

Short Communication

## Micropropagation of the River Lily, *Crinum variable* (Amaryllidaceae)

CW Fennell<sup>1\*</sup>, NR Crouch<sup>2</sup> and J van Staden<sup>1</sup>

<sup>1</sup> Research Centre for Plant Growth and Development, School of Botany and Zoology, University of Natal Pietermaritzburg, P/Bag X01 Scottsville 3209, South Africa

<sup>2</sup> Ethnobotany Unit, National Botanical Institute, PO Box 52099, Berea Road, Durban 4007, South Africa

\* Corresponding author, e-mail: fennel@nu.ac.za

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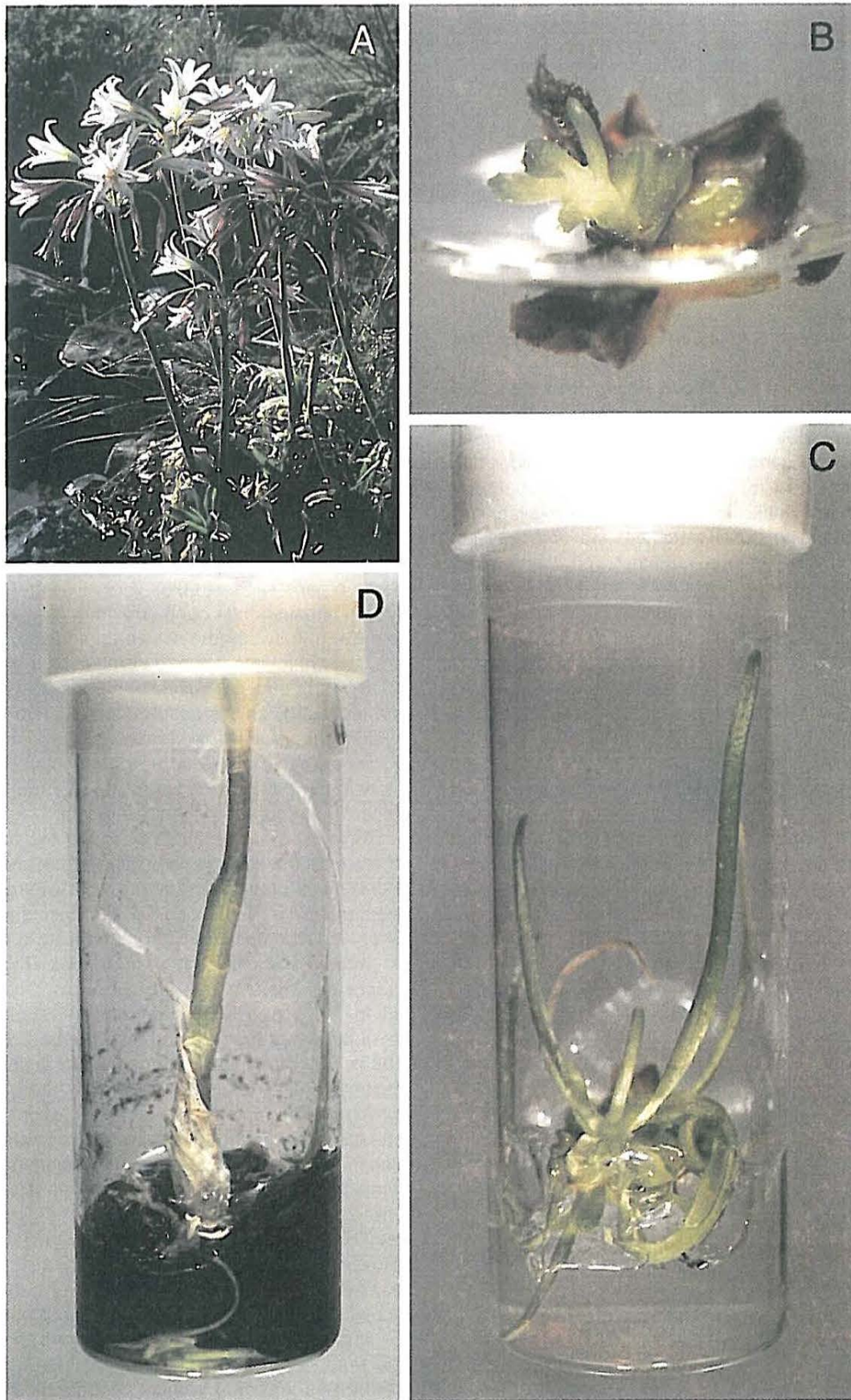
*Crinum variable* (Jacq.) Herb. is restricted to the Namaqualand, Bokkeveld Mountains and western Karoo regions of South Africa. Although its potential as a horticultural subject has been recognised, factors relating to the biology of the plant limit large scale propagation. These include short-lived seed viability and vegetative offsets that are produced too slowly or infrequently. As an alternative to conventional methods, *C. variable* was successfully propagated *in vitro* using twin-scale explants. Shoots developed in the axes of twin-scales when placed on a Murashige and Skoog (MS) medium.

