Effects of Reduced Humidity and Paclobutrazol on Acclimatisation of Tissue-Cultured Plants

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INTRODUCTION

Plant tissue culture is now a widely used propagation method. Its main advantage is the ability to rapidly multiply selected cultivars and to produce genetically uniform plants. The techniques range from simple micropropagation using a bud, node or leaf segment, to cell or protoplast culture. Commercial production mainly involves micropropagation. Whatever the technique used, the final step involves the transfer of the cultured plants from the protected environment of the culture vessel to the nursery bench. The process of the plant adapting to this change in environment is known as acclimatization.

Under in vitro conditions of low light intensities and in the presence of carbohydrate in the medium, plants are heterotrophic i.e., they are unable to photosynthesise to meet their carbohydrate requirements. The high humidity in the culture vessel also results in plants unable to control water loss through transpiration (Brainerd and Fuchigami, 1981; Capellades et al., 1990; Grout and Aston, 1978; Smith et al., 1986; Grout and Millam, 1985). During acclimatization, the plants must survive the water stress long enough to re-establish photosynthesis and controlled transpiration.

To maximise the potential of tissue culture applications in the nursery, it is necessary to increase the survival of plants during the acclimatisation procedure and reduce the time that this procedure requires. One approach is to control the humidity either before or after the plants are planted-out (Whish et al., 1992). Another is the use of plant growth retardants (PGR) to reduce the wilting of plants when transferred to the nursery. Increasing the light intensity or reducing the sugar supply in the medium may also promote photosynthesis (Kozai, 1991).

Paclobutrazol (PAC) (PP333, ICI) is a broad spectrum, xylem mobile PGR which reduces growth by inhibiting gibberellin biosynthesis (Lever, 1986). PAC does not block the activity of existing exogenous or endogenous gibberellin (Lever, 1986). The morphological effects of PAC application include reduced leaf size of apples (Curry and Williams, 1983) while the anatomical effects include increased cell thickness, number of mesophyl layers, sunken narrower stomates, increased chloroplast size, and an increase in epicuticular wax deposition (Gao et al., 1987).

The reduction of the humidity in the culture vessel has improved the survival of several species including *Ptilotus* sp. (Whish et al., 1992) and *Allium* sp. (Fari and Nemeth, 1987). It was found that the reduction of humidity in the head space led to increased rooting, higher leaf dry matter, and profuse wax production in onion (Fari and Nemeth, 1987). Survival of *Ptilotus* after deflasking was better if roots were produced in vitro (Whish et al., 1992).

This paper reports the response of two species, *Rosa* 'Red Cascade' and kangaroo paw (*Anigozanthos bicolor*) to reduced humidity or PAC application during the final stage of micropropagation.

MATERIALS AND METHODS

Culture Media. Both species were cultured on de Fossard (1976) medium with high minerals and organic supplements and 30 g/litre^{-1} sucrose. The agar concentration was 8 g/litre^{-1} for kangaroo paw and 6 g/litre^{-1} for rose. The pH was adjusted to approximate 5.8 prior to adding agar and autoclaving in 200 ml aliquots for 25 min at 121C and 103 kPa.

Humidity Treatments. Plants of both species were exposed to four relative humidities (RH), 86%, 90%, 95%, and 100%. The 100% RH was the control simulating a normally sealed culture vessel. Humidity was controlled by placing the open culture vessels over saturated salt solutions within larger chambers. The theory behind this method is that in a confined space, at equilibrium, the atmosphere above a saturated solution will have a particular RH (Table 1). The salt solutions were autoclaved for 15 min at 121C and 103 kPa, then 50 ml dispensed into each sterile Chanrol M30 Polypropylene chamber.

Table 1. Selected salts and the theoretical equilibrium RH.

