

## EFFECT OF DIFFERENT PROPAGULE TYPES, GROWING MEDIA AND ROOTING HORMONES ON INITIAL SPROUTING OF THE MEDICINAL PLANT *CYPERUS ROTUNDUS* L.

K.K.S.K. De Alwis, K.U. Tennakoon\*, C.V.S. Gunatilleke  
and I.A.U.N. Gunatilleke

Department of Botany, Faculty of Science, University of Peradeniya,  
Peradeniya, Sri Lanka.

### ABSTRACT

This study examined the effect of propagule type [full tuber with or without mother plant, tuber cutting (disc) with or without a visible bud], growth medium (water medium or coir dust and sand mixed in equal proportion) and rooting hormones [Indole Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA)] on the propagation success of *Cyperus rotundus* L. Percentage sprouting (95%) and the means of total biomass per plant and leaf number per plant were highest in plants raised from propagules comprising the full tuber without the mother plant treated with IBA. The means of plant height (27 cm) and leaf length per plant (14 cm) were highest in the same propagule type grown in coir and sand medium, but the number of buds per plant was highest (1.7) when grown in water.

### Key words

*Cyperus rotundus*, Medicinal plants, Propagation, Growth media

### INTRODUCTION

About 75% of world population depends on traditional medicine for their primary health care needs (Anon., 1996). A recent survey conducted by the World Health Organization (WHO) has revealed that about 70% of the population in Sri Lanka still relies on the indigenous system of medicine, based mainly on plant formulations (Fernando, 1999). The indigenous flora of Sri Lanka comprises around 3,350 species of higher plants, of which nearly a half is used for medicinal purposes (Fernando, 1999). Nevertheless, 60 percent of the medicinal plants required for the preparation of medicines are imported, mostly from India (Anon., 1996). Initiatives are now being taken to promote the cultivation of widely used medicinal plants in Sri Lanka.

Among the large number of medicinal plant species used in Ayurvedic medicine in Sri Lanka, *Cyperus rotundus* L. is widely used by the local people (Koyama, 1985). The tubers of this species contain effective compounds such as cyperene, cyperone, cyperol and 1-pinene (Koyama, 1985). It also contains glycerides of oleic, palmitic and linolic acids with small quantities of essential oil

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\* Corresponding Author's E-mail: kushant@pdn.ac.lk

(Loutfy, 1983). The tubers are used as a remedy for spasms, as an emenagogue or as an astringent and antiseptic. A paste made with lime-juice is applied to acne, scorpion stings and ulcers with beneficial effect. Internally, the tubers act as a stomachic, carminative and cholagogue with astringent properties and are used in anorexia, acute dyspepsia, diarrhoea, dysentery and congestion of the liver (Jayaweera, 1980).

In the year 2000, the amount of herbal material used in Sri Lanka was 2,218,075 kg, valued at Sri Lanka Rs. 176,334,895.15 (Anon., 2001). This same year about 18,000 kg of *C. rotundus* had been used by the Ayurvedic Department for the preparation of a range of herbal drugs; of this amount thirty-one percent (5,723 kg) was used as fresh herbal material and 68.9% (12,654 kg) as dry herbal material. The local supply of medicinal plants for the preparation of Ayurveda medicines is inadequate to meet the current demand. According to sources in the Ayurveda Department, nearly 20 metric tons of *C. rotundus* have been imported from India. The annual report of the Sri Lanka Customs (Anon., 1996) further documents that the amount of *C. rotundus* imported to Sri Lanka was 23 metric tons valued at Rs. 1.1 million in 1993 and 9 metric tons valued at Rs 0.9 million in 1994. According to a study carried out by the Sri Lanka Conservation and Sustainable use of Medicinal Plants Project in year 2000, the national demand for *C. rotundus* for the preparation of herbal medicines in Sri Lanka is 60.5 metric tons per annum valued at Rs. 4.9 million (Anon., 2001). These statistics confirm the importance of *C. rotundus* to the herbal medicine industry in Sri Lanka.

