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The relationship between genotype, tissue age and endogenous cytokinin levels on adventitious bud formation on leaves of *Lachenalia*

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Abstract

The relationship between genotype, tissue age and endogenous cytokinin levels on adventitious bud formation on *Lachenalia* leaf tissue were investigated. The genotypes studied, showed a variation in bud formation. The hybrid explants responded differently to factorial combinations of BA and NAA. The growth regulators could not substitute for the regeneration potential of the genotype. Tissue age had a pronounced effect on regeneration potential. Young tissue formed the largest number of buds. An interaction between tissue age and genotype was detected. Cytokinin levels in young leaf tissue were higher than in older tissue. In young tissue no relationship was observed between the cytokinin level and the number of buds formed. However, in older tissue it appears as if a relatively low endogenous cytokinin level enhanced bud formation.

Abbreviations: BA – benzyladenine, NAA – naphthalene-1-acetic acid, Z – zeatin, ZR – ribosylzeatin

Introduction

Lachenalia is a bulbous plant in which BA and NAA stimulate adventitious bud formation on explants in vitro [6]. It has been reported that the response of different hybrids and tissue from different positions on the leaf varies greatly [14].

Because of the role of hormones in plant growth and development, and the importance of exogenous auxins and cytokinins on organogenesis in vitro, it seems probable that the levels of endogenous hormones in explants play a regulatory role in adventitious bud formation. From the literature there is considerable indirect evidence that the levels of endogenous hormones may play an important regulatory role in tissue when in culture. The gradient in regeneration potential which exists within an organ as a result of different developmental stages [11, 17] can be cited as an example. Furthermore, the differential responses of species or cultivars to culture conditions have been attributed to different levels of endogenous hormones within different genotypes [4, 16].

The purpose of this study was to investigate the effect of different levels of applied BA and NAA, genotype, and tissue age on adventitious bud formation in *Lachenalia* and to determine whether these showed any relationship to the endogenous cytokinin levels in explant material.

Materials and methods

Plant material

Leaf tissue of *Lachenalia* species and hybrids was used in this study. All bulbs were of a similar age and were planted in a potting mixture consisting of equal parts of loam, compost and bark. The plants

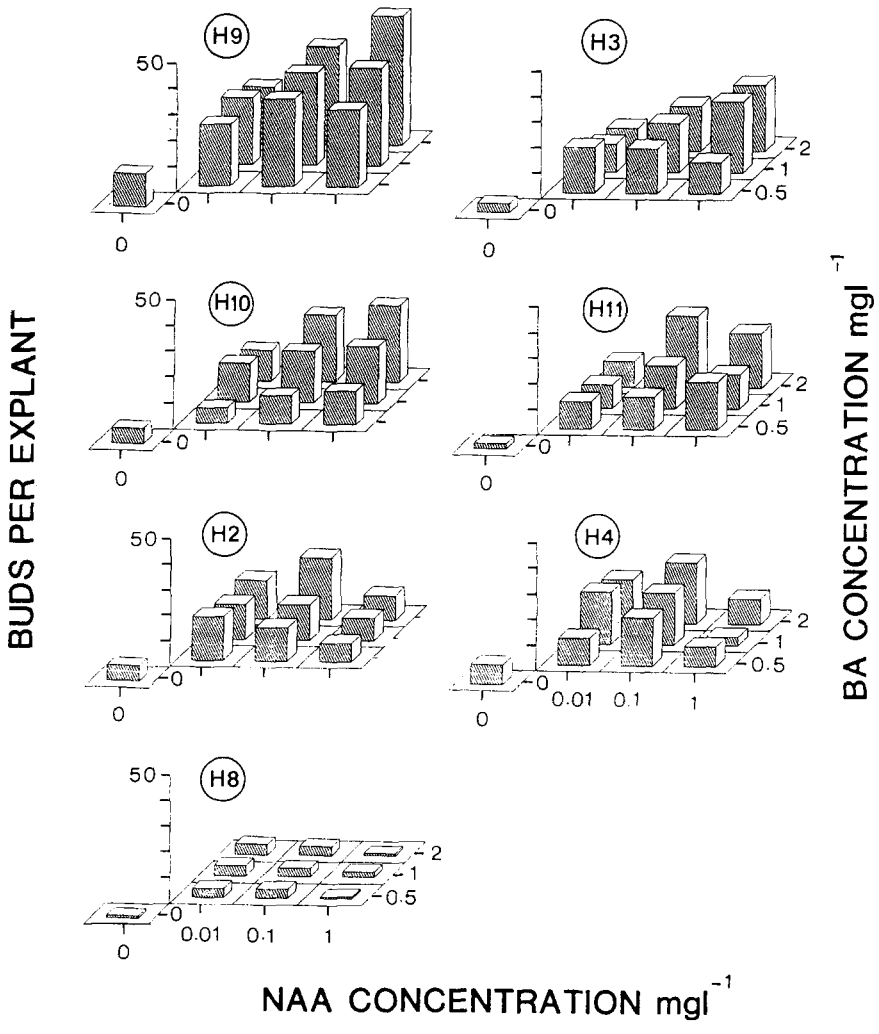

Fig. 1. Bud formation on leaf tissue of *Lachenalia* hybrids cultured on factorial combinations of BA and NAA.