Humidity	86%	90%	95%	100%
Saturated salt	*Potassium* chloride	*Zinc* sulphate	*Dibasic* sodium phosphate	*Deionised* water

To prevent water evaporation from the gel, the surface was covered with aluminium foil. Small incisions were made in the aluminium foil and the plants were inserted through to the medium. The culture vessel lid was removed and the open vessel placed into the humidity chamber which was then sealed and kept in a culture room at either 22C for rose or 25C for kangaroo paw, with 16-h day and 8-h night and a light intensity of 25 ìmol m^{-2} sec^{-1}. After 4 weeks in the culture rooms, the humidity in the chambers was recorded using a hand held humidity meter (Hanna, HI 8564, Thermo Hygrometer). This was achieved by partially removing the lid of the chamber and inserting the probe to the level of the plant then wrapping the chamber and the probe with plastic wrap.

Paclobutrazol Treatments. Five concentrations of PAC (0, 1, 2, 3, and 4 mg litre^{-1}) were incorporated after the rooting media had been autoclaved and cooled to 60C. The medium composition and culture room conditions were the same as described above.

Acclimatization. For acclimatization, the plants were removed from the media and the roots washed to remove excess gel. Root length and fresh weight of the plants was recorded before they were planted into a potting mix of 1 vermiculite : 1 perlite : 1 peat (by volume) then subjected to the following regime: 4 days under mist and shade cloth; 3 days under mist only; 4 days in a white washed glasshouse; then 2 weeks in a normal glasshouse.

At the conclusion of acclimatization, the root length, survival index and growth index were recorded. The survival index was a simple ranking of 1 for alive and 0

for dead. The growth index included 5 levels over the range 1= no growth, 3= average growth, and 5= vigorous growth. Plants were then oven dried for 48 h at 80C to determine total dry weight.

RESULTS AND DISCUSSION

Effects of Humidity. At the end of the 4-week culture period the RH in the chambers was around 90% to 95 % irrespective of the humidity treatment applied and there was no difference between species (data not presented).

For kangaroo paw, plant dry weight tended to be greater with the 86% RH treatment (Fig. 1) however the difference between the humidity treatments was small, only 0.22 g between the highest and the lowest. This small response may be due to the stabilising of the RH at approximately 90% to 95% in all chambers by the end of the four weeks in culture. Evaporation of water from the gel and condensation into the salt solutions may have gradually raised the RH and reduced the treatments effect. The aluminium foil did not prevent evaporation from the medium.

Roots on plants kept at 86% RH were, on average, 65 mm long while plants at 100% RH had 30-mm roots. Why is there an increase in root growth at lower humidities? The roots were extensively branched and healthy in all cases. At 86% RH, kangaroo paws were usually well developed with even, dark green leaves and healthy appearance. At higher RH plant growth was less with only a few shoots present.

The reduced humidity in vitro increased the growth of kangaroo paw and improved its overall appearance while not effecting the number surviving; however, the plants could be ready for market after a short time in the glasshouse.

The rose cultivar did not respond to RH in any consistent way. The biomass was greatest at 90% RH but with no difference between the humidity treatments. The reduced humidity allowed the plants to establish ex vitro in shorter time compared to the higher humidities. The 90% RH caused a slight decrease in root length and this was correlated with a reduced growth index. The increase in total biomass was probably due to thickening of the roots since shoot growth and root length were reduced.

Plant Growth Retardant Application. There was a noticeable difference in the height and the habit of the plants when they were removed from culture. Plants treated with the higher concentration of PAC, namely 4 mg/litre^{-1}, leaves were reduced in size and darker green in colour. This was uniform for both species.

After acclimatization, the response of plant dry weight to PAC application rate was inconsistent (Figs. 1 and 2). In both species growth was promoted with 1 mg/litre^{-1} PAC. Kangaroo paw was inhibited by 2 mg/litre^{-1} and rose promoted at 4 mg/litre^{-1}.

Visual ranking of plant growth gave a more consistent response with growth suppressed at 2 mg/litre^{-1}.