A large number of species having medicinal properties are common weeds (Fernando, 1999). *C. rotundus* is a common weed in Sri Lanka and it is further known as the 'world's worst weed' (Mabberley, 1989). Since *C. rotundus* is a weed, it is important to collect tubers of *C. rotundus* free from weedicides and pesticides in order to use them as medicinal ingredients. However, due to the rapid increase of weedicides and pesticides in cultivated lands in Sri Lanka it has become increasingly difficult to obtain uncontaminated tubers of *C. rotundus* very often growing as a weed in these lands. Therefore, it has become necessary to grow *C. rotundus* in a systematic manner in areas not contaminated with weedicides and pesticides in order to obtain organically grown quality tubers for the preparation of herbal medicine.

Another very common problem encountered with the collection of *C. rotundus* is the difficulty in identification of the correct species among a large number of similar or related taxa (150 in number; Senaratne, 2001) in the same family. The typical character of *C. rotundus* that can be used to differentiate it from other closely resembling species is the positioning of the bracts at the base of the mature inflorescence. The bracts of *C. rotundus* originate from the base of the inflorescence and are as long as or shorter than the mature inflorescence (Koyama, 1985).

As the study species is considered to be a common weed in many tropical countries, most research on *C. rotundus* has been carried out to develop methods to control its spread (Bhargava and Sobti, 2000; Blum *et al.*, 2000; Pandey and

Padhiar, 1999; Rajendran and Lourduraj, 1999; Reddy *et al.*, 1998; Sathyavelu *et al.*, 1999; Marambe, 1998). Studies related to the methods of propagation, nursery establishment and agronomical practices of this species are not reported very often.

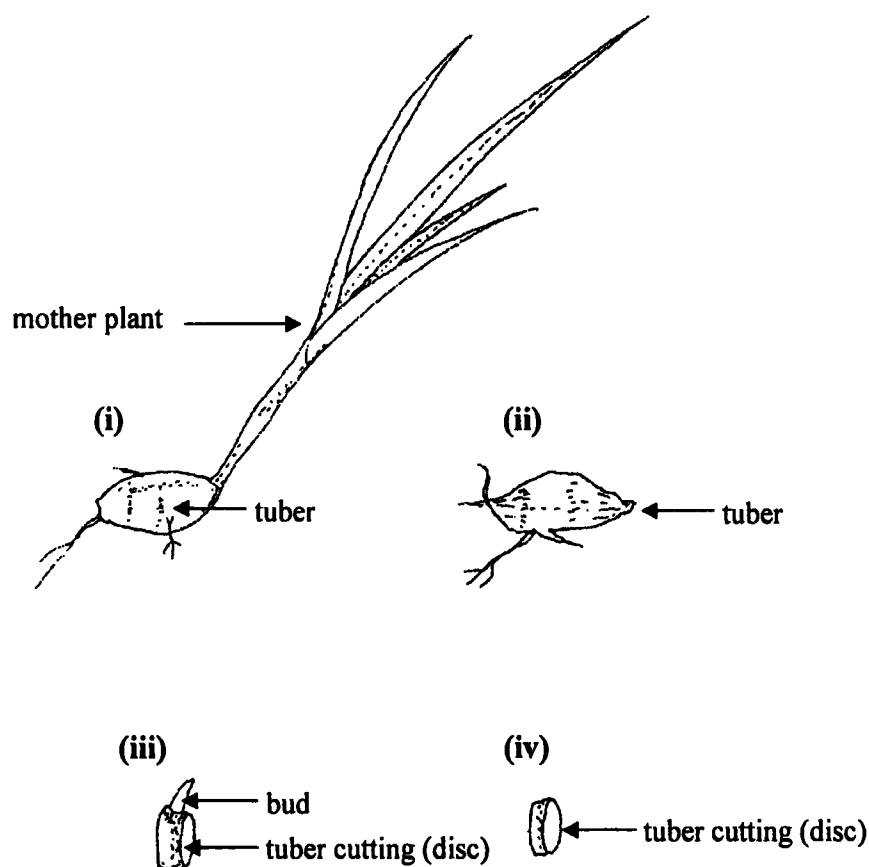
In this paper, we report simple and cost effective vegetative propagation techniques that could be used to raise a large number of *C. rotundus* plants and the growth conditions required in order to meet the demand of organically grown plants free of pesticides and other hazardous chemicals. We specifically examined the following: (i) The propagation success of the species using four different propagule types, *viz.*, the full tuber with or without the mother plant and horizontal slices of the tuber (disc) with or without a visible bud. (ii) The effect of two different growth media (water medium or coir dust and sand mixed in equal proportion) and two rooting hormones, Indole Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA), on the propagation success of the different propagule types of *C. rotundus*.