Plant growth regulators were not required for the induction of shoots but benzyladenine (BA), in the absence of naphthalene acetic acid (NAA), promoted their outgrowth. The inclusion of 5g l<sup>-1</sup> activated charcoal improved development by increasing the bulblet size and frequency with which shoots formed bulblets. These bulblets were used to initiate further cultures by splitting them in half. Such a system provided for the continuous multiplication of *C. variable* bulblets which readily transferred to the soil.

Few South African crinums are as restricted in distribution as *Crinum variable* (Jacq.) Herb., known from only a handful of localities around Garies, Nieuwoudtville and Sutherland. Such is its localisation that following its description by Jacquin in 1804, as *Amaryllis variabilis* Jacq., it was not relocated in the wild until 1961, when Mr Dave Hardy of the Botanical Research Institute found a population in the Groen River near Garies. In flower (Figure 1A), the River Lily as it is known, may reach 1m in height and produce six to 12 flowered umbels. Plants bloom between February and April in early Autumn, during extremely hot and dry conditions (Verdoorn 1964). This is unlike other drought-adapted crinums which flower only after rain (Lehmiller 1987). Accordingly, *C. variable* should prove to be a useful horticultural subject in the drier regions of South Africa. The heads of narcissus-scented (Manning and Goldblatt 1997) flowers typically mature from white to a deep rose-pink; this colour progression gave rise to its specific epithet. The large trumpet-shaped flowers open wider as they mature, reaching up to 80mm across, revealing the white anthers. *Crinum variable* is one of only three southern African crinums whose funnel-shaped flowers have segments longer than the perianth tube, the others being *C. lineare* L.f. and *C. macowanii* Bak. (Verdoorn 1973). The closest relative of *C. variable* is considered to be the rare *C. lineare* L.f., which is restricted to the Eastern Cape Province (Verdoorn 1973). During flowering, the leaves are normally brown and shriv-

elled; the fresh lettuce-green leaves generally only appear following the onset of the winter rains. The leaves, which are strap-shaped and channelled, are at first stiffly erect but arch and broaden as they age, reaching 35cm in length and 4cm in breadth (Verdoorn 1964). The margins are minutely toothed (Manning and Goldblatt 1997). In habitat amongst riverbed rocks, the plants benefit from the deep waters that gather during the winter months, some specimens being temporarily inundated. Verdoorn (1973) recorded that the ovoid bulbs reached 26cm in length and 9cm in diameter, narrowing gradually towards the apex and so without a distinct neck. However, subsequent field observations (Lehmiller 1987) have reported on the existence of bulbs with diameters of up to 20cm. Offshoots often sprout from the axils, and from around the bulb (Verdoorn 1964).