There is no obvious explanation for this irregular pattern of response. Root length was not significantly affect by PAC (data not shown) although root systems were seen to be compact and thickened compared to the untreated controls. This thickening of the roots has previously been seen in chrysanthemum treated with PAC in vitro (Smith et al., 1991). It may be that the end results are superimposed on different rates of recovery from the treatments. Plants were initially noticeably

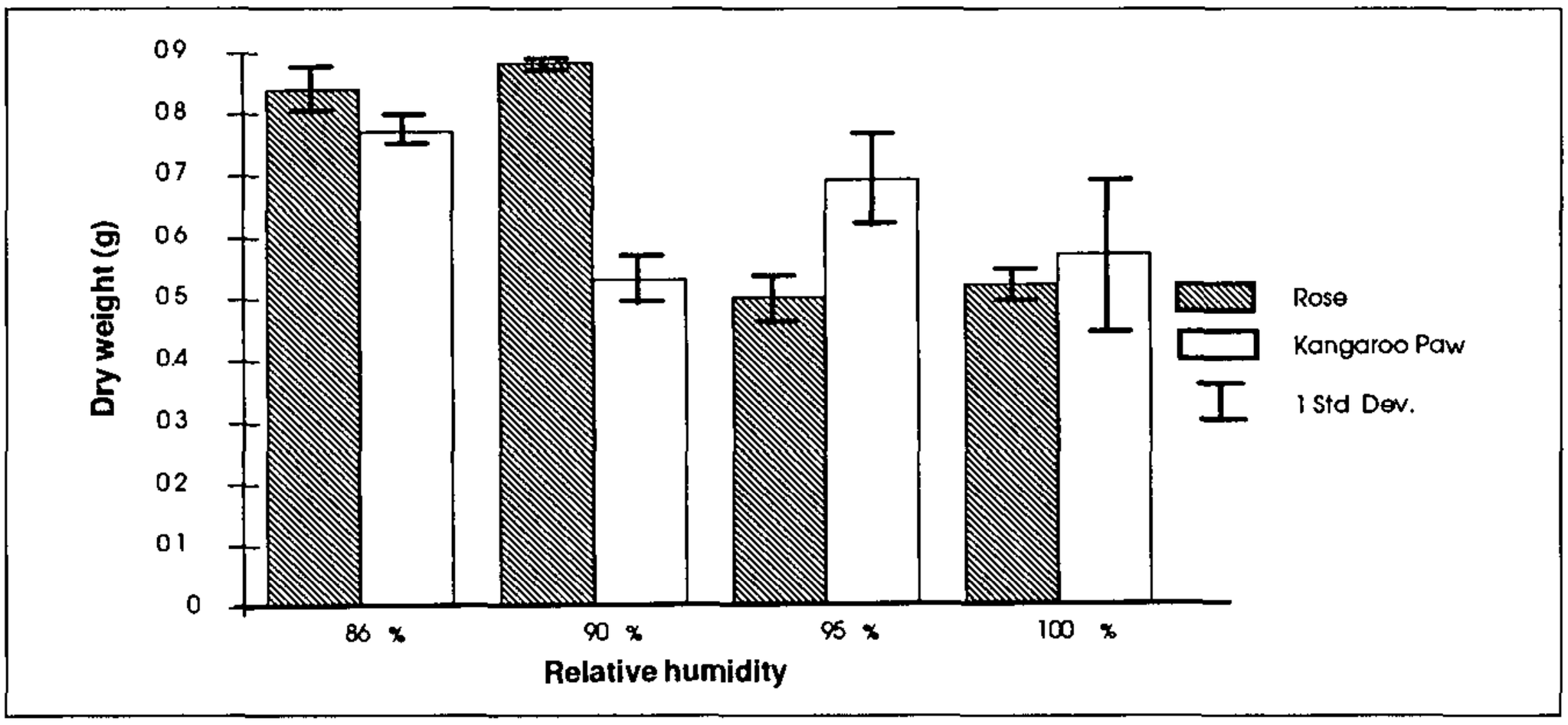

Figure 1. Comparative dry weights of kangaroo paw and *Rosa* 'Red Cascade' under reduced humidity in vitro.