## MATERIALS AND METHODS

Propagation of *C. rotundus* can be done either vegetatively or reproductively (Hartmann *et al.*, 1997). In this study we considered the vegetative propagation techniques that could be used to raise a large number of plants of *C. rotundus*. Propagation by tubers can be done either by planting the entire tubers or by cutting them in to sections, each containing one or more axillary buds or "eyes" (Hartmann *et al.*, 1997). A tuber is a special kind of swollen, modified stem structure that functions as an underground storage organ (Hartmann *et al.*, 1997).

The experiments on vegetative propagation of *C. rotundus* using tubers were established in May 2001 using four types of propagule types *viz.*, (i) full tuber with mother plant, (ii) full tuber without the mother plant, (iii) tuber cutting (disc) with a bud and (iv) tuber cutting (disc) without a visible bud (Fig. 1). The plant material used for this study was obtained from Katugastota in the Kandy district. The average size of the tubers with or without the mother plants used as propagules were about 1-3 cm in length and 0.5-1.5 cm in width. The size of the tuber cuttings (discs) with or without a visible bud, used as propagules were 0.5 – 1.5 cm diameter and 2-3 mm in length. All four propagule types were then subjected to different substrate treatments to find out the best treatment type to obtain new plantlets from them. One set (60 propagules) each of the propagule types was planted in Styrofoam trays containing equal parts of sand and coir dust as the medium. Another set of the propagule type was grown in shallow trays, which contained water as the growth medium. A third set of propagule type was treated with 3000 mg/l of the commercially available rooting hormone Naphthalene Acetic Acid (Secto) and finally the fourth set with 3000 mg/l Indole Butyric Acid (IBA). These were maintained in a plant house at an average temperature of 27 °C and a relative humidity of 80%. The maximum instantaneous light intensity received by all plants was 890  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The study was set out

as a factorial experiment, which included two main factors (the different propagule types and the different growth media), each at four levels.



**Figure 1.** Four propagule types used in the vegetative propagation experiments of *Cyperus rotundus*. i) Full tuber with mother plant, ii) full tuber without mother plant, iii) tuber cutting (discs) with a bud and iv) tuber cutting (discs) without a visible bud.

#### **Assessment of sprouting and growth performance of newly emerged plants**

Sprouting of each propagule type and the growth performance of the new plants, in terms of plant height, number of leaves per plant, average leaf length per plant were recorded only for a period of two weeks as our objective was to find out the initial propagation success of the propagules and the growth performance of newly emerged plants. The Number of new buds that emerged from each propagule type was counted. In the newly emerged plants, plant height was measured from the base to the tip of the longest leaf and leaf length from top of the leaf sheath to the leaf tip. After growth measurements were recorded, the whole plants were uprooted and oven dried ( $80^{\circ}\text{C}$ ) until a constant weight was obtained. Total above ground and below ground biomass of new plants were determined separately.

The number of new buds that emerged and allometric measurements of the new plants were analyzed using analysis of variance (ANOVA) in the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS) version

6.12. All data on percentage sprouting were arcsine transformed prior to analysis. Analyses were tested for differences among propagule types [full tuber with mother plant, full tuber without mother plant, tuber cuttings (discs) with a bud, tuber cuttings (discs) without a visible bud], differences among growth media (water substrate, substrate comprising coir dust and sand mixed in equal proportions, propagules treated with Secto and IBA and then planted in the medium of coir dust and sand mixed in equal proportions) and the interactions between propagule types and the growth media. Appropriate means of the raw data were used to plot the graphs.

## RESULTS

### Percentage sprouting

The proportion of sprouting in *Cyperus rotundus* was significantly different ( $P < 0.001$  level) among the four propagule types tested, among the substrate treatments and their interaction ( $P < 0.05$  level) (Table 1).

Table 1

Variance ratios (F-values) and significance (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ , ns - not significant) following two way analysis of variance on measurements of four types of propagules after two weeks of growth under different treatments. Measurements were taken under plant house conditions (mean temperature 27 °C, mean relative humidity 80% and the maximum instantaneous light intensity 890  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ).

