However, where the space occupied by each plant is taken as circular then:

$$D = \frac{10\,000}{\pi(S/2)^2}$$

and

$$S = 2\sqrt{\frac{10000}{\pi D}}$$

Table 2 The Plant Number Scale showing plant count, cover symbols, percentage cover, class limits and class intervals

Count	Symbol	% Cover	Class limits	Class interval
0	+	0.01	> 0.000–0.049	0.049
1	1	0.10	0.050–0.249	0.199
2	2	0.40	0.250–0.654	0.404
3	3	0.91	0.655–1.259	0.604
4	4	1.61	1.260–2.064	0.804
5	5	2.52	2.065–3.074	1.009
6	6	3.63	3.075–4.284	1.209
7	7	4.94	4.285–5.694	1.409
8	8	6.45	5.695–7.314	1.619
9	9	8.16	7.315–9.129	1.814
10	A	10.08	9.130–11.139	2.009
11	B	12.19	11.140–13.354	2.214
12	C	14.51	13.355–15.769	2.414
13	D	17.03	15.770–18.389	2.619
14	E	19.75	18.390–21.214	2.824
15	F	22.67	21.215–24.239	3.024
16	G	25.80	24.240–27.459	3.219
17	H	29.12	27.460–30.884	3.425
18	I	32.65	30.885–34.514	3.629
19	J	36.38	34.515–38.344	3.829
20	K	40.31	38.345–42.374	4.029
21	L	44.44	42.375–46.609	4.234
22	M	48.78	46.610–51.044	4.434
23	N	53.31	51.045–55.679	4.634
24	O	58.05	55.680–60.519	4.839
25	P	62.99	60.520–65.559	5.039
26	Q	68.13	65.560–70.799	5.239
27	R	73.47	70.800–76.284	5.484
28	S	79.10	76.285–81.929	5.644
29	T	84.75	81.930–87.729	5.799
30	U	90.70	87.730–93.774	6.044
31	V	96.85	93.775–98.424	4.649
32	W	100.00	> 98.425	1.575

With plant spacing known, as calculated according to Acocks's formula, data can be converted to Acocks's density classes for direct comparison.

The method described provides a simple means of determining both canopy cover and density as actual values or as Plant Number Scale classes, as well as indicating plant size in terms of mean crown spread. Mean plant spacing, which is not easily measurable, can be derived from density. The method also includes more variation than is included with the Plant Number Scale method and is, therefore, suitable for plants with low canopy cover. It is also suitable for comparing Acocks' density classes over time.

Acknowledgements

The support of the ARC for this work is gratefully acknowledged as well as the encouragement of the the Director of the Range and Forage Institute, Dr Amie Aucamp.

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Effect of a seaweed concentrate on acclimatization of *in vitro* grown plantlets of *Kniphofia pauciflora* and *Scilla krausii*

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Received 26 March 1998; revised 29 May 1998

Kelpak, a seaweed concentrate prepared from *Ecklonia maxima* (Osbeck) Papenfuss, applied as a soil drench following the planting out of *in vitro* grown plantlets of *Scilla kraussii* Bak. and *Kniphofia pauciflora* Bak. significantly increased root growth and promoted plantlet establishment. It is suggested that seaweed concentrate can be used successfully and economically to aid in the acclimatization of *in vitro* grown plantlets.

Keywords: Acclimatization, Kelpak, *Kniphofia pauciflora*, *Scilla kraussii*.

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An important stage in tissue culture is the hardening off, or acclimatizing of the *in vitro* grown plantlets to the conditions outside the culture flask. A loss at this late stage has serious financial implications, especially when one considers all the resources that are put into producing the *in vitro* plantlets.

Many reports indicate that seaweed products improve plant

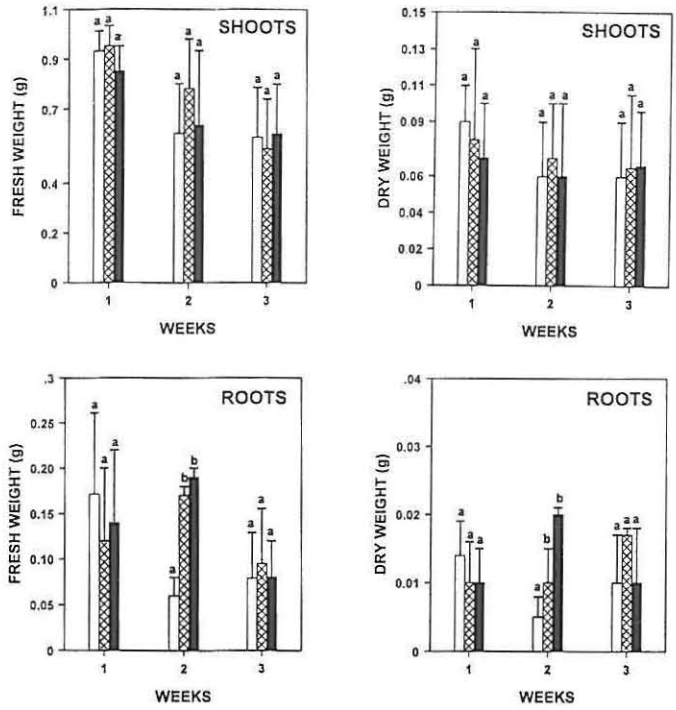

Figure 1 Shoot and root growth of *in vitro* grown plants of *S. kraussii* 1, 2 and 3 weeks after planting out, treated with ❒ water, ⊠ 0.5% or ■ 1.0% Kelpack. Bars with the same letter are not significantly different using a multiple range test ($P < 0.05$).

