

## ABSTRACT

Comparative studies on *in vitro* regeneration  
of some varieties of *Anthurium andraeanum* Lind.  
from leaf explants

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Leaf explants of several cultivars of *Anthurium andraeanum* were tested for their capacity to regenerate. Cultivars examined were from Hawaiian, Dutch and Local stock. The procedure involved 1) callus induction 2) callus subculture and shoot regeneration 3) shoot multiplication 4) rooting of individual shoots 5) hardening of plantlets.

Investigations made during callus induction revealed fungicide pre-treatment was beneficial in the reduction of the contamination level. Benlate with systemic action proved to be most effective. Explant orientation was important as excised leaves placed on adaxial surface were more receptive during induction. The age of the explant was critical, with almost mature leaves displaying the highest survival rate and callus production. Of the media tested full strength Nitsch was most appropriate. Other combinations tested responded poorly. The substitution of inorganic ammonium nitrate with organic allantoin or hypoxanthine was detrimental to the survival of explants. The effect of 2,4-D (2,4-dichlorophenoxyacetic acid) on callus production was species

specific with good callus production occurring between 0.05 and 1.0 mg L<sup>-1</sup>; 5 mg L<sup>-1</sup> and above was detrimental to the survival of the explants. Some cultivars were extremely sensitive (Lunette), some were less sensitive (Red/White, Tropical Pink, Cuba) while others (Tulip, Ozaki, Peach Obaki) were intermediate in response. The condition of the mother plant also determined the response to 2,4-D observed, with explant obtained from plants in active growth phase requiring a lower dose of 2,4-D for callus induction. Factorial experiments with 2,4-DxBA (benzyl adenine) showed that the effect of 2,4-D was enhanced by BA, however, BA could be used as the sole hormone.

Experiments performed during callus subculture and differentiation revealed that ammonium nitrate at low levels (200 mg L<sup>-1</sup>) enhanced callus survival, however, no significant difference in callus differentiation was detected. Comparisons made between cultivars showed that the effect of BA on growth and differentiation was species specific, with some cultivars responding best at low levels (0.5 mg L<sup>-1</sup>) (Nitta, Cuba) while others were less specific (Red 100, Tulip). Further, light was observed to enhance shoot differentiation in cultivar Tulip.

In shoot proliferation experiments six cultivars were extensively compared; these were Tropical Pink (local), Premium Red (local), Red/White (local), Cuba (Dutch), Tulip (Dutch) and De Weese (Hawaiian). Experiments were performed investigating the effect of BA on proliferation. Results indicated a species specific

response. In the first subculture three groups were observed suggesting a correlation between bloom color and response to BA. 1) White cultivars, responded best at high BA levels (beyond  $1.0 \text{ mg L}^{-1}$ ). 2) Pink cultivars responded best between 0.7 and  $1.0 \text{ mg L}^{-1}$  BA while 3) The red cultivar was most sensitive with best results between 0.5 and  $0.9 \text{ mg L}^{-1}$  BA. The second subculture revealed a loss in effectivity of BA. Three groups, according to source of breeding, were also established. 1) Local cultivars showed increased root growth. 2) Dutch cultivars showed no significant difference. 3) Hawaiian cultivars revealed inhibited root growth. Further, the effectivity of BA as a proliferation agent was enhanced in absence of light. In etiolated shoots loss of effectivity of BA was less on the second subculture, but drastically reduced by third.

Experiments during development of isolated shoots varying the ammonium nitrate level revealed a complex response pattern. Linear, quadratic, and cubic regressions were fit to growth factors. Three groups were observed and division was according to bloom color. In pink cultivars, growth was enhanced, in red, inhibited, while white showed no significant difference.

In the hardening stage, of the media tested, coir gave continuously best results. Other media or media combinations tested were intermediate in response except for peat, which proved to be lethal.

Histological investigations on callus initiation and development, using cultivars Tropical Pink and De Weese, were performed. A distinct meristematic zone was obvious by day 12 in both cultivars. Meristematic activity was initiated in the spongy mesophyll. This activity was increased in the presence of vascular tissue. By day 24 in cv. Tropical Pink and day 36 in cv. De Weese, meristematic activity in outer callus bulges could be associated with the formation of meristemoids.

Gel electrophoresis comparisons of proteins, esterase and peroxidase were studied between parent plant, callus and mature and immature regenerated plant of cultivars Tropical Pink, De Weese and Tulip. Callus produced a simple banding pattern in most cases, similarly, the immature regenerated plants. The banding pattern of parent plant and mature regenerated plant were similar, suggesting that no genetic aberrations had occurred.