	F- values and significance level	
	Percentage sprouting	No. of new buds that emerged
<b>Two way analysis</b>		
Propagule type	35.31***	8.65***
Substrate treatment	15.44***	8.35***
Replicate	0.46 <sup>ns</sup>	3.49*
Propagule X substrate treatment	2.97*	1.68 <sup>ns</sup>
<b>One way analyses</b>		
<i>Differences among substrate treatment within a single propagule type</i>		
Full tuber with mother plant	2.35 <sup>ns</sup>	4.17**
Full tuber without mother plant	3.4 <sup>ns</sup>	3.5*
Tuber cuttings (discs) with a bud	10.2*	2.12**
Tuber cuttings (discs) without a bud	9.82**	13.19 <sup>ns</sup>
<i>Differences among propagule types within a single substrate treatment</i>		
water	4.66 <sup>ns</sup>	4.64**
Coir : sand (1:1)	5.79*	1.65 <sup>ns</sup>
Secto treated and established in coir dust and sand medium	40.95**	1.21 <sup>ns</sup>
IBA treated and established in coir dust and sand medium	7.10*	7.19***

Among the growth media, the differences in the percentages of sprouting observed by tubers with or without mother plants were not significant. On the other hand, the percentage sprouting of the different propagule types in each of the substrates was significant, except in water (Table 1).

Among the four propagule types grown in each substrate, full tubers without mother plants gave the highest percentage sprouting; those transplanted in coir dust and sand medium after treatment with IBA showed 95% sprouting and that without any rooting hormone 83%. Percent sprouting was least when discs of both types were treated with Secto (15%) and grown in the sand and coir (1:1) medium (Fig. 2). Except when the full tuber with the mother plant was used, all other propagule types had significantly lower percentage sprouting when treated with Secto and grown in coir dust and sand medium (Table 2).

**Table 2**

**Statistical differences in percentage sprouting of each propagule type of *Cyperus rotundus* grown in the four different substrate treatments. Letters qualitatively (a>b>c) indicate row-wise, the significant differences among treatments for each propagule type at P<0.05 level.**

Propagule type	Propagules grown in water	Propagules planted in coir and sand medium	Secto treated propagules planted in coir dust and sand medium	IBA treated propagules grown in coir dust and sand medium
Full tuber without mother Plant	ab	ab	b	a
Full tuber with mother Plant	a	a	a	a
Tuber cuttings (discs) with a bud	a	a	b	a
Tuber cuttings (discs) without a visible bud	a	b	c	ab

### **Number of new buds formed from propagules**

The mean number of new buds that formed per propagule varied significantly among the different propagule types and among the different treatments but the interaction between propagule types and treatments was not significant (Table 1).