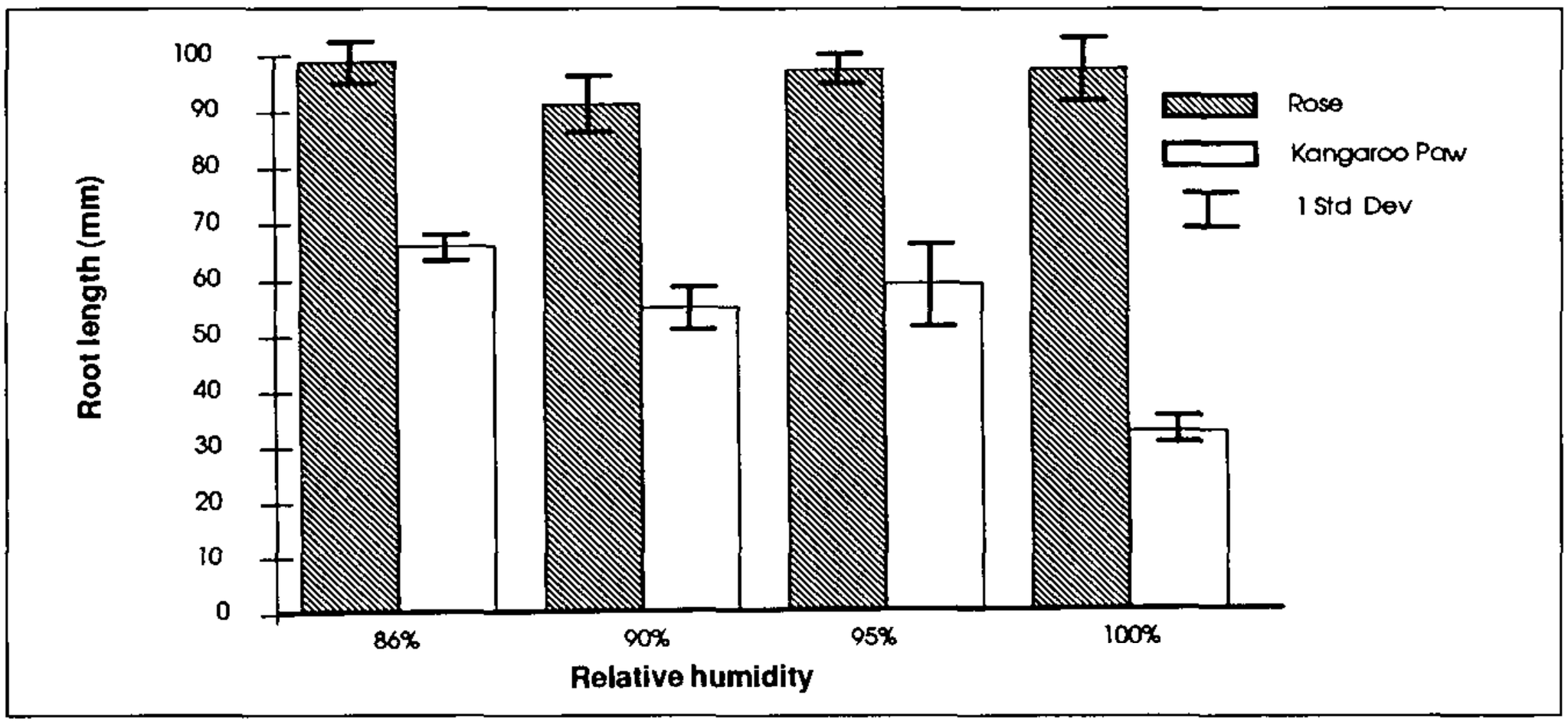

Figure 2. Comparative root lengths of kangaroo paw and *Rosa* 'Red Cascade' under reduced humidity in vitro.

