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In Vitro Rooting and Greenhouse Acclimatization of *Lachenalia* Shoots

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Abstract. **Shoot formation was obtained from *Lachenalia arbuthnotiae* W.F. Barker, L. *bulbifera* (Cyrillo) Engl., and *L. purpureo-coerulea* Jacq. leaf tissue explants cultured on Murashige and Skoog (MS) medium supplemented with sucrose at 30 g·liter^{-1}, 8.87 μM BA, and 0.44 μM K-NAA. Shoots of all three species rooted on subculture to MS medium supplemented with 0.0, 4.14, or 8.29 μM K-IBA or 0.0, 4.46, or 8.92 μM K-NAA. Maximum percent rooting was ≈81% from treatment with 4.14 μM K-IBA for *L. arbuthnotiae* and with 8.29 μm K-IBA for *L. purpureo-coerulea*; it was 59% from treatment with 8.92 μm K-NAA for *L. bulbifera*. Rooted and nonrooted shoots were acclimatized in a greenhouse. Survival of rooted plants was 93% for *L. arbuthnotiae*, 95% for *L. bulbifera*, and 94% for *L. purpureo-coerulea*. Survival of nonrooted shoots was 71% for *L. arbuthnotiae* and 91% for *L. bulbifera*. Chemical names used: 6-benzyladenine (BA); potassium salt of indole-3-butyric acid (K-IBA); potassium salt of 1-naphthaleneacetic acid (K-NAA).**

Lachenalia is a genus of bulbous geophytes endemic to South Africa. The genus includes many species with excellent horticultural merit (Duncan, 1988). *Lachenalia* species can be propagated from seed and bulb cuttings, and some from leaf cuttings (Duncan, 1988) and through tissue culture (Klesser and Nel, 1976; Nel, 1983; Niederwieser and Vcelar, 1990). The effects of explant-tissue age and orientation, sucrose, BA, and NAA on adventitious bud formation from leaf tissue have been investigated (Niederwieser and van Staden, 1992; van Staden and Drewes, 1994; van Rensburg and Vcelar, 1989). Klesser and Nel (1976) and Nel (1983) have published limited information on the in vitro rooting and greenhouse acclimatization of *Lachenalia* tissue-culture shoots. Neither report includes survival rates of rooted shoots after acclimatization or a protocol for greenhouse acclimatization. Therefore, I report on the effect of IBA and NAA on the in vitro rooting of three *Lachenalia* species and on their greenhouse acclimatization and survival, none of which, to my knowledge, have been reported to be propagated by tissue culture.

Materials and Methods

Three species were investigated: *L. arbuthnotiae*, *L. bulbifera* (formerly *L. pendula*), and *L. purpureo-coerulea* . The tissue culture technique used for shoot regeneration was similar to that previously reported for *Lachenalia* (Nel, 1983; Niederwieser and Vcelar, 1990). Leaves were cut off just above the bulbs from greenhouse-grown plants near full floral anthesis. Leaves were rinsed in running tap water for 5 min, surface-disinfected in a solution of 1.0% sodium hypochlorite and 0.1% Tween 20 for 12 min, then rinsed twice in sterile distilled water 5 min each time. Individual leaf explants, each 5 × 10 mm, were placed horizontally, abaxial side down, in 25 × 150-mm culture tubes containing 10 ml of medium, which consisted of MS (Murashige and Skoog, 1962) basal salts and vitamins, sucrose at 30 g·liter^{-1}, 8.87 μM BA, and 0.44 μM K-NAA. Medium pH was adjusted to 5.7 before adding Sigma A 1296 agar (Sigma Chemical Co., St. Louis) at 6.0 g·liter^{-1}. Culture tubes were sealed with polypropylene caps and autoclaved at 121C for 15 min.

After explant placement, culture tubes were sealed with parafilm, then placed upright in 40-tube racks in an incubator (model 818; Precision Scientific, Chicago). Cultures were maintained at 22C and given a 16-h photoperiod with a photosynthetic photon flux of 30 to 44 μmol·m^{-2}·s^{-1} provided by two 40-W broad-spectrum fluorescent lamps (Verilux, Greenwich, Conn.). Photosynthetic photon flux was measured with a quantum sensor (model LI-190SA; LI-COR, Lincoln, Neb.). Shoots were subcultured every 5 to 10 weeks to the same medium to obtain sufficient shoots for the rooting study.

