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The in vitro culture of *Gerbera aurantiaca*

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Abstract. Multiple shoot cultures were obtained from elongating axillary buds of *Gerbera aurantiaca* Sch. on the medium of Murashige & Skoog (MS) supplemented with 5 μM benzyladenine. Shoots rooted in vitro on MS medium supplemented with 5 to 10 μM indolebutyric acid or 5 to 10 μM α-naphthaleneacetic acid. Plantlets were hardened in a mist bed and transferred to soil.

Introduction

Of the 16 species of the genus *Gerbera* (Asteraceae) occurring in southern Africa, two species are listed as rare. *Gerbera aurantiaca*, which is endemic to the eastern parts of southern Africa, is considered as a vulnerable plant. This species can become endangered if the causal factors of its decline continue to operate [1]. The natural habitat of *Gerbera aurantiaca* is rocky areas within the grasslands of Natal, where it grows in large but sparsely spread colonies. The flower morphology resembles that of *Gerbera jamesonii* Bolus. The colors of the flowers vary from dark red to orange. *Gerbera jamesonii* has been cultured in vitro from axillary buds [4], capitula [2, 6, 7] and ovules [3]. This is the first report of the in vitro culture of *Gerbera aurantiaca*. This work was done in an effort to prevent the further decline of the species.

Materials and methods

Seed of *Gerbera aurantiaca* was collected from its natural habitat near Pietermaritzburg. Achenes from the ray florets were surface-sterilized for 5 min with 3.5% NaOCl. The aseptic achenes were then rinsed in sterile distilled water using 3 × 100 ml water per 20 achenes. The achenes were

placed on a medium containing half-strength Murashige & Skoog (MS) medium [5] solidified with 0.8% agar. Germination took place in aseptic conditions at 25 ± 2 °C and continuous light at an irradiance of 20 W m^{-2}. The seedlings were grown to a height of 8 to 10 cm before being utilized as explant material. Explants consisted of a 2 to 3 mm segment of the compact shoot including two axillary buds and a small segment of the petiole. Unopened capitula collected from the wild were also used as explants. The capitula were surface-sterilized for 20 min in 3.5% NaOCl. Five capitula were then rinsed in 3 volumes of 200 ml sterile distilled water. The capitula were cut into 4 equal-sized explants. The shoot and capitulum explants were placed on 10 ml of MS basal medium dispensed in 10 mm × 200 mm culture vials. 3% sucrose was added to the basal medium and it was solidified with 0.8% agar. The pH of the medium was adjusted to 5.7 after which it was autoclaved at 121 °C for 20 min. The shoot induction medium was supplemented with 0.1, 0.5, 1, 5 and 10 μM of benzyladenine (BA). Shoots which developed on the shoot induction medium were subcultured on basal MS medium containing 5 and 10 μM indolebutyric acid (IBA) or 5 and 10 μM α-naphthaleneacetic acid (NAA). Axillary bud explants were also put on the basal medium containing 5 μM BA and 1 μM NAA or 5 μM BA and 1 μM 2,4-dichlorophenoxyacetic acid (2,4-D). The cultures were incubated for 60 days without transfer at 25 ± 2 °C in continuous light of 20 W m^{-2} irradiance.

Rooted plantlets were removed from the culture vials. After the agar had been removed by washing, the plantlets were planted in washed sand with a pebble size of 0.5 to 0.85 mm. The plants were hardened over a period of 2 weeks in an enclosed mistbed which delivered an intermittent mist of 15 sec per 3 min interval. The ambient temperature varied between 26 °C and 30 °C during the day and 14 °C and 20 °C at night.

The shoot induction treatments were replicated 15 times and 20 replications were used for each rooting treatment. One explant was used per replication. The results were statistically analysed and the differences between treatment means were determined with the Duncan multiple range test at 95% level of confidence [8]. All experiments were repeated twice.

Results and discussion

Multiple bud production and shoot elongation from axillary bud explants

At all the BA concentrations tested (0.1 to 10 μM) one axillary bud per explant elongated to form a leafy shoot. The formed axillary shoots were 5

Table 1. The effect of BA on axillary shoot formation and subsequent growth of *Gerbera aurantiaca* explants after 60 days of culture in vitro. Mean ± standard error.

BA concentration (μM)	Explants with axillary shoots (%)	Mean number of axillary shoots per explant	Mean length of axillary shoots (mm)
0.1	0	0	0
0.5	0	0	0
1	0	0	0
5	90 ± 15	5.1 ± 0.9	5.4 ± 1.0
10	95 ± 10	5.6 ± 1.1	5.7 ± 0.9

to 20 mm long at the end of the 60-day incubation period. The axillary buds from these axillary shoots elongated on the basal medium which contained 5 to 10 μM BA (Table 1). No significant differences were found between the number of axillary buds which elongated or the length attained by these buds (Table 1). At a concentration of 10 μM BA, 20% of the cultures were vitrified. No vitrified cultures were observed at lower concentrations of BA.

