

Review on Adventitious Bud Formation in *Lachenalia*

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Abstract

Lachenalia varieties are propagated vegetatively through adventitious bud formation. In this report, published and unpublished results and observations are reviewed. Adventitious buds are formed on leaves, peduncles, bulb-scales and axillary meristems. In leaf tissue, buds form from epidermal cells and more than 90% originate from single cells. The regeneration potential of species and varieties varies amongst species and this factor thus became an important evaluation criterion in the breeding programme. Tissue age affects both the number of buds formed, as well as the size of bud/bulblet. Young tissue proved to be the best source of tissue in several studies. The physiological stage of the mother plant affected bud formation on bud forming tissue. Once plants enter the post anthesis stage, the regeneration potential decreases markedly. This effect is less evident when plant growth regulators (PGR's) are used in vitro. PGR's stimulate bud formation markedly and an interaction between the regeneration potential, tissue age and the optimal PGR concentration was shown. Propagation through adventitious bud formation will continue to play an important role in the production of the crop as lachenalias are not resistant to virus diseases. Growers therefore have to adhere to a propagation scheme with different phases and limited generations in each phase.

INTRODUCTION

Some *Lachenalia* species (Duncan, 1988) and hybrids (Roodbol and Niederwieser, 1998), form supernumary bulblets around the basal plate of the bulb and they multiply relatively fast. However, most species do not and techniques for mass propagation through adventitious bud formation on leaf tissue have been developed after the genus was first used in a hybridisation programme. *Lachenalia* is susceptible to viral diseases (Klesser and Nel, 1976). Therefore, in vitro propagation of nuclear stock precedes propagation through leaf cuttings in a plant propagation scheme (Niederwieser, 1996). This paper reviews published and unpublished research on the development and application of the techniques as well as the factors that affect the process of adventitious bud formation.

MATERIALS AND METHODS

Lachenalia species and hybrids were used for studies at Roodeplaat, workers in other countries used mostly species. At Roodeplaat bulbs were planted in autumn in well drained growing media and cultivated in a commercial green house with average minimum of 5 °C and average maximum of 21 °C. The day length followed the natural seasonal pattern for the Pretoria region. The basal tissue culture medium used, consisted of the inorganic salts of Murashige and Skoog 1962, 5% sucrose, 0.1 mg/l of NAA and 2 mg/l of BA. For tissue culture, leaves were generally cut into explants of 1cm² and cultured with the abaxial surface on the medium. Leaf cuttings were placed upright in composted pine bark medium and grown in a commercial greenhouse. EM and light microscopic observations were made in anatomical studies. Epidermal strips were made

to determine the origin of buds.

Origin of Adventitious Buds

Adventitious buds form on leaves (Klessner and Nel, 1976; Nel, 1983; Niederwieser and Vcelar, 1990; Niederwieser and Van Staden, 1990 a & b; Perrignon, 1992; Ault, 1995; Suh, Roh and Lee, 1997) bulb-scales, peduncles (unpublished) and axial meristems (Roodbol and Niederwieser, 1998).

Microscopic observation indicated that cell division takes place in epidermal and parenchyma cells of a leaf explant shortly after tissue has been removed from the plant. Adventitious buds, however, originate from epidermal cells only (Niederwieser and Van Staden, 1990a). More than 95% of buds were observed to originate from derivatives of primary stomatal mother cells, particularly the guard mother cells. Although buds form on the adaxial and abaxial surface of leaf tissue *in vitro* and on leaf cuttings, the majority of buds form on the adaxial surface (Niederwieser and Vcelar, 1990; Ndou, 2000). Bud formation shows a strong tendency to take place along the proximal, transverse end of cultured tissue (Niederwieser and Vcelar, 1990; Niederwieser and Van Staden, 1990b) and leaf cuttings (Perrignon, 1992; Ndou, 2000).

Recently, we carried out an experiment to investigate the interactions between the various tissue types in leaf tissue of *Lachenalia* (unpublished results): Leaf explants from the basal parts of leaves of four hybrids were used. For one third of the explants, the adaxial epidermis with some underlying parenchyma tissue was isolated from the rest of the tissue without damage to the vascular bundles. The resulting explants were each placed on culture medium with the damaged, internal tissue on the medium. For another third of the explants, the abaxial epidermis with underlying parenchyma cells was removed. Intact explants served as the control. For three of the four hybrids used, isolated adaxial epidermal tissue was able to sustain the formation of buds (Table 1). Abaxial epidermis of Hybrids no 1 & 2 was able to do so provided some underlying parenchyma cells were present. Buds on the isolated epidermal tissue formed over the whole surface of the tissue and not predominantly near the proximal surface. This clearly supports the theory of polar transport of auxins in leaf tissue as suggested by Paterson (1983) and VanAartrijk and Blom-Barnhoorn (1983). Results obtained with Hybrid 1, showed that bud formation was stimulated by isolation of the adaxial and abaxial tissue as the number of buds on both the adaxial and the abaxial explants was higher than on the control explants. The results also indicate however, that the regeneration potential of the abaxial epidermis remained lower than that of the adaxial one despite the isolation from other tissue. Hybrid 1 may be an interesting genotype for further study on the relationships between different tissues in leaves. The leaves are thicker than that of other hybrids and are relatively easy to handle.

