

concentration whereas 2iP had no effect on the number of these shoots.

BA at 2, 8, 32, and 64 μM was then combined with 0.01, 0.1, and 1.0 μM NAA. The addition of NAA to the medium containing 32 or 64 μM BA increased the number of shoots produced when compared to media containing only BA (Fig. 3). The number of shoots ≥ 5 mm was greatest at 0.1 μM NAA, with 13.7 shoots produced in 64 μM BA, and 13.4 produced with 32 μM BA added. The most shoots ≥ 10 mm were produced with 64 μM BA + 1.0 μM NAA, (8.5 shoots) (Fig. 4). However, 0.1 μM NAA + BA at 32 or 64 μM produced 8.2 and 8.3 shoots, respectively.

Individual shoots 5 mm or longer were removed from shoot clusters and stuck in a soilless potting mixture of 1 sphagnum moss peat: 1 vermiculite (v/v) in styrofoam trays (19.5 \times 13.5 \times 7.5 cm). Styrofoam trays

were sealed in plastic bags and placed in the greenhouse for 4 weeks while rooting occurred. The cuttings rooted 100% without auxin treatment. A one-week acclimation period with a gradual opening to ambient humidity was utilized before transfer to the greenhouse. Plants were grown to flowering and no differences from the type cultivar was observed.

These results indicate that the potential for micropropagation of *Vinca minor* exists. Shoots of 'Bowlesii' vinca produced in vitro with 32 or 64 μM BA and 0.1 μM NAA can be rooted in a soilless medium under high humidity with 100% success.

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In Vivo Pollen Germination of *Aglaonema* Affected by Relative Humidity

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Abstract. *Aglaonema crispum* (Pitcher & manda) Nicols., *A. pictum* (Roxb.) Kunth and *A. commutatum* Schott 'Treubii' inflorescences pollinated at high (100%) relative humidity (RH) showed excellent in vivo pollen germination. Inflorescences pollinated at low (40% to 50%) RH revealed significantly less germination than those pollinated at high RH. Pollen exposed to low RH for 4 hr before pollination did not germinate.

Aglaonema species, members of the family Araceae, are important tropical ornamental plants because of their attractive variegated foliage and tolerance of low humidity and light levels indoors. Although previous breeding programs produced 4 important hybrids (2), no information has been published on factors affecting seed production in *Aglaonema*. Pollinations of *Aglaonema*, as part of the foliage breeding program at the Agricultural Research and Education Center, Apopka, have resulted in poor seed production. A previous report demonstrated that 100% relative humidity was essential for in vivo pollen germination and seed set in *Diefenbachia* (1).

This study was initiated to determine if

relative humidity affected in vivo pollen germination in *Aglaonema*.

Aglaonema crispum 'Chartreuse Halo', *A. commutatum* 'Treubii', and *A. pictum* were used in this study (2). Fresh pollen was collected in 5 cm petri dishes before 10:00 AM from plants growing in a shaded greenhouse with a maximum light intensity of $250 \pm 10 \mu\text{mol s}^{-1} \text{m}^{-2}$. Petri dishes were taken into a lab and either placed in a plastic bag with a wet paper towel and sealed (high RH pollen treatment) or left open (low RH pollen treatment). RH of the air-conditioned lab varied from 40% to 50%. Simultaneously, newly opened inflorescences were harvested and placed in the lab with their peduncles in H_2O and spadix bagged with a wet towel (high RH inflorescence treatment) or left unbagged (low RH inflorescence treatment). Pollen and inflorescences were allowed to equilibrate at the high or low RH for 4 hr before pollination. Pollinations were made with a one-half cm wide camel hair brush using sufficient pollen to insure coverage of each stigmatic surface. Pistillate flowers were harvested from each spadix 24 hr after pollination. The flowers were fixed in a solution of 1 acetic acid: 8 ethanol: 1 formalin (by

volume) for 18–24 hr, softened in 8 N NaOH for 12–24 hr, and stained in 0.1% analine blue in 0.1 N K_3PO_4 for 24 hr (3). The flowers were placed on a slide and gently squashed under a cover slip in a drop of glycerine. Pollen germination on the stigmatic surface was observed using a Blak-Ray longwave ultra-violet light (Model B-100A) and a dissecting microscope. The number of pollen tubes present per flower was rated on a scale of 1–6 where 1 = 0, 2 = 1–4, 3 = 5–9, 4 = 10–19, 5 = 20–49 and 6 = 50+. Tests were conducted on days when sufficient inflorescences and pollen were available. Statistical analyses for each test were done separately.