were maintained in a fibre glass greenhouse for the duration of the growing season (winter). The average maximum temperature was 21°C and the average minimum, 5°C. Day length followed the natural seasonal pattern in the Southern Hemisphere. The plants were watered twice weekly and no additional nutrients were supplied.

Tissue culture procedures

The leaves of plants when in full bloom were used as explant material. Leaves were removed from the donor plants by cutting them immediately above the bulb. Surface sterilization was achieved by rinsing the leaves in a non-toxic 0.1% soap solution for 10 min before gently shaking them in 1% NaOCl for 30 min. After each step the leaves were rinsed in sterile water. In all cases, pieces of tissue (80–100 mm) were cultured in a horizontal position with the abaxial surface on the medium. The explants were maintained on a basal medium (BM) containing Murashige & Skoog [8] salts, 0.5 mg l^{-1} thiamine, 25 mg l^{-1} NaFeEDTA and 5% sucrose. Unless stated otherwise, 1.0 mg l^{-1} each of BA and NAA was added to the BM. The medium was solidi- was solidified with 0.6% agar after the pH was

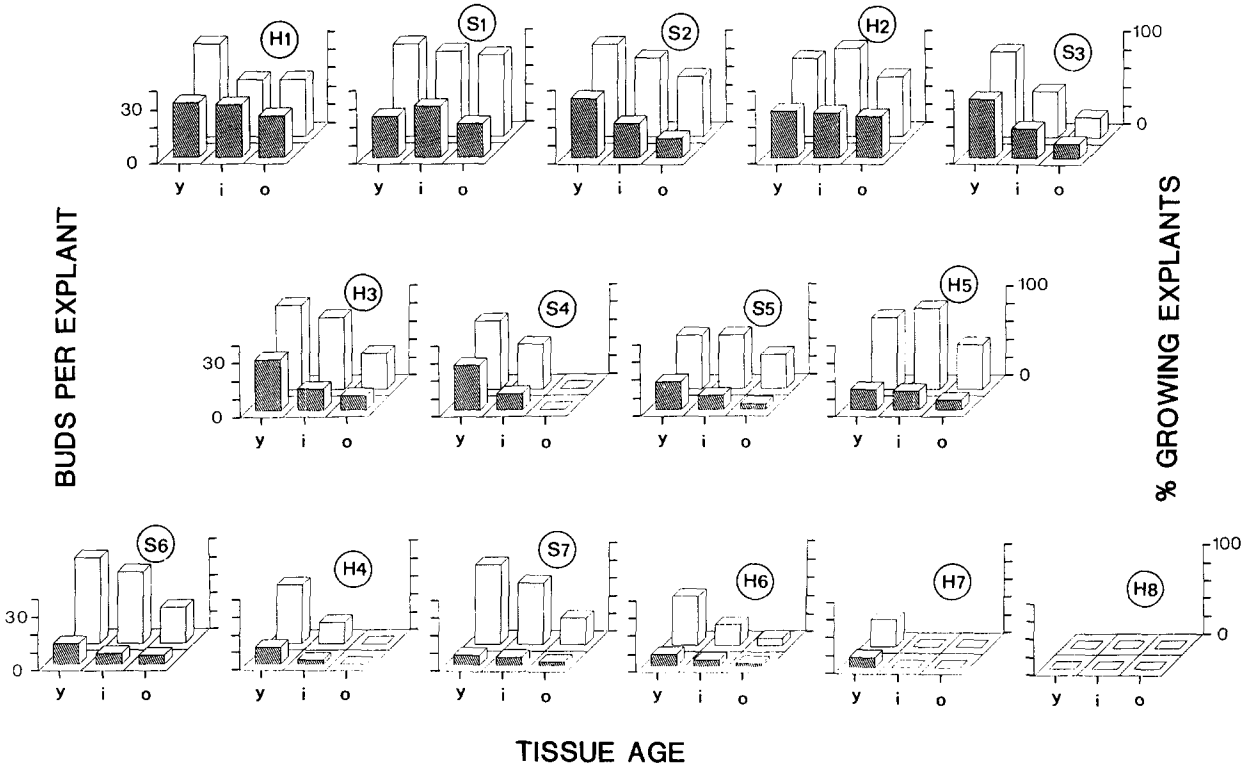

Fig. 2. The effect of genotype and tissue age (y = young; i = intermediate; o = old) on bud formation [□] and explant growth [▨] on leaf tissue of *Lachenalia* species and hybrids.

adjusted to 5.8 with NaOH. Ten ml aliquots were dispensed into 19 × 125 mm culture tubes and these autoclaved at 121°C for 15 min. Cultures were maintained at 24°C under a 12 h light period with an intensity of 95 $\mu E\, m^{-2} s^{-1}$. Eight weeks after the tissue was placed on the culture medium, the number of buds per explant was recorded.