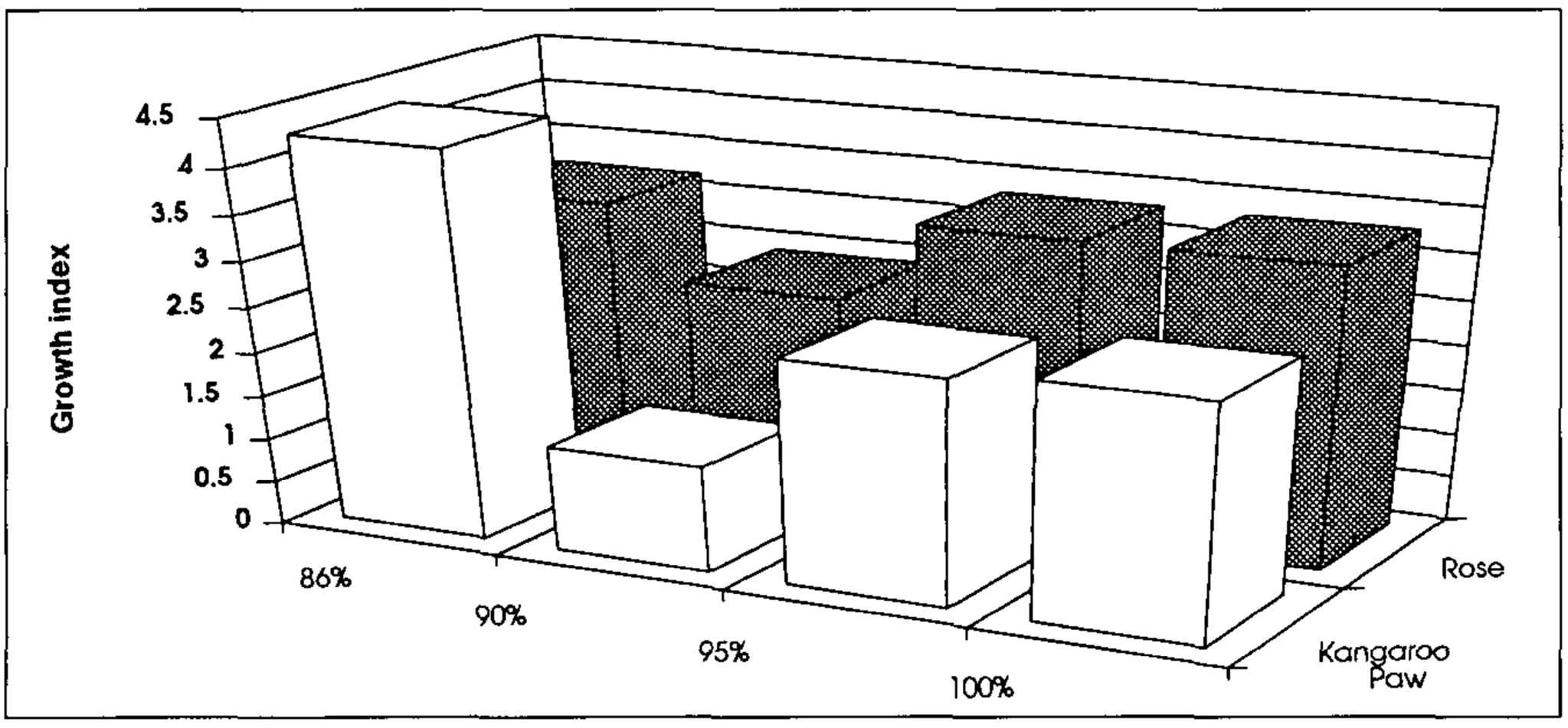

Figure 3. Comparative growth index for kangaroo paw and *Rosa* 'Red Cascade' under reduced humidity in vitro.