A. pictum pollen, held 4 hr at high RH and used for selfing or crossing with *A. commutatum* 'Treubii' flowers at high RH showed excellent germination (Table 1). In contrast, the same crosses made entirely under low RH conditions yielded no germination. *A. crispum* 'Chartreuse Halo' flowers self-pollinated at high RH with pollen held at high RH showed mean germination ratings ranging from 4.2 to 5.5 in 4 separate tests (Table 2). Incubating flowers 4 hr at low RH followed by pollination at high RH did not lower germination. However, flowers held 4 hr at low or high RH and then pollinated at low RH with pollen held at high RH, had significantly reduced mean germination ratings, ranging from 1.8 to 2.3 (Table 2). Fresh pollen kept 4 hr at low RH before pollination did not germinate under either RH condition tested.

Optimum in vivo germination of *Aglaonema* pollen occurred when both pollen and pistillate flowers were maintained at high RH before and after pollination. Pollination of styles held at low RH 4 hr before, but incubated at high RH after pollination, also produced good germination. Pollen was particularly sensitive to drying; a 4 hr exposure to low RH eliminated its ability to germinate even at high RH.

The small amount of pollen germination observed on stigmas incubated at low RH indicates that 100% RH following pollina-

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Table 1. In vivo germination of *Aglaonema pictum* pollen, on *A. pictum* and *A. commutatum* 'Treubii' stigmas, following exposure of pollen and pistillate flowers (stigmas) to low (40% to 50%) or high (100%) RH for 4 hr before pollination and 24 hr after pollination at $27^{\circ} \pm 1^{\circ}\text{C}$.

Pollination	RH before pollination		RH after pollination stigma	Mean germination rating ^z	Total no. observations inflor./stigmas
	Pollen	Stigma			
<i>A. pictum</i> ♂	High	High	High	5.5 ± 0.8	3/30
<i>A. pictum</i> ♂	Low	Low	Low	1.0 ± 0.2	3/30
<i>A. c.</i> 'Treubii' x <i>A. pictum</i>	High	High	High	6.0 ± 0.0	4/16
<i>A. c.</i> 'Treubii' x <i>A. pictum</i>	Low	Low	Low	1.0 ± 0.2	4/16

^zVisual rating where 6.0 = 50+ pollen tubes present; 5.0 = 20–49 tubes; 4.0 = 10–19; 3.0 = 5–9; 2.0 = 1–4 and 1.0 = no pollen germination.

Table 2. In vivo germination of *Aglaonema crispum* 'Chartreuse Halo' pollen following exposure of pollen and pistillate flowers (stigmas) to low (40% to 50%) or high (100%) relative humidity (RH) for 4 hr before pollination. Stigmas were incubated 24 hr after pollination at low or high RH at constant $27^{\circ} \pm 1^{\circ}\text{C}$. Data is presented from 4 separate tests.

Pollen	Stigma	RH after pollination (stigma)	Mean pollen germination rating ^z				Total no. observations inflor./stigmas
			Test 1	Test 2	Test 3	Test 4	
High	High	High	5.5 a ^y	4.2 a	4.9 a	5.2 a	9/97
High	Low	High	---	4.5 a	---	---	3/27
High	High	Low	2.3 b	---	---	---	3/36
High	Low	Low	1.8 b	---	2.1 b	1.8 b	6/70
Low	High	High	---	---	1.0 c	1.0 c	3/34
Low	Low	Low	---	---	1.0 c	1.0 c	3/34

^zVisual rating where 6.0 = 50+ pollen tubes present; 5.0 = 20–49 tubes; 4.0 = 10–19; 3.0 = 5–9; 2.0 = 1–4 and 1.0 = no pollen germination.

^yMean separation within columns by Duncan's multiple range test, 5% level.

tion is not essential for germination of *Aglaonema* pollen. Maintaining RH at 100%, however, significantly increased germination in all tests. In Central Florida, greenhouse RH commonly drops to 50% during the warm parts of the day. Based on this study, we now routinely wrap newly pollinated *Aglaonema* inflorescences with moist

paper towels and enclose them in small plastic bags for 24 hr following pollination. In addition, fresh pollen is collected early in the morning and used immediately or kept at high RH until used later the same day. Using these methods, seed production on *Aglaonema* stock plants increased dramatically, and seed set failures are now rare.

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