Crinums have a long history of cultivation (Bryan 1989). Yet there is still much scope for the selection and development of cultivars among African species (Stirton 1980) for which the centre of diversity lies south of the Sahara (Fangan and Nordal 1993). Their potential development as pot plants has been recognised by Jansen Van Vuuren *et al.* (1993) and Lehmiller (1996) as they rarely or only sporadically produce offsets. Conventionally, offsets or seeds are used to propagate the plants. There are inherent difficulties with both techniques because natural rates of vegetative propagation are slow and the recalcitrant seeds, which are fleshy, germinate readily and without water (Verdoorn 1973)



**Figure 1:** Stages in the micropropagation of *Crinum variable*; (A) *C. variable* in flower, a source of twin-scale explants; (B) Shoot production in the axis of a twin-scale; (c) Multiple shoot production induced by  $1\text{mg l}^{-1}$  BA; (D) Bulblet formation on MS medium supplied with activated charcoal



**Table 1:** Decontamination and regeneration potential of different explants of *Crinum variable*

Explant	Decontamination (%)			Explants producing shoots (%)
	Benlate pretreatment + NaOCl (35 mins)	NaOCl (20 mins)	NaOCl (30 mins)	
Bulb scale + basal plate	—	67	—	0
Bulb scale	—	75	67	0
Twin-scale	32	78	79	66
Leaf	—	100	—	0

such that their viability is short-lived. *In vitro* techniques are thus worth considering for *Crinum variable*: one of just two species represented in the Cape flora.

A voucher (Crouch 777, NH), collected on the Groen River to the south of Garies in Namaqualand, has been lodged for verification purposes.

Bulbs were transferred to pots containing a mixture of fine river sand (2 parts) and peat (1 part) and grown in the greenhouse where they were watered, intermittently, during the growing season. As the plants had not reached flowering size, only bulb and leaf material was used as a source of explants, such that insufficient replicates were available for statistical analysis. The bulbs were washed and then cut lengthwise into halves. The upper, tunicate, portion was discarded. Both bulbs and the basal parts of emerging leaves were pretreated by rinsing in 1% Sporekill™ (17–30min) and 70% ethanol (30 sec–1 min). This was followed by sterilization in 3.5% NaOCl for either 20 or 30min. To rid plants of endogenous fungal contaminants, potted plants were also watered with a Benlate™ solution and then soaked in 0.2% Benlate (15min) prior to sterilisation in NaOCl.

The following explants were dissected from sterilised material under aseptic conditions: (1) single bulb scales with part of the basal plate, (2) single bulb scales with no basal plate tissue, (3) twin-scales, consisting of two adjacent scales joined by the basal plate, and (4) basal leaf segments. Each was placed on an MS basal medium (Murashige and Skoog 1962) with 2% sucrose and 0.2% Gelrite™ and grown in the light (70.7  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ; 16 hours light and 8 hours dark) at a temperature of 25°C.

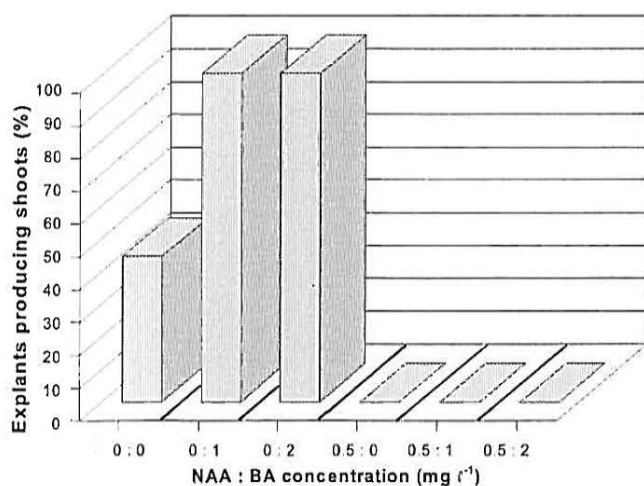
To optimise shoot induction from twin-scales, the hor-

mones benzyladenine (BA) and naphthaleneacetic acid (NAA) were used in the range 0–2mg l<sup>-1</sup>. Bulb development was monitored on the MS medium with or without 5g l<sup>-1</sup> activated charcoal. The propagules were subsequently split, longitudinally, in half for use as secondary explants and placed on media containing BA, in the concentration range 0–10mg l<sup>-1</sup>.

Mature bulblets were dipped in a systemic fungicide before planting in sterilised soil, containing one part each of sand, peat and fine bark chips, and placed in the misthouse to acclimatise.