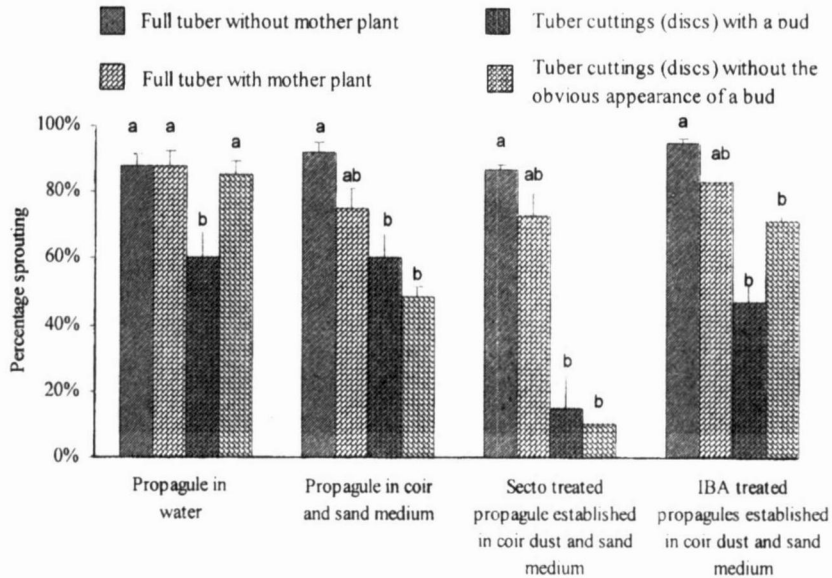


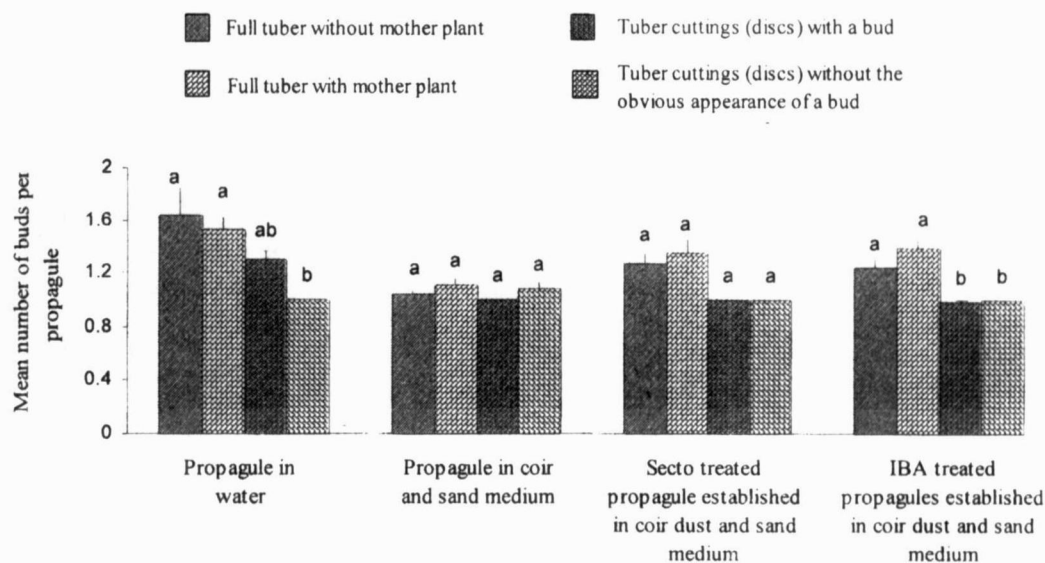
Figure 2. Percentage sprouting of *Cyperus rotundus* using four different propagule types and grown in four different substrate treatments. Letters qualitatively (a>b>c) indicate the significant differences among planting material within each treatment at P < 0.05 level.

Comparing the performance of each propagule type in the different substrata, the number of new buds that emerged in all propagule types, except in cuttings without a visible bud, was significantly different (Tables 1 and 3). Among the four propagule types grown in water and in those treated with IBA and then established in coir dust and sand, the number of new buds that emerged differed significantly. In both these media, full tubers with and without the mother plant grew significantly higher number of buds compared to that arising from discs without a visible bud. All the propagule types treated with or without Secto and then established in coir dust and sand did not show any significant differences (Table 1, Figure 3).

Table 3

The formation of new buds of *Cyperus rotundus* using four different propagule types grown in four different substrate treatments. Letters qualitatively (a>b>c) indicate row-wise significant differences among substrates within each propagule type at P < 0.05 level.

Propagule type	Propagule grown in water	Propagule grown in coir and sand medium	Secto treated propagule grown in coir dust and sand medium	IBA treated propagule grown in coir dust and sand medium
Full tuber with out mother Plant	a	b	ab	ab
Full tuber with mother Plant	a	b	a	a
Tuber cuttings (discs) with a bud	a	b	b	b
Tuber cuttings (discs) without a visible bud	a	a	a	a



**Figure 3.** The formation of new buds from different types of *Cyperus rotundus* propagules grown in four different substrate treatments. Letters qualitatively (a>b>c) indicate the significant differences among planting material within each treatment at  $P < 0.05$  level.

### Growth performance of new plants

All the growth variables of new plants (mean plant height, mean leaf length and mean biomass) were significantly different among propagules, among different treatments and their interactions, except the interaction for biomass (Table 4). Comparison of the performance of the new plants that emerged from each propagule type grown in the different substrates, showed significant differences in mean plant height, mean number of leaves per plant and mean leaf length. Mean biomass was only significantly different among the substrates only in plants raised from full tubers with the mother plants and in tuber cuttings with a bud.

The different propagule types showed significant differences in all allometric measures, except mean biomass, when grown with and without Secto and transplanted to coir dust and sand. Performance of propagules treated with IBA and transferred to coir dust and sand was significantly different for all measures except plant height, and those grown in water showed a significant difference only for biomass (Table 4).