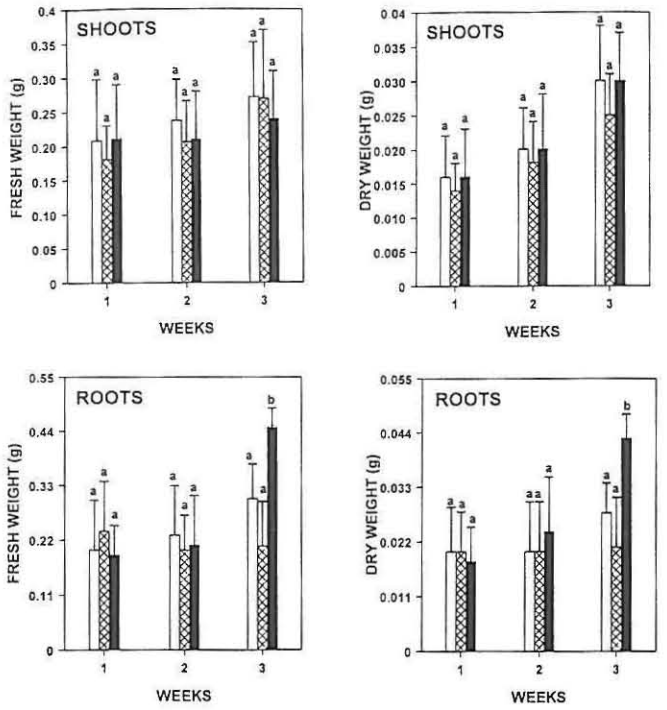

Figure 2 Shoot and root growth of *in vitro* grown plantlets of *K. pauciflora* 1, 2 and 3 weeks after planting out, treated with ❒ water, ⊠ 0.5% or ■ 1.0% Kelpak. Bars with the same letter are not significantly different using a multiple range test ($P < 0.05$).

growth (Metting *et al.* 1990). It has been suggested that organic compounds rather than mineral elements are responsible for yield increases. The cytokinins, which occur at relatively high concentrations in various seaweeds and commercial seaweed preparations (Penderson 1973; Blunden & Wildgoose 1977; Featonby-Smith & van Staden 1984b and Tay *et al.* 1985), have been suggested as causal agents (Tay *et al.* 1987).

The application of seaweed concentrate (SWC) to plants significantly increase root initiation and growth (Featonby-Smith & van Staden 1984a; Beckett & van Staden 1989). Since cytokinins are considered to inhibit rooting (van Staden & Harty 1988) it is unlikely that the observed increase in root growth is due to these hormones. Both endogenous and synthetic auxins stimulate rooting (Jackson & Harney 1970; Hartman & Kesler 1975). Auxins have been isolated from Kelpak (Crouch *et al.* 1992) and may be responsible for the increased root growth.

This paper reports on the increase in growth of roots during acclimatization of plantlets of *Scilla kraussii* Bak. and *Kniphofia pauciflora* Bak. following SWC application.

The SWC used in this study is marketed as Kelpak and is prepared by a cell burst process from the brown alga *Ecklonia maxima* (Osbeck) Papenfuss (Featonby-Smith & van Staden 1983).

A micropropagation system for *Kniphofia pauciflora* was developed by McAlister and van Staden (1996). *In vitro* shoot formation was initiated using the apical regions of a stolon. Murashige and Skoog (1962) (MS) medium supplemented with 100 mg l^{-1} myo-inositol, 3% sucrose and solidified with 0.8% agar was used. Kinetin (2 mg l^{-1}) and NAA (1 mg l^{-1}) were used to obtain shoot development. Shoots were subsequently placed onto a hormone-free medium for rooting (McAlister & van Staden 1996). *In vitro* shoot formation of *Scilla kraussii* was initiated from leaf explants on MS medium supplemented with 100 mg l^{-1} myo-inositol, 20 mg l^{-1} glycine, 2% sucrose, 0.2% gelrite and 1 mg l^{-1} IAA.