Despite the fact that twin-scales were more difficult to sterilise than the leaves (Table 1), they were the only explants to regenerate shoots (Table 1 and Figure 1B). High contamination rates are often observed in cultures initiated from soil-borne organs such as bulbs (Hol and Van Der Linde 1992). This is because their open structure allows microorganisms to move in between the scales. In addition, VA mycorrhizae are known to infest the outer leaves (Iqbal and Baren 1986). Their endogenous habit means that surface sterilants are ineffective unless used in combination with alcohols or systemic fungicides, like Benlate. Even then, there may be no consistency in the degree of decontamination achieved, as was the case for *C. variable*. This may relate to differences in the quality of the parent bulb.

Twin-scales are frequently used in the micropropagation of amaryllidaceous species (Van Aartrijk and Van Der Linde 1986) since meristems already exist where the leaves join the basal plate (Thompson 1989). These give rise to shoots. Shoot induction occurred spontaneously in *C. variable*, that is, without the addition of hormones (Figure 2). For *C. macowanii*, the average number of plantlets that regenerated in the absence of plant growth regulators compared favourably with the best hormone treatments (Slabbert *et al.* 1993). By cutting into the basal plate, apical dominance is destroyed. This effectively stimulates the outgrowth of pre-existing axillary meristems. However, shoot initiation can be enhanced by adding cytokinins to the medium (Figure 1C). The involvement of cytokinins in apical dominance has been demonstrated in other bulbous species (Hussey 1976). But unlike the Iridaceae, liliaceous and amaryllidaceous species require higher concentrations of BA to promote branching (Hussey 1976). The combination of auxin and cytokinin, however, reduced shoot initiation (Figure 2). This was accompanied by abnormal organogenesis. In other studies, BA and NAA, in interaction, were found to adversely affect the numbers of shoots produced (McAlister *et al.* 1998), sometimes inhibiting plantlet regeneration (Slabbert *et al.* 1993). The addition of charcoal to the medium resulted in a greater number (92%) of explants forming bulblets compared to the control (41%). The bulblets were, on average, 3.9mm in diameter (Figure 1D), whereas those grown on

**Figure 2:** Interactive effect of NAA and BA on *in vitro* shoot induction in *Crinum variable*

**Table 2:** Effect of BA on shoot production from halved bulblets of *Crinum variabile*

	BA Concentration (mg l <sup>-1</sup> )			
	0	0.1	1	10
Explants producing shoots (%)	66	0	75	50

charcoal-free media, were smaller at 2.3mm. Charcoal is routinely used in the propagation of some bulbs as it reduces browning and the decay of twin-scale bases (Steinitz and Yahel 1982) and strongly influences bulblet regeneration and size (e.g. *Narcissus tazetta* L.; Amaryllidaceae) (Steinitz and Yahel 1982, Peck and Cumming 1986). Its ability to regulate these processes is thought to be linked to the adsorption of substances likely to antagonise cytokinin activity or even the regulation of cytokinin activity itself (Takayama and Misawa 1980). The development of bulblets as propagules has several benefits, including: the elimination of a rooting stage and the need for hardening-off (Ziv 1997). They can also be used as a source of secondary explants by splitting them longitudinally in half. Bulblets of *C. variabile* which were divided in this manner produced new shoots even without the addition of BA (Table 2). At concentrations of 1 mg l<sup>-1</sup>, however, shoot regeneration was enhanced. Greater amounts of BA reduced shooting. Cytokinins like BA, thus have a stimulatory effect on shoot regeneration from both twin-scales and bulblets that develop *in vitro*. Similar effects have been noted in *Agapanthus*, *Alstroemeria*, *Freesia* (Hussey 1980), *Gladiolus* (Dantu and Bhojwani 1995) and *Nerine* cultivars (Custers and Bergervoet 1992).

Bulblets of *C. variabile* transferred to the soil showed no dormancy, with 100% survival and leaf elongation during the period of acclimatization in the misthouse. Aspects of the pot cultivation requirements of this taxon have been dealt with by Lehmillier (1996). The benefits of the micropropagation scheme, outlined above, include the potential for large scale production of a new horticultural subject. For example, a single bulb (measuring 4cm in diameter) may yield approximately 23 plantlets in 17 weeks, or double this number in 6 months.

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