Means of plant height, leaf length and biomass of new plants were significantly lower in those raised from tuber cuttings (discs) with or without a visible bud grown in water, compared to the corresponding values recorded for the other planting materials (Fig. 4). Growth performances of new plants, in terms of mean plant height, mean leaf length and mean biomass were significantly higher ( $P < 0.001$  level) when raised from full tubers without mother plant and grown in the coir dust and sand medium. The performance of plants raised from both types

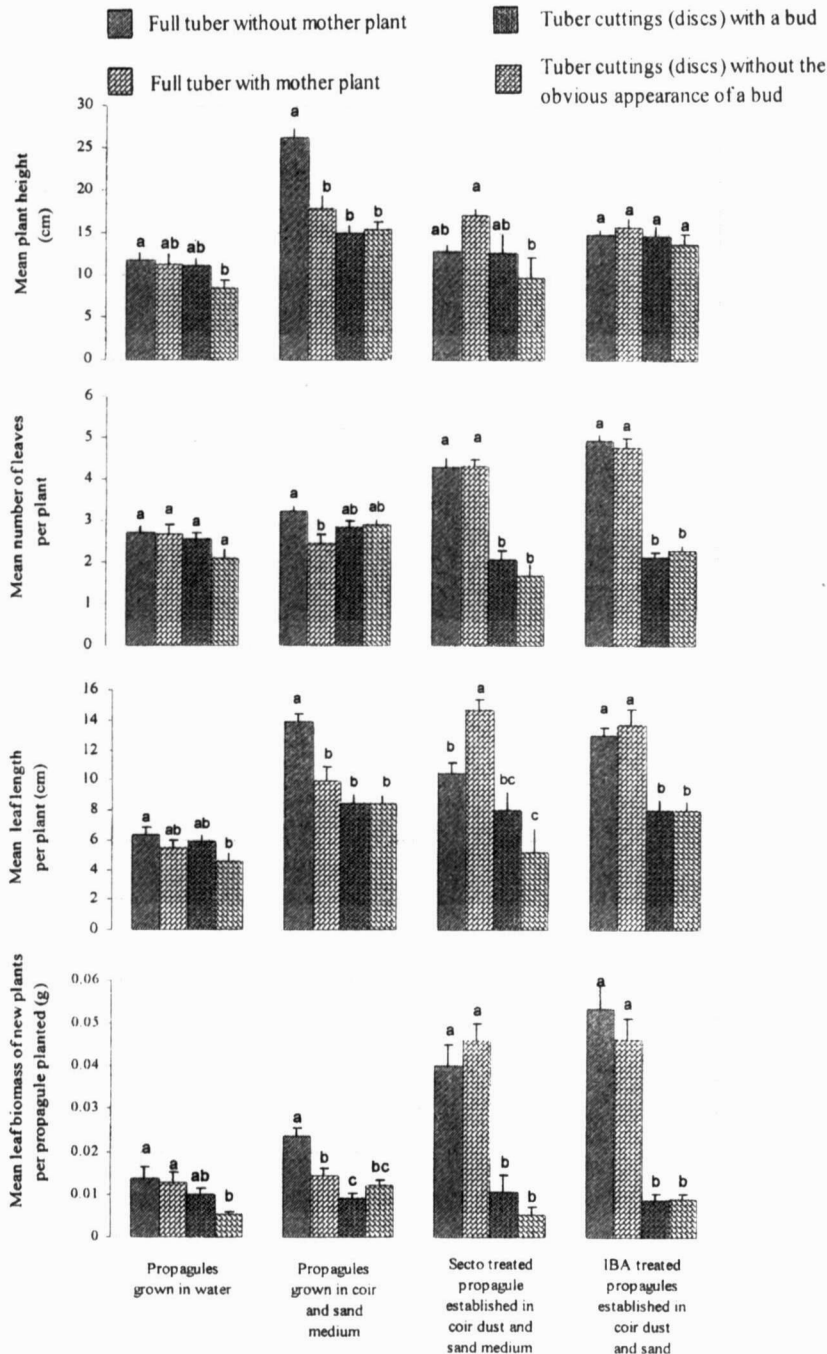
Table 4

Variance ratios (F-values) and significance levels (\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ , ns-not significant) following two way analysis of variance on measurements of four types of propagules after two weeks growth under different substrate treatments. Measurements were taken under plant house conditions (mean temperature 27 °C, mean relative humidity 80% and the maximum instantaneous light intensity of 890  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ).