For rooting, nonrooted shoots, 2 to 7 cm tall, of each species were cultured individually on MS medium supplemented with 30 g sucrose/liter and 0.0, 4.14, or 8.29 μM K-IBA, or 0.0, 4.46, or 8.92 μM K-NAA. Percent rooting was recorded after 9 to 10 weeks. Shoots with at least one root ≥1.0 cm long were considered rooted. Rooted shoots of *L. purpureo-coerulea* and rooted and nonrooted shoots of *L. arbuthnotiae* and *L. bulbifera* then were removed from tissue culture for acclimatization in the greenhouse. Shoots were planted in 72-cell plug trays, each cell containing ≈40 cm^3 medium of 3 sand : 1 peatmoss (v/v). Trays were covered with clear plastic domes, then placed under 50% shadecloth in the greenhouse. The greenhouse averaged 18.6C with a maximum of 25.0C and a minimum of 5.6C during the acclimatization period. The plastic domes were propped up ≈3 cm on one side after 2 weeks and completely removed after 3 weeks. The shoots received ≈9.5 h of natural photoperiod. Shoot survival was recorded 8 weeks after the shoots were removed from tissue culture.

For shoot initiation, 80 leaf explants were used for *L. bulbifera* and 120 each for the other two species. For the rooting study, individual shoots of each species were removed from the proliferation medium and were assigned randomly to each of the five rooting treatments. The number of shoot explants used for each rooting treatment was 30 for *L. arbuthnotiae* and 40 for *L. bulbifera* and *L. purpureo-coerulea*. The rooting study was conducted once. Rooting percentage was analyzed with the G statistic, comparing the individual auxin treatments to the control for each species.

Results

Shoots were evident on the leaf explants within 3 weeks for *L. bulbifera* and *L. arbuthnotiae* and within 5 weeks for *L. purpureo-coerulea* after culture initiation. Shoots subcultured to fresh proliferation medium readily formed additional shoots. *Lachenalia arbuthnotiae* and *L. bulbifera* produced callus from most subcultured shoots, whereas *L. purpureo-coerulea* rarely produced callus. Callus was excised and discarded each subculture. Explants on proliferation medium did not produce roots.

Shoots of all three species rooted on all rooting media. Three of the four auxin treatments significantly increased rooting percentage for *L. arbuthnotiae* and *L. bulbifera* compared to the nontreated cuttings (Table 1). Conversely, the auxin treatments had no effect or significantly decreased rooting percentage for *L. purpureo-coerulea* compared to the control. Maximum percent rooting was ≈81% from treatment with 4.14 μM K-IBA for *L. arbuthnotiae* and with 8.29 μM K-IBA for *L. purpureo-coerulea*; it was 59% from treatment with 8.92 μM K-NAA for *L. bulbifera*. Klesser and Nel (1976) reported 65% rooting for one *Lachenalia* hybrid cultured on medium containing 5.37 μM NAA; subsequently, Nel (1983) reported that 9.80 μM IBA resulted in better rooting than treatment with NAA, but did not include any data.

Lachenalia species appear to vary in shoot rooting response according to the type and concentration of auxin used to stimulate rooting. Niederwieser and van Staden (1992) similarly reported that *Lachenalia* hybrids vary in their optimal cytokinin requirements for bud stimulation. Therefore, for micropropagation of each *Lachenalia* genotype, it may be necessary to test several concentrations of various growth regulators for optimal shoot production and rooting.

For this study, survival of rooted shoots

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Table 1. Auxin effects on the rooting percentage of *Lachenalia arbuthnotiae*, *L. bulbifera*, and *L. purpureo-coerulea* after 9 to 10 weeks of culture on rooting medium.

Auxin (μM)		Rooting (%)[z]		
		Species		
K-IBA	K-NAA	*L. arbuthnotiae*	*L. bulbifera*	*L. purpureo-coerulea*
0.0	0.0	43.3	22.0	76.2
4.14	0.0	81.3**	36.4	57.4
8.29	0.0	68.6*	43.9*	81.3
0.0	4.46	71.4*	52.5**	53.5*
0.0	8.92	60.6	58.8**	54.3*

[z]Significance within each species based on the comparison of individual auxin treatments with the control (no hormone) using the G statistic.
*, **Significant at $P \leq 0.05$ or 0.01, respectively.

after removal from tissue culture was 93% (96 of 103) for *L. arbuthnotiae*, 95% (170 of 179) for *L. bulbifera*, and 94% (123 of 131) for *L. purpureo-coerulea*. Survival of nonrooted shoots was 71% (20 of 28) for *L. arbuthnotiae* and 91% (170 of 186) for *L. bulbifera*. These results compare favorably to Klesser and Nel's (1976) finding of 50% survival in a greenhouse for nonrooted shoots of a *Lachenalia* hybrid. Therefore, the labor and cost of rooting shoots in vitro might be eliminated, and instead, nonrooted shoots might be established directly in a greenhouse.

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