Axillary bud cultures on the basal medium with 5 or 10 μM BA developed a limited amount of callus. More callus was formed in the presence of 10 μM BA than with 5 μM BA. The callus was compact, green and morphogenetic. Buds and leafy structures developed on the callus. Callus with a similar morphogenetic appearance was formed on the basal medium containing 5 μM BA and 1 μM NAA. A non-morphogenetic callus was formed on the basal medium containing 5 μM BA and 1 μM 2,4-D.

Morphogenesis of capitulum explants

The incubation of capitulum explants on the basal medium containing 0.1 to 10 μM BA resulted in the formation of 6 shoots at 5 μM BA. Only one

Table 2. The effect of IBA and NAA on the initiation and growth of roots on *Gerbera aurantiaca* shoots after 60 days of culture in vitro. Mean ± standard error.

IBA and NAA concentration (μM)	Plants rooted (%)	Mean number of roots per plant	Mean length of roots per plant (mm)
IBA			
5	95 ± 15	2.6 ± 0.6	9.5 ± 2.2
10	100	5.1^{a} ± 0.7	15.2^{b} ± 1.5
NAA			
5	95 ± 10	5.0^{a} ± 0.8	11.2^{c} ± 0.9
10	95 ± 15	6.1^{a} ± 1.3	8.9 ± 0.6

^{a,b,c} Significant at a 95% level of confidence.

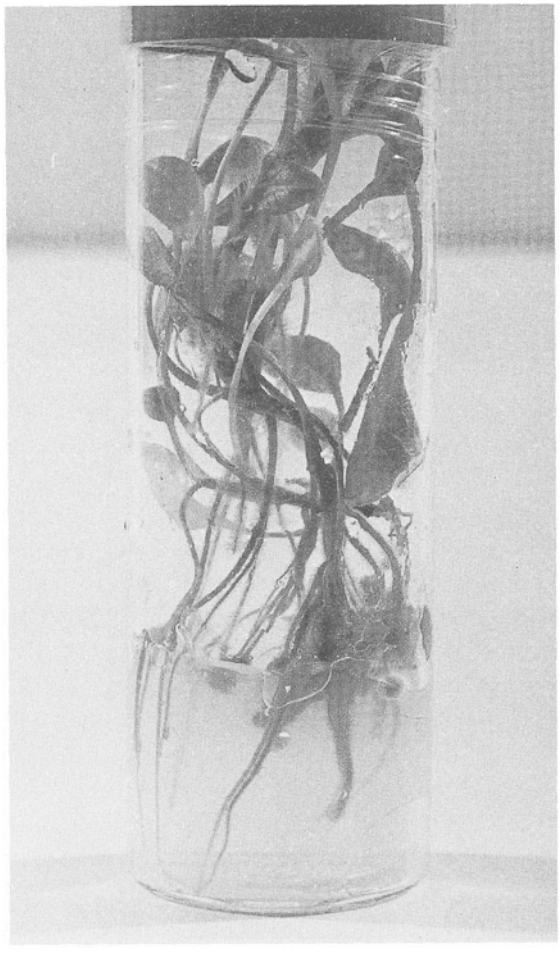

Fig. 1. In vitro cultured plantlet of *Gerbera aurantiaca* with roots on MS medium containing 10 μM IBA.

out of 20 capitulum explants formed shoots. Most explants died after extensive browning occurred. The formed shoots were chlorotic and died after three transplants on the basal medium containing 5 μM BA. In the follow-up experiments, less chlorotic shoots were obtained on the same medium but the shoots did not grow and died.

Root induction and growth

No significant difference was found between the number of shoots that rooted on the different concentrations of IBA and NAA used for the rooting

Fig. 2. Gerbera aurantiaca plant derived from in vitro culture after five months of growth since hardening.

treatments (Table 2). More roots formed in the presence of 10 μM IBA and 5 and 10 μM NAA than at 5 μM IBA (Table 2). The best root growth occurred in the presence of 10 μM IBA (Table 2). The roots that formed on the basal medium supplemented with IBA or NAA were initiated at the base of the shoot or at the base of the petioles (Fig. 1).

The plantlets which were grown in vitro were hardened in a mist bed as described earlier. The duration of the mist period was not altered during the hardening treatment. Of the 50 plants which were subjected to the hardening treatment, 85% survived. The plants were planted out into loam potting soil and grown under 40% shade. After 6 months growth these plants looked similar to plants in their natural environment (Fig. 2).

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