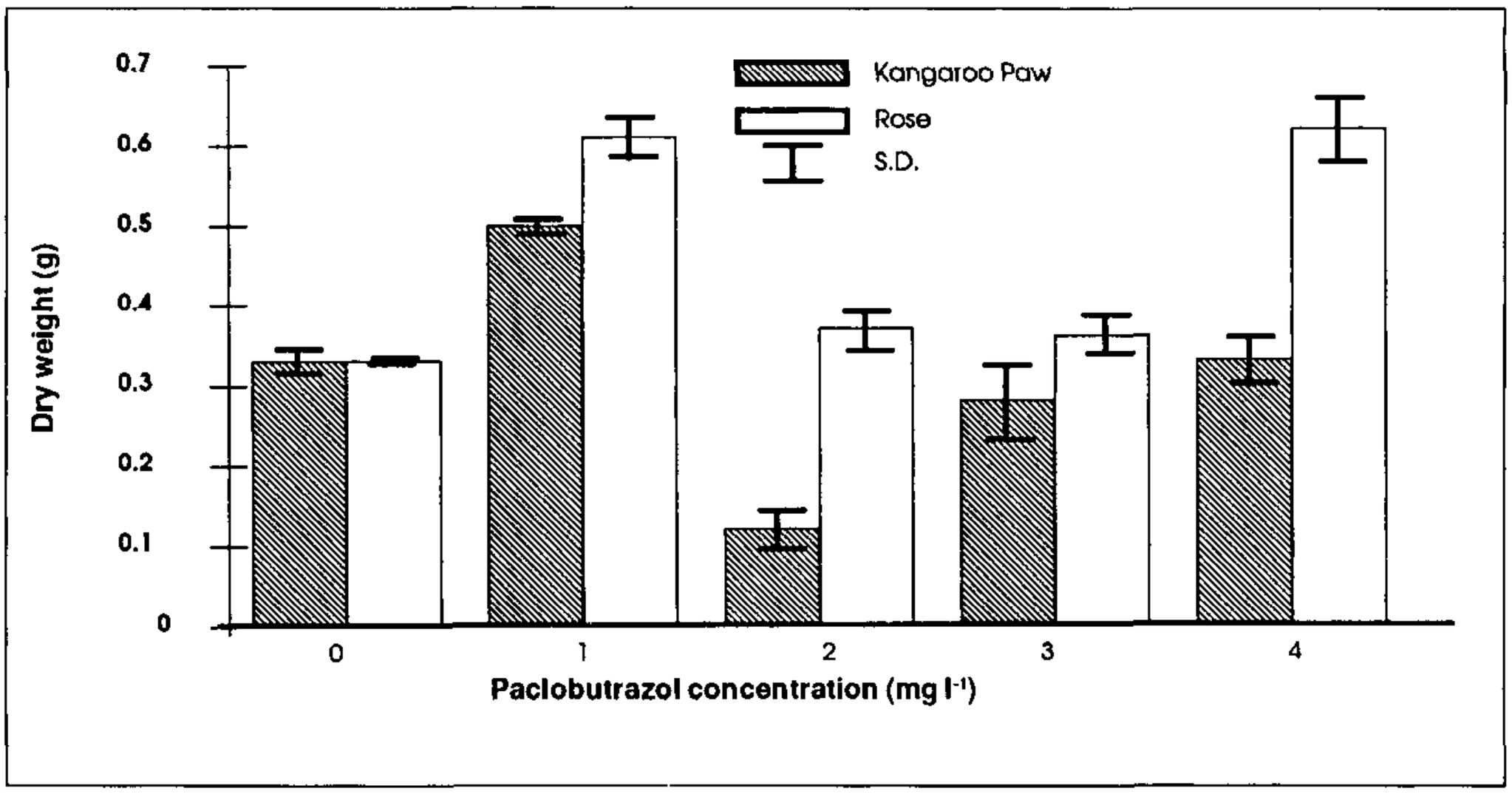

Figure 4. Comparative dry weights of kangaroo paw and *Rosa* 'Red Cascade' under different paclobutrazol concentrations in vitro.