Ndou (2000) showed that meristematic activity in cells surrounding the vascular bundles, resulted in root formation, and not bud formation as reported by Suh, Roh and Lee (1997). By using epidermal strips of cultured leaf explants, Niederwieser and Van Staden (1990b) showed that more than 90% of buds have a single cell origin. It can be assumed that bud formation on bulb-scales follows the same pattern as on leaf tissue as scales are swollen leaf bases. The origin of adventitious buds formed from the axial meristem was not studied. It is thus not known whether those buds have a single-cell origin.

Factors Affecting the Formation of Buds

1. Genotype. The genotypic effect and its interactions with most of the other factors affecting bud formation, were reported by most workers. The most evident effect of the genome is on the regeneration potential. Some genotypes formed as much as 35 buds per leaf explant *in vitro* whereas others formed a few buds per explant (Niederwieser and Vcelar, 1990; Niederwieser and Van Staden, 1990, 1992a). Similar genotypic differences were shown for leaf cuttings (Perrignon, 1992; Ndou, 2000).

2. Tissue Age. Tissue age has a pronounced effect on the regeneration potential of leaf

tissue in vitro (Niederwieser and Vcelar, 1990; Niederwieser and Van Staden, 1990a; Niederwieser and Van Staden, 1992a) and in vivo (Perrignon, 1992; Ndou, 2000). Young tissue generally has the highest regeneration ability, with a tendency to decrease as the age of the tissue increases (proximal, centre and distal parts of leaves). Exceptions were observed for some genotypes with a high regeneration potential in vitro (Niederwieser and Van Staden, 1990a) and in vivo (Ndou, 2000). In the study of Niederwieser and Van Staden (1992a) the effect of tissue age was shown to be statistically significant for tissue within the proximal 5 cm of leaves for 5 of the 6 hybrids used. For some of these hybrids, tissue age showed a significant interaction with the BA and/or NAA concentration.

3. Physiological Stage of Donor Plants. The first report on the effect of the physiological stage indicated that the maximum number of buds is formed when plants had entered anthesis (Niederwieser and Vcelar, 1990). Recent work by Ndou (2000), however, indicated that the regeneration ability of leaf cuttings decreases towards anthesis. In both studies, an interaction between genotype and physiological stage was shown. Supernumary bulblets (adventitious buds formed from axial meristem) of the cv Romelia, are first observed on meristems dissected one month after planting (Roodbol and Niederwieser, 1998). The rate of supernumary bud formation increases until approximately one month before anthesis. No buds form after anthesis. It is tempting to speculate that the physiological condition in *Lachenalia* plants before anthesis, is favourable for adventitious bud formation, but that this situation changes as the plants enter anthesis when the inflorescence starts to elongate. Metabolites are possibly relocated to the inflorescence to prepare for flowering. The difference in the response of tissue in vitro and leaf cuttings can possibly be related to the availability of plant growth regulators that are supplied by the culture medium.

4. Metabolites within Leaf Tissue. The importance of metabolites within leaf tissue on the growth of buds once formed, has been demonstrated by the observation that the number of buds on explants is independent of explant size, whereas the growth of these buds is better on large than on small explants (Niederwieser and Vcelar, 1990). Niederwieser and Van Staden (1990a and 1992b) did some preliminary studies on cytokinin levels and bud formation. No relationship was found between the level of endogenous cytokinin and the regeneration potential as determined by the genotype. Some relationship appears to exist between tissue age and hormone level. Young tissue generally has higher levels of cytokinin than older tissue.

In genotypes tested, no starch was observed in leaf tissue in vivo, but starch was present abundantly in bulb parts and in the young inflorescences (Ndou, 2000). This is in contrast to an in vitro study as many starch grains were observed in leaf explants after seven days in culture (Niederwieser, 1990). It is possible that sucrose was not utilised by leaves in vivo to produce starch, but translocated to bulb tissue for starch formation as in *Narcissus* (Chen, 1969). In vitro, sucrose was probably available from the medium. Under such conditions sucrose may be used for starch production.