Seven *Lachenalia* species (designated S-1 to S-7) and 11 hybrids (designated H-1 to H-11), were cultured. Leaves were divided transversely into two and only the proximal part was used in the experiments concerned with genotypic effects.

The effect of BA and NAA in factorial combinations (BA at 0.5; 1; and 2 $mg\, l^{-1}$ and NAA at 0.01; 0.1 and 1 $mg\, l^{-1}$) on bud formation on leaves of 7 hybrids (Fig. 1) was determined. The proximal 7 cm of a leaf (youngest part) from 4 different plants was used for each treatment. Each leaf was divided longitudinally into 2 equal sections and the sections used for 2 different treatments. Forty explants were used for each treatment and the exact origin of the explants on the leaves recorded. The results were subjected to an analysis of variance in order to determine the effect of genotype, growth regulator combination, and explant position on the leaf, on bud formation.

The effect of explant age and the possible interaction thereof with the genotype on bud formation on leaf tissue was determined. Depending on leaf size and the number of plants available, between 6 to 15 leaves were used for each genotype. At the time of culture each leaf was divided longitudinally into 2 equal halves. One half was used for tissue culture and the other retained for cytokinin analysis. As *Lachenalia* leaves continue growing from a basal meristem throughout their growing season, each leaf consists of tissue at different developmental stages. To investigate the effect of leaf age on bud formation each leaf half was divided into 3 equal sections; proximal, middle and distal sections.

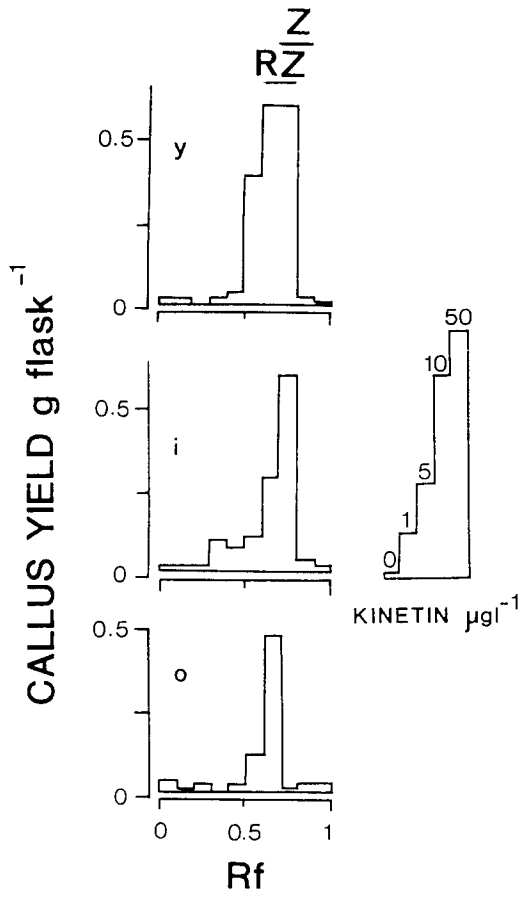

Fig. 3. Cytokinin-like activity in young (y), intermediate (i) and old (o) *Lachenalia* (H3) leaf tissue.

These were designated as young, intermediate and old. The sections from each position were pooled and 2 to 5 explants, dissected from each leaf section, were cultured. Between 30 to 40 explants (depending on available material) were cultured for each leaf position for every genotype used. In addition to the number of buds formed per explant, the percentage of explants on which adventitious buds formed was recorded. Data were subjected to an analysis of variance to determine the significance of tissue age and genotypic effects.

Cytokinin extraction and determination

For cytokinin extraction and determination plant material was collected at the time of tissue culture as outlined earlier. Lots of 5 g tissue were frozen in liquid nitrogen, crushed and then freeze dried prior to storage at − 20°C. The method of extraction and separation by paper chromatography was as described earlier [10]. Cytokinin-like activity was determined using the soybean callus bioassay [7].

Cytokinin levels were expressed as kinetin equivalents and were obtained by plotting the fresh mass of soybean callus from fractions showing cytokinin activity against a standard kinetin curve. By adding these values, single kinetin equivalents were obtained for each treatment.

