In vitro propagation of a number of South African Oxalis species

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Plantlets and bulbs of four herbaceous South African Oxalis species (O. reclinata Jacq., O. variifolia Steud., O. helicoides Salter and O. gracilis Jacq.) were regenerated in vitro from stem internode explants on a modified Murashige & Skoog basal medium. Phytohormone addenda were either 5 mg l⁻¹ α-naphthaleneacetic acid (NAA) and 0.5 mg l⁻¹ 6-(benzylamino)purine, or 2 mg l⁻¹ NAA and 0.1 mg l⁻¹ 6-(furfurylaminopurine. Organogenesis was much better at 10°C than at 25°C. At the lower temperature, plantlets of O. helicoides flowered in vitro.

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Keywords: Bulbs, internode explants, micropropagation, Oxalis, plantlets.

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The genus Oxalis L. (Oxalidaceae) consisting of some 800 species worldwide (Heywood 1978) has centres of diversity in both South America and South Africa. In South Africa this dicotyledonous group is represented by about 150 temperate species that exhibit a wide range of herbaceous forms and inflorescence structures (Salter 1944). All native species are geophytes that bear a variety of perennating organs, including tubers, stolons, bulbils, aerial bulbils and bulbs. The trifoliar leaves of plants emerge and flowers are produced during the cold and wet winter months. Under brightly lit conditions, flowers of Oxalis open in a wide range of hues, varying from white through yellow to scarlet. Bicoloured tubular or bell-shaped flowers occur, mostly with yellow throats. In many species spotting of leaves and petals is evident. A few South African members are naturalized and widespread in the Mediterranean region (Marshall 1987), because of their propensity for bulbil propagation. However, many species have a highly localized distribution, suggesting that not all taxa possess this attribute. Observations on greenhouse material over a three-year period indicate widely varying capacities for bulbil reproduction within the genus. Accordingly, tissue culture holds potential for rapid vegetative propagation of the less vigorous species. To our knowledge, South African members of this large genus have not previously been propagated in vitro. Many of these plants have horticultural potential. We report on the regeneration of plantlets of four species, O. reclinata Jacq. (light rose pink flowers), O. gracilis Jacq. (pink flowers with a yellow throat), O. variifolia Steud. (persian rose flowers), an O. helicoides Salter (white flowers with a dark yellow throat). O. helicoides (Figure 1) and O. variifolia (Figure 2) are particularly attractive.

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Figure 1 The dense-flowering habit of Oxalis helicoides.

Voucher Specimens: Stirton 12028 (O. reclinata), Stirton 12050 (O. gracilis), Stirton 11679 (O. variifolia) and Stirton 12009 (O. helicoides) are housed in the Compton Herbarium, National Botanical Institute, Private Bag X7, Claremont, Cape Town 7735, South Africa. Specimens were identified by J.P. Roux, Manager: Herbarium Collections.

Pot-grown plants were harvested mid-way through the winter flowering period, when stem internode sections, 3 cm in length and 1 mm in diameter, were excised. Following surface sterilization in 1.75% NaOCl for 4 min, sections were rinsed three times with sterile distilled water for 5 min. Damaged stem ends were removed before 3-mm explants were prepared for transfer to a sterile medium. Modified Murashige & Skoog (1962) medium (no glycine added) was supplemented with 30 g l⁻¹ sucrose. Before adjusting the pH to 5.7, either NAA (5 mg l⁻¹) and 6-(benzylamino)purine (BA) (0.5 mg l⁻¹) or NAA (2 mg l⁻¹) and 6-(furfurylaminopurine) potassium salt (KIN) (0.5 mg l⁻¹) (Ochatt et al. 1989) were included in the medium. Aliquots of 10 ml were dispensed to 25 × 150-mm rimless tubes and solidified with Unilab agar (8 g l⁻¹). After transfer of the explant (long axis in contact with the agar), culture vessels were supplied with cool white fluorescent light (44.5 μmol
m$^{-2}$s$^{-1}$) at 25 ± 3°C under a 16-h light regime. Twenty explants from each of the species were cultured for each treatment.

Callus production was evident in some explants after only seven days; within 21 days, 70% of the explants of all species formed calluses. The developing tissues were heterogenous mixtures of white, green, yellow and (in the case of O. reclinata) red calluses. Initially, shoot organogenesis was stimulated; shoots, roots and, most importantly, bulbs developed (Table 1). Plantlets (together with bulbs) of all four species were regenerated on at least one of the two media investigated (Table 1). In the case of O. reclinata, plantlets were regenerated on both media. Roots usually formed before shoots (Table 1), except in the cases of O. reclinata and O. helicoides plantlets generated on a medium containing NAA (5 mg l$^{-1}$) and BA (0.5 mg l$^{-1}$). Overall vitality of developing plantlets improved, perhaps partly in consequence of lower phenolic exudation into the culture media. Flowering of O. helicoides in vitro (Figure 3) occurred after repeated subcultures at 10°C.

**Oxalis pes-caprae** L. from South Africa is a winter-flowering species in those Mediterranean regions where it has naturalized (Marshall 1987). Conceivably, other South African Oxalis species could thrive under north temperate climatic conditions. In this regard, it is significant that culture temperatures affected organogenesis in vitro. The lower culture temperature which more closely parallels the natural growing conditions of these geophytes, best promoted the development of plant organs. In previous reports on the tissue culture of Oxalis (Khan et al. 1988; Ochatt et al. 1988), plantlets only developed in 2-phase systems involving changes in phytohormone addenda. However, in our investigations, only a temperature shift was required. To our best knowledge, Oxalis bulb formation in vitro represents a novel finding. Oxalis bulbs and plantlets propagated in vitro, as described, should provide horticulturalists with a means of capitalizing on this under-exploited and worthwhile genus.

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**References**