5. Medium Components. The optimal sucrose concentration for bud initiation in *Lachenalia* appeared to be relatively high (50 – 70 g/l) (Van Rensburg and Vcelar, 1989). Elongation of the shoots that grow from the buds, however, is inhibited by a high sucrose concentration. The general tissue culture medium used at Roodeplaat contains 0.1 mg/l NAA and 2 mg/l BA. Factorial experiments on 6 hybrids showed that NAA was clearly critical for adventitious bud formation, apparently more so than BA (Niederwieser and Van Staden, 1992a). An interaction exists between the genotype and the optimal concentration of NAA and BA. The optimal concentrations for genotypes with a high regeneration potential was higher than for genotypes with a low regeneration potentials. It was proposed that the highly regenerative genotypes may be better able to metabolise NAA and BA than genotypes with a low regeneration potential (Niederwieser and Van Staden, 1992a).

6. Application of Techniques. Most *Lachenalia* cvs show a low rate of propagation through daughter bulbs. Adventitious bud formation on leaf tissue in vitro and in vivo, is thus used for propagation. As lachenalias are highly susceptible to Ornithogalum Mosaic

Virus, a propagation scheme based on those recommended for lilies and daffodils (European and Mediterranean Commission for Plant Improvement) has been developed during the past seven years. Strict adherence to this scheme is proving to be the key success factor for *Lachenalia* production in South Africa, especially in areas where *Ornithogalum* is also grown. Virus-tested nuclear plants (Phase I) are propagated through adventitious bud formation on leaf tissue in tissue culture (Phase II). Phase III bulbs, obtained through leaf cuttings of Phase II bulbs are supplied by Roodeplaat to specialist propagators who propagate bulbs through Phase IV by means of leaf cuttings and natural propagation through daughter bulbs. Growing on and preparation of bulbs for forcing is done in Phase V. A problem that needs to be addressed is the size of bulblets produced on leaf cuttings.

Low temperature treatment of shoots formed from adventitious buds in vitro, leads to bulb formation (Slabbert and Niederwieser, 1999). This basic technique needs to be adapted for commercial production as its application may prove to be more advantageous than the one in current use.

As a result of the interaction between genotype and other factors affecting bud formation, growers need to determine the effect of important factors such as tissue age and physiological stage of donor plants for the most important cultivars for optimal production planning.

CONCLUSIONS

Extensive work has been done on the process of adventitious bud formation as well as on the application of the technique. Currently, adventitious bud formation on leaf tissue in vitro and on leaf cuttings, is the major method of *Lachenalia* propagation. Optimisation can now proceed through on-farm research.

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Literature Cited

- Ault, J.R. 1995. In vitro rooting and greenhouse acclimatization of *Lachenalia* shoots. HortSci. 30, 1304 – 1305.
- Chen, S. 1969. Carbohydrate metabolism in the *Narcissus* leaf. J. Exp. Bot. 20, 302-316.
- Duncan, G.D. 1988. The *Lachenalia* handbook. Ann. Kirstenbosch Bot. Gardens Vol17. Ed. J.N. Eloff.
- Klessler, P.J. and Nel, D.D. 1976. Virus diseases and tissue culture of some South African flower bulbs. Acta Hort. 59, 71-76.
- Murashige, T and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 15:473-479.
- Ndou, AM. 2000. Optimization of bulblet production by leaf cuttings in *Lachenalia*. M. Inst. Agrar. Dissertation, University of Pretoria, South Africa.
- Nel, D.D. 1983. Rapid propagation of *Lachenalia* hybrids in vitro. S.Afr. J. Bot. 2, 245-246.
- Niederwieser, J.G. 1990. Adventitious bud formation of *Lachenalia* Jacq. Leaf tissue in vitro. Ph.D Thesis. University of Natal.
- Niederwieser, J.G. 1996. Propagation techniques for a new flower bulb crop. (*Lachenalia*). Comb. Proc. Intern. Plant Prop. Soc. 46, 114-115.
- Niederwieser, J.G. and Van Staden, J. 1990a. The relationship between genotype, tissue age and endogenous cytokinin levels on adventitious bud formation on leaves of *Lachenalia*. Plant Cell Tis. Org. Cult. 22, 223-228.
- Niederwieser, J.G. and Van Staden, J. 1990b. Origin of adventitious buds on cultured *Lachenalia* leaves. Beitr. Biol. Pflanzen. 65, 443-453.
- Niederwieser, J.G. and Van Staden, J. 1992a. Interaction between benzyladenin,