Results and discussion

Effect of genotype on bud formation

The regeneration potential of proximal leaf tissue of different *Lachenalia* genotypes varied considerably. Two hybrids (H-1, H-2) and three species (S-1, S-2, S-3) produced 20–30 buds per explant while three hybrids (H-5, H-6) and two species (S-6, S-7) produced 1–10 buds per explant. No buds were produced by one hybrid (H-8).

Because of the importance of the genotype in regulating regeneration in vitro, the choice of suitable genotypes for such studies is critical [5, 13]. In the application of tissue culture to a breeding programme however, there is often no choice in the tissue from which growth is required [2].

Effect of BA and NAA on bud formation

The average number of buds per explant, as well as the response to the BA and NAA combinations, varied for the different hybrids (Fig. 1). The most responsive hybrid, H-9, formed an average of 33 buds per explant and H-8, the least responsive hybrid, an average of 3.6 buds per explant.

It was noticeable that bud production by explants in the absence of hormones closely mirrored the response of hybrids to the hormone grid experiments, and clearly indicated that genotypic effects appeared unresponsive to hormonal influences over the concentrations tested.

The present results indicate that *Lachenalia* plants (hybrids and species) with a high regeneration potential (and high optimum NAA requirement) were able to utilize NAA better than hybrids

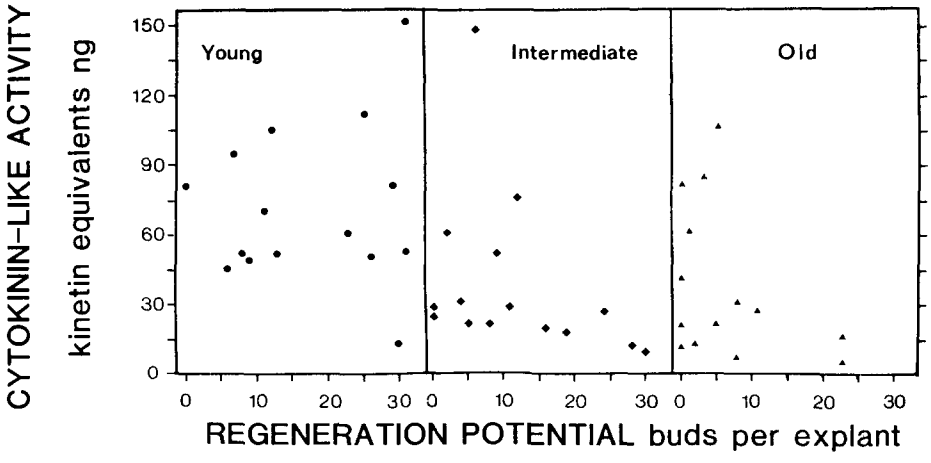

Fig. 4. The relationship between the endogenous cytokinin levels and adventitious bud formation of young, intermediate, and old leaf tissue of 15 *Lachenalia* genotypes.

with a low regeneration potential and a low optimum NAA requirement. A substantial number of *Lachenalia* species and hybrids were used in this study and while it is recognised that other factors such as metabolism, transport, storage, compartmentation, and interactions between growth regulators [3, 12, 15] could be of importance it may be reasonably assumed that the responses observed are representative of this genus.

The effect of tissue age on bud formation

The age of explant tissue had a significant effect on bud formation and, in general, the highest number of buds per explant were formed on young tissue and the least on old tissue (Fig. 2). For convenience these results have been presented in three response categories. In the first two, the relationship between buds per explant and growth are quite consistent, while in the last, growth outstrips bud formation (S-6, H-4, S-7, H-6, H-7).

Endogenous cytokinin levels in explant material

Bioassays of all samples indicated that cytokinin-like activity co-chromatographed with zeatin (Z) and ribosylzeatin (RZ) (Rf 0.6–0.8) (Fig. 3). Cytokinin activity in leaves of different genotypes varied. No relationship was found between the effect of the genotype on bud formation and the endogenous cytokinin level.

Young tissue generally contained higher cytokinin levels than intermediate and old tissue. In young tissue, cytokinin levels showed no relationship with the number of buds formed (Fig. 4). There does however, appear to be a relationship between endogenous cytokinin level and regeneration potential in intermediate and old tissue. Tissue of intermediate age which formed relatively high numbers of buds contained low levels of cytokinins. In old tissues, with a relatively low regeneration potential, only a few genotypes formed a high number of buds, they were the ones with low cytokinin levels (Fig. 4). This may provide evidence in support of the hypothesis of Narasimhulu & Chopra [9] that regeneration is governed by two sets of determinants viz. genetic and environmental, with genetic factors working at a primary level and environmental factors at a secondary level. We propose that the term 'environmental' could be replaced by 'epigenetic' to accommodate ontogenetic factors [1] which also appear to function at a secondary level and which may involve endogenous hormones.

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