Uniform plantlets of *S. kraussii* and *K. pauciflora* were planted in trays with thirty replicates per treatment. The potting medium used was compost:bark:sand (2:2:1). Control plants were treated with distilled water, whilst 0.5% or 1.0% SWC was applied as a soil drench. The SWC application was applied to plantlets at transplanting. Thereafter, plantlets were watered daily with water. Trays were placed in plastic covered enclosures (166 cm × 120 cm × 37 cm) in a greenhouse. After one week the enclosures were gradually opened to reduce the humidity level to that of the greenhouse.

Ten plants were harvested 1, 2 and 3 weeks after transplanting. Shoot and root fresh and dry weight was recorded. Data for each harvest were statistically analysed using a one way analysis of variance and a multiple range test ($P < 0.05$).

Plantlets of *S. kraussii* showed a significant increase in root fresh and dry weights two weeks after treatment with 0.5% and 1.0% Kelpak (Figure 1). No significant difference in shoot fresh or dry weights of *S. kraussii* at either of the treatments was observed. A significant difference in root fresh and dry weights of *K. pauciflora* was only observed three weeks after treatment with 1.0% Kelpak (Figure 2). There was no significant difference in shoot fresh and dry weight of *K. pauciflora* at either of the treatments.

The survivial rate was 100% for all treatments in both species after 3 weeks. Loss during acclimatization of *ex vitro* plantlets mostly happens within the first week. Besides the ability of plantlets to withstand waterloss due to evaporation under conditions with a lower humidity than in the culture flask, early onset of rooting is important in the establishment of the plantlets. In this study SWC induced rooting at an earlier stage than in control plants.

The use of SWC in reducing transplant shock of vegetables and ornamentals has been examined and it was suggested that SWC aids in the establishment of these plants by increasing both size and vigour (Aldworth & van Staden 1987). Increased root growth following SWC application is well documented (Finnie & van Staden 1985; Crouch & van Staden 1991). Such increases had been attributed to endogenous auxins in the SWC. Crouch

and van Staden (1992) and Atzmon and van Staden (1994) reported that SWC applied as a soil drench aids in seedling establishment. This is important as root development will affect acclimatization in the greenhouse and seedling establishment in the field. It is suggested that SWC can be successfully used to aid in acclimatization of *in vitro* grown plantlets.

Acknowledgements

The University of Natal Research Fund and the Foundation for Research and Development are thanked for financial support. Cultured plants were provided by Mrs L. van Staden.

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In vitro culture of *Eulophia* species

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Received 6 February 1998; revised 3 June 1998

Seeds of *Eulophia cucullata, E. streptopetala* and *E. petersii* were placed in aseptic culture and germinated on Murashige and Skoog medium supplemented with 3% sucrose and 0.01% *myo*- inositol. *E. cucullata* and *E. petersii* germinated after 3 months whereas *E. streptopetala* took 6 months to germinate. *E. streptopetala* and *E. petersii* had a high percentage of seed germination whereas with *E. cucullata* only about 15 seeds germinated from a pod. Multiplication of *E. cucullata* was obtained by cutting the protocorms in half, from which new shoots then developed. These were split further so that numerous plants were obtained. Rooting of *E. petersii* and *E. streptopetala* was increased with the addition of 0.4% activated charcoal to the Murashige and Skoog medium. The rooted plants were acclimatized successfully in either bark or a sand:soil mixture.

Keywords: *Eulophia*, seeds, *in vitro* propagation, orchids.

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Eulophia is a very large genus of terrestrial orchids with over 200 species distributed throughout the tropical and sub-tropical regions of the world (Hawkes 1977). In South Africa there are 36 species (Schlepe 1966). *E. cucullata* is a large showy terrestrial species, one metre or more tall. The pseudobulbs are rounded. The leaves are tufted and lanceolate and are 25–30 cm long. The inflorescence appears before the leaves. The flowers are pink in colour and are about 3.5 cm in length. This plant grows in grasslands and on poor pastures, it is widely distributed in tropical Africa (Bechtel *et al.* 1981) .

E. streptopetala is a robust plant up to 1.5 m tall with well developed pseudobulbs (Stewart & Hennessy 1981). The leaves are lanceolate and ribbed, 50 cm long and 8 cm wide. The inflorescence is many-flowered. The sepals are green blotched with brownish purple inside and the petals are bright yellow outside and pale cream inside with the spur being a purple red. *E. streptopetala* grows in woodlands and *Eucalyptus* plantations and in long grass at the edge of thickets and amongst rocks in montane grassland. It flowers from September to December (Onderstall 1984).

E. petersii is also a robust plant with large pseudobulbs mostly above ground (La Croix *et al.* 1991). The leaves are stiffly succulent and are grey green with finely but sharply serrated margins (Willianson 1977). Sepals and petals are green tinged with purple-brown markings. The lip is white with purple lamellae with the side leaves green with purple veins. *E. petersii* grows in riverine thickets in hot dry areas, usually on a bank well above a river. This plant flowers in November and December.

As *Eulophia* species are sold on the traditional medicine markets in South Africa they are becoming rare and steps must be