- naphthalene acetic acid and tissue age on adventitious bud formation on leaf sections of *Lachenalia* hybrids. S. Afr. J. Bot. 58, 13-16.
- Niederwieser, J.G. and Van Staden, J. 1992b. Metabolism of 6-benzyladenine by leaf explants of *Lachenalia* during adventitious bud formation. S. Afr. J. Bot. 58, 236-238.
- Niederwieser, J.G. and Vcelar, B.M. 1990. Regeneration of *Lachenalia* species from leaf explants. HortSci. 25, 684-687.
- Paterson, K.E. 1983. Polarity of regeneration in excised *Crassula argentea*. II. Auxin transport inhibitors and auxin-cytokinin interaction. Can. J. Bot. 61, 1064-1071.
- Perrignon, R.J. 1992. Bulblet production in vivo from leaves of *Lachenalia*. Acta Hort. 325, 341-346.
- Roodbol, F. and Niederwieser, J.G. 1998. Initiation, growth and development of bulbs of *Lachenalia* 'Romelia' (Hyacinthaceae). J. Sth. Afr. Soc. Hort. Sci. 8, 18-20.
- Slabbert, M.M. and Niederwieser, J.G. 1999. In vitro bulblet production of *Lachenalia*. Pl. Cell Rep. 18, X
- Suh, J.K., Roh, M.S. and Lee, J.S. 1997. Leaf cutting propagation, growth and flowering of *Lachenalia*. Acta Hort. 340, 369-376.
- Van Aartrijk, J. and Blom-Barnhoorn, G.J. 1983. Adventitious bud formation from bulb-scale explants of *Lilium speciosum* Thunb. In vitro. Effects of wounding, TIBA, and temperature. Zeitschrift Pflanzenphysiol. 110, 355-363.
- Van Rensburg, J.G.J. and Vcelar, B.M. 1989. The effect of the sucrose concentration on the initiation and growth of adventitious buds from *Lachenalia* leaf tissue. S. Afr. J. Bot. 54, 196-202.

Tables

Table 1. The average number of adventitious buds formed per explant of four *Lachenalia* hybrids as affected by the isolation of epidermal tissue from the explant.

| H Y B R I D | Leaf Number | CONTROL | | TREATMENT 1 | | TREATMENT 2 | |
|----------------------------|----------------|-------------------------------------|-------------------------------------|---|--|--|---|
| | | Adaxial surface of explant | Abaxial surface of explant | Adaxial Epidermis + parenchyma | Abaxial epidermis + parenchyma + vascular tissue | Adaxial epidermis + parenchyma + vascular tissue | Abaxial epidermis + parenchyma |
| 1 | 1 | 3.5 | 0.0 | 23.3 | 1.0 | 5.5 | 4.3 |
| | 2 | 10.0 | 2.0 | 25.5 | 10.6 | 30.5 | 4.0 |
| | 3 | 11.0 | 2.8 | 34.8 | 16.3 | 20.5 | 7.0 |
| | 4 | 14.0 | 3.5 | 42.5 | 7.5 | 11.0 | 5.5 |
| | Average | 9.6 | 2.0 | 31.5 | 8.8 | 16.8 | 5.2 |
| 2 | 1 | 14.5 | 0 | 16.0 | 0.5 | 9.3 | 0 |
| | 2 | 7.0 | 0.5 | 4.5 | 1.5 | 9.0 | 0 |
| | 3 | 20.8 | 0.5 | 3.8 | 0 | 12.3 | 3.3 |
| | 4 | 16.0 | 0 | 4.8 | 2.0 | 5.7 | 0 |
| | 5 | 4.5 | 1.5 | 5.3 | 1.3 | 4.0 | 0 |
| | Average | 12.6 | 0.5 | 6.9 | 1.1 | 8.1 | 0.7 |
| 3 | 1 | 16.3 | 0 | 16.0 | 0.5 | 9.3 | 0 |
| | 2 | 12.3 | 0 | 4.5 | 1.5 | 9.0 | 0 |
| | 3 | 12.5 | 0 | 3.8 | 0 | 12.3 | 0 |
| | 4 | 12.3 | 0 | 4.8 | 2.0 | 5.7 | 0 |
| | 5 | 8.9 | 0 | 5.3 | 1.3 | 4.0 | 0 |
| | Average | 12.4 | 0 | 6.9 | 1.1 | 8.1 | 0 |
| 4 | 1 | 12.3 | 0 | 0 | 0 | 0 | 0 |
| | 2 | 6.0 | 0 | 0 | 0 | 0 | 0 |
| | 3 | 8.8 | 0 | 0 | 0 | 0 | 0 |
| | 4 | 8.3 | 0 | 0 | 0 | 0 | 0 |
| | Average | 8.8 | 0 | 0 | 0 | 0 | 0 |

Treatment 1: The adaxial epidermis and a thin layer of underlying parenchyma cells were removed from the explant. The resulting adaxial and abaxial explants were cultured separately.

Treatment 2: The abaxial epidermis and a thin layer of underlying parenchyma cells were removed from the explant. The resulting adaxial and abaxial explants were cultured separately.

Control: Intact leaf explants cultured with the abaxial epidermis in the medium.