	F- Values and significance level			
	Mean plant height	Mean leaf number per plant	Mean leaf length per plant	Mean biomass per plant
<b>Two way analysis</b>				
Propagule type	11.02***	56.09***	36.87***	7.03***
Substrate treatment	45.86***	36.76***	57.23***	6.22**
Replicate	11.11***	5.7**	4.17*	3.15**
Propagule x substrate treatment	7.42***	16.01***	8.28***	1.21ns
<b>One way analysis</b>				
<i>Differences among substrates within a single propagule type</i>				
Full tuber with mother plant	6.45**	29.04***	29.86***	30.40***
Full tuber without mother plant	61.18***	36.22***	34.94***	2.46 <sup>ns</sup>
Tuber cuttings (discs) with a bud	9.08***	6.35**	10.10***	8.12***
Tuber cuttings (discs) without visible bud	4.17**	3.3*	3.43*	0.36 <sup>ns</sup>
<i>Differences among propagule types within a single substrate treatment</i>				
water	1.8 <sup>ns</sup>	1.71 <sup>ns</sup>	1.98 <sup>ns</sup>	2.18*
Coir : sand (1:1)	19.16***	4.45**	14.13***	16.42***
Secto treated and established in coir dust and sand medium	4.63**	17.82***	10.75***	0.86 <sup>ns</sup>
IBA treated and established in coir dust and sand medium	0.88 <sup>ns</sup>	79.13***	20.72***	31.7***

of tubers treated with IBA or Secto and transplanted in the coir dust and sand showed significantly higher performance for all measurements, except plant height, compared to their performance in the two remaining substrate media (Fig. 4).

Comparison of the performance of propagules types in each of the substrate treatments (Table 5) showed the following: mean plant height was significantly higher ( $p < 0.001$  level) in plants raised from all propagule types treated with or without IBA and grown in coir dust and sand, compared to those grown in water. Mean number of leaves on the other hand, was significantly



**Figure 4.** Growth performance of two week old *Cyperus rotundus*, plants raised from four types of propagules and grown in four different substrates. Letters qualitatively (a>b>c) indicate the statistical differences in performance among different types of propagules within each substrate treatment at P<0.05 level (n = 60).

higher in plants raised from full tubers treated with hormones and grown in coir dust and sand. Plants raised from tuber cuttings in contrast, produced significantly more leaves in the coir dust and sand medium. Leaf length of new plants raised showed a trend similar to that of leaf number. Biomass of plants raised from each of the propagule types, except from tuber cuttings with a visible bud, varied significantly ( $p < 0.001$  level) among the substrate media (Table 5).

Table 5

Statistical differences in growth performances of the newly emerged plants of *Cyperus rotundus* two weeks after propagules were planted. Four different types of propagules were grown in different substrate media. Letters qualitatively (a>b>c) indicate row-wise statistical differences in performance among media for each propagule type at  $p < 0.05$  level ( $n = 60$ ).

Propagule Type	Propagules grown in water	Propagules grown in coir dust and sand	Secto treated and grown in coir dust and sand	IBA treated and grown in coir dust and sand
<b>Mean plant height</b>				
Full tuber with out mother Plant	c	a	bc	b
Full tuber with mother Plant	b	a	a	a
Tuber cuttings (discs) with a bud	b	a	b	a
Tuber cuttings (discs) without a visible bud	b	a	ab	a
<b>Mean number of leaves per plant</b>				
Full tuber with out mother Plant	d	c	b	a
Full tuber with mother Plant	b	b	a	a
Tuber cuttings (discs) with a bud	b	a	b	b
Tuber cuttings (discs) without a visible bud	ab	a	b	b
<b>Average leaf length</b>				
Full tuber with out mother Plant	c	a	b	a
Full tuber with mother Plant	c	b	a	a
Tuber cuttings (discs) with a bud	b	a	ab	ab
Tuber cuttings (discs) without a visible bud	b	a	ab	ab
<b>Mean Leaf biomass</b>				
Full tuber without mother Plant	b	a	b	ab
Full tuber with mother Plant	b	b	a	a
Tuber cuttings (discs) with a bud	b	a	ab	b
Tuber cuttings (discs) without a visible bud	a	a	a	a