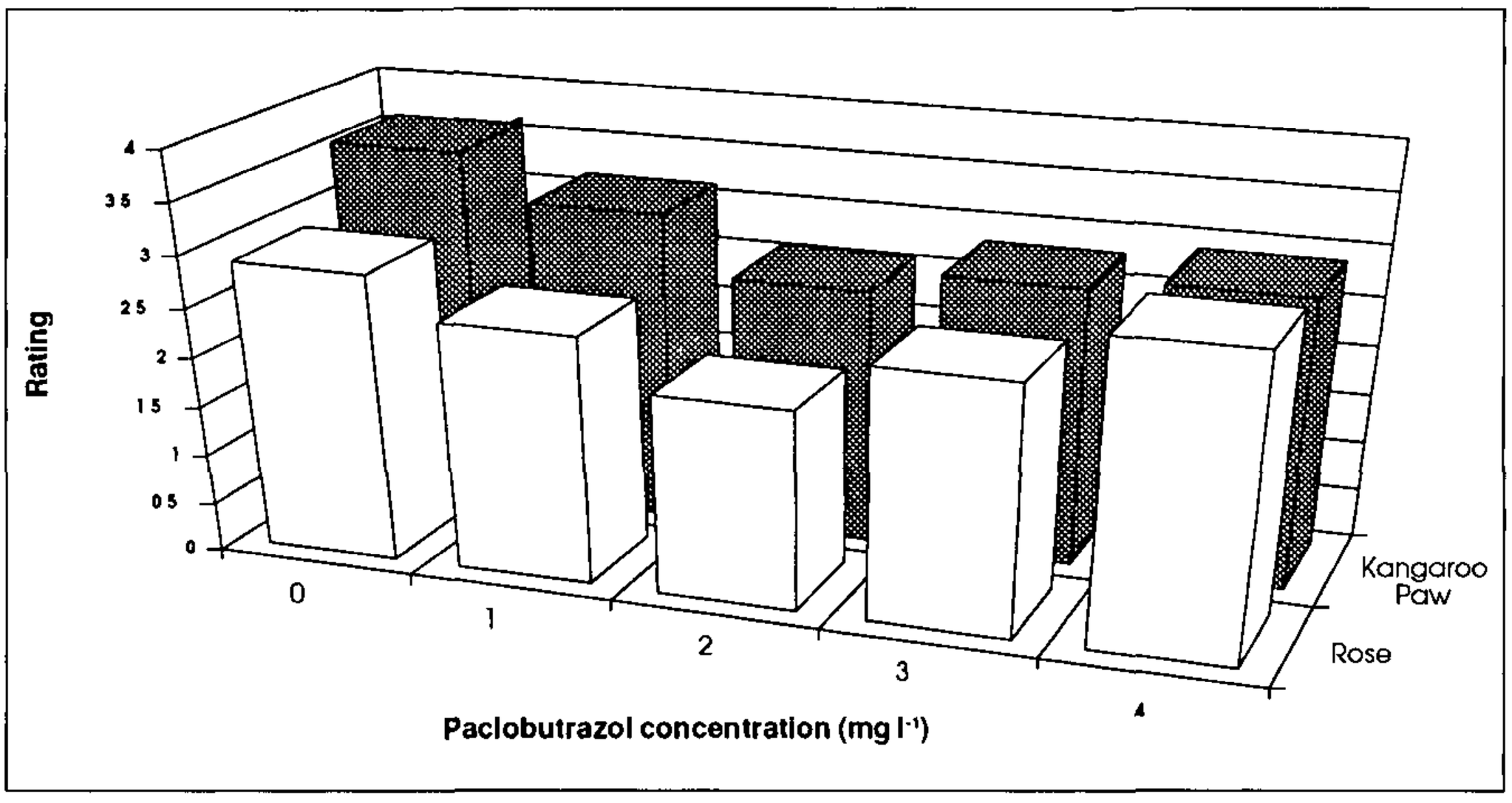

Figure 5. Comparative growth rating index for kangaroo paw and *Rosa* 'Red Cascade' after paclobutrazol treatment in vitro.

bushy at the higher concentrations; however, following 25 days of glasshouse environment, growth had begun to return to normal in all treatments.

CONCLUSIONS

Survival and growth of rose was not improved by the reduction of humidity in vitro. Kangaroo paw growth was increased by reducing in vitro humidity to 86% to 90% RH and root length increased at RH below 100%. Growth responses to PAC were erratic.

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