
1.0	100	4.6	100	81.2
10.0	94.1	9.1	92.3	78.3

Table 2. Effects of paclobutrazol and gibberellic acid on weaned *Salvia greggii* variegated.

Growth regulator (mg·L^{-1})	Shoot length (mm)	Internode length (mm)	Nodes per explant (no.)
0 PAC[1]	162	17.0	9.1
0.1 PAC	161	17.15	9.3
1.0 PAC	161	16.4	9.9
2.0 PAC	163	16.3	9.7
1.0 GA_3	164	16.7	9.7
1.0 PAC &1.0 GA_3	157	16.65	9.1
P Value	P= 0.619	P=0.547	P=0.496

[1] Paclobutrazol (PAC), gibberellic acid (GA_3)

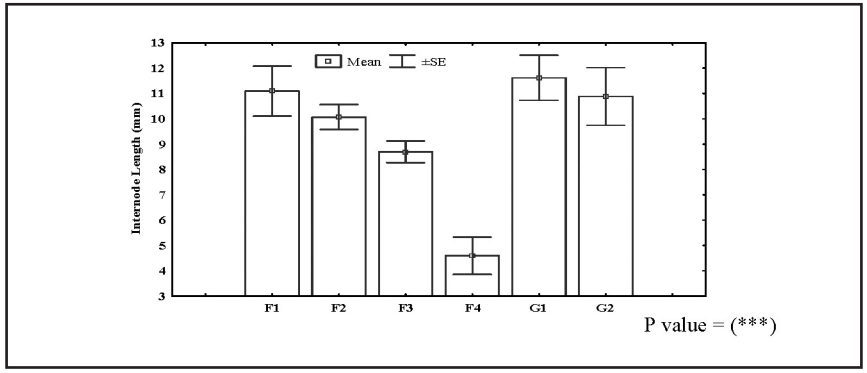

Figure 1. Effects of paclobutrazol and giberellic acid on internode length of *Salvia greggii* variegata in vitro.

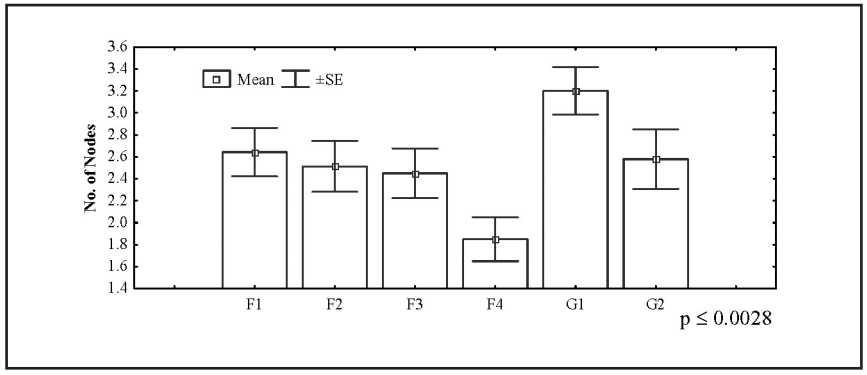

Figure 2. Effects of paclobutrazol and giberellic acid on the number of nodes produced by *Salvia greggii* variegated in vitro.

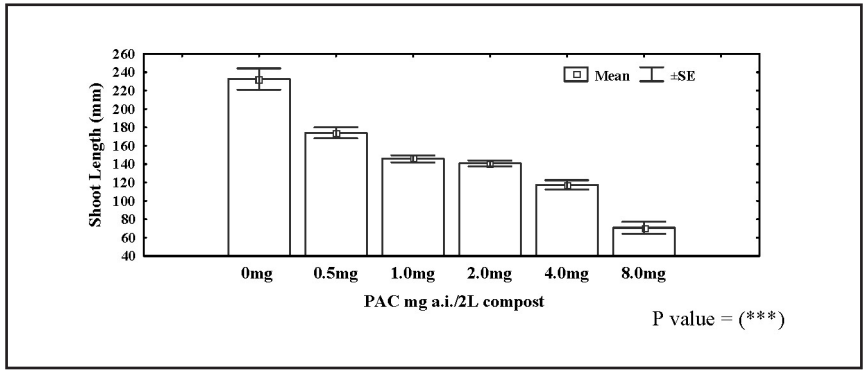

Figure 3. Effects of paclobutrazol applied as a drench on shoot length of weaned *Salvia greggii* variegated

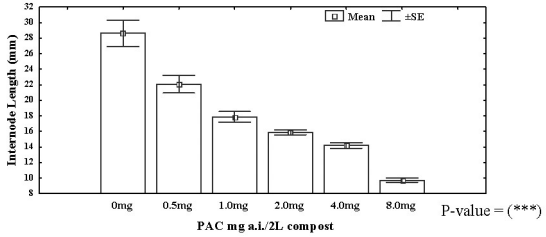

Figure 4. Effects of paclobutrazol applied as a drench on internode length of weaned *Salvia greggii* variegated

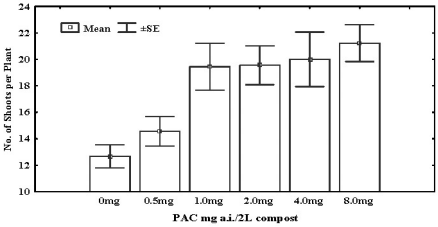

Figure 5. Effects of paclobutrazol applied as drench on shoot number of weaned *Salvia greggii* variegated

Figure 6. Effect of paclobutrazol concentration on stem height of *Salvia greggii*. Left to right : no treatment, 0.5 mg, 1.0 mg, 2.0 mg, 4.0 mg and 8.0 mg a.i. /2 L compost as a soil drench.

ments, the number of nodes produced on treated weaned plants was not significantly different to that of untreated plants (P=0.3816).

Generally paclobutrazol has been known to have little effect on root and shoot growth. In our salvias it induced bud break, with shoot numbers increasing as we increased dose above 0.5 mg a.i. (Fig. 5). This enabled us to produce compact plants with a dense structure (Fig. 6) and highlights the value of using this growth regulator to obtain shorter plants (Smith et al., 1990). Survival of plants during treatment was 100% with the maximum loss of variegation at 19%. No loss in variegation was observed in plants treated with the two highest levels of paclobutrazol.

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