## DISCUSSION

The results demonstrate that the percentage sprouting and mean number of sprouted buds from all four propagule types were high when grown in water, but subsequent growth (in terms of plant height, leaf number and length and biomass) was relatively poor in water. When full tubers were placed in water, all the conditions favorable for bud formation were met except for the full supply of nutrients. However, nutrients are required from the substrate and at the same time

the substrate also anchors the growing plant. Adequate levels of all nutrients are not present in water unless they are supplemented. Hence, it can be assumed that food resources in the tubers are solely made available for the newly emerged plants to grow. A combination of all these factors may be responsible in retarding the subsequent growth of *C. rotundus* plants in water.

According to Salisbury and Ross (1985) potato tubers (underground stem), exhibit stem characteristics such as 'eyes' (axillary buds). These buds remain inactive in response to the presence of the apical bud. When the apical shoot of the potato is removed to produce seed pieces (propagules), the apical dominance is lost and new shoots emerge from the axillary buds. Similar to potato tubers, axillary buds of *C. rotundus* tubers grow when the tubers are cut in to discs. The principle of the sprouting mechanism of *C. rotundus* tuber cuttings (discs) is also very much similar to this phenomenon.

In general, percentage sprouting and bud formation are low when all the propagule types were treated with hormones (Secto and IBA) before they were transplanted to the medium comprising coir dust and sand in equal proportions. However, the subsequent growth performance is high in these treatments.

The use of synthetic auxins to induce root formation on cuttings has now been a standard practice in horticulture for over 40 years (Luckwill, 1981). Salisbury and Ross (1985) have reported that, similar compounds synthesized artificially could also cause many physiological responses. As in many other instances reported for *Alnus nitida* Endl., *Tectona grandis* Linn.f., *Glycine max*, *Artocarpus heterophyllus* Lam., *Quercus leucotrichophora*, *Cerbera manghas*, *Pelargonium graveolens*, (Singh and Singh, 1996; Kajornsrichon *et al.*, 1998; Liu *et al.*, 1998; Sengupta and Thakur, 2000; Sushma *et al.*, 2000; and Thatoi *et al.*, 2000) *C. rotundus* tubers too, positively responded to NAA and IBA.

However, in this study full tubers, compared to tuber cuttings (discs), of *C. rotundus* treated with hormones showed a high percentage sprouting. In the small tuber cuttings, the external application of hormones might have induced an inhibitory effect due to ethylene formation as reported for pea and sunflower by Burg and Burg (1966). Ethylene in turn causes the swelling and inhibition of the growth of subsequent tissues.

The number of buds that emerged from propagules grown in water was higher than that in coir dust and sand. This may be due to the marginally lower pH (6.84) value of the medium compared to that in water (pH 7.2). However, the subsequent better performance in coir dust and sand may be mainly due to the provision of a suitable medium for anchorage of the plant. Also the coir dust might have also provided supplementary nutrients required for these plants.

The plantlets that emerged from propagules treated with Secto and IBA hormones and subsequently placed on coir and sand medium showed much better performance than those grown in water medium. Taiz and Ziegler (1998) have reported that the cell walls of auxin treated plants have greater capacity for growth

in acidic media than in neutral media. A similar phenomenon may be attributed to the enhanced growth of plants raised from auxin treated propagules raised in coir dust and sand medium, where pH is slightly low (pH= 6.4) due to the presence of organic acids in coir dust.

From these findings, we can conclude that the best method to obtain a large number of *C. rotundus* plants is by using full tubers without the mother plant. Percentage sprouting and number of buds that emerged from this propagule type were high when water was used as the growth medium. However, the growth performance of plants was low when they were allowed to grow for longer periods in the same water medium. Therefore, we recommend that, the newly emerged plants should be transplanted in to a medium such as coir dust and sand mixed in equal proportions. According to results of our ongoing experiments (results not shown here) preliminary plants with a height of about 9 cm or above is the best stage for subsequent transplanting in to the growth medium. However, in case of a short supply of tubers, tuber cuttings (discs) with or without a bud could be used as propagules to obtain a large number of plants over a short period of time.

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