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Clonal Propagation of Lemon Grass (*Cymbopogon citratus* STAPF) through Shoot Tip Culture

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A tissue culture method for rapid clonal propagation of lemon grass (*Cymbopogon citratus*) is described. Shoot cultures were induced by culturing small excised shoot apices on the LS basal medium supplemented with 0.1 μM NAA and 10 μM BA. Multiple shoots were divided and cultured on a basal medium containing 1 μM NAA for rooting. The regenerated plantlets were successfully transferred to soil.

Lemon grass (*Cymbopogon citratus* STAPF), a perennial herb belonging to Gramineae, has been widely cultivated in tropical and subtropical countries to prepare lemon grass oil, which contains citral as a major essential oil component and is used as a flavoring.[1] Although the propagation of lemon grass has been conventionally carried out by suckering, this method is time-consuming and the rate of propagation is rather low. Rapid propagation *in vitro* could alleviate this problem.

Tabata *et al.*[2] reported the regeneration of lemon grass from callus cultures. However, it has been well recognized that plant cells grown in culture undergo extensive genetic changes and that variant traits may sometimes appear among the plants regenerated from cultured cells.[3] Here we describe the micropropagation of lemon grass by shoot tip culture, which has been considered more suitable for clonal propagation of plants with genetic stability.

Materials and Methods

Linsmaier and Skoog (LS) medium[4] containing 3% sucrose and 0.9% agar was used as a basal medium throughout the present investigation. Silicone-sponge plugged test tubes (30×125 mm) containing 20 ml of medium were used for multiple shoot formation and rooting of shoots. All the cultures were incubated at 25°C in a photoperiod of 16 hr/day of fluoresent light (about 3,000 lx).

Shoot tips were isolated from the field-grown plants and surface sterilized successively in ethanol (quick dip) and in 2% solium hypochlorite solution for 10 min. Then, shoot meristems of about 0.5 mm size along with leaf primordia were excised aseptically under a stereoscope and immediately placed onto the culture medium.

The propagated shoots were divided into 5-6 pieces, which were inoculated on agar media containing various kinds and concentrations of auxins for rooting. The rooted plantlets thus obtained were transferred to potting mixtures and cultivated for two months in the greenhouse before transplanted to the field.

Results and Discussion

Induction of multiple shoots

We examined combinations of 1-naphthaleneacetic acid (NAA ; 0.1, 1.0 μM) and either benzyladenine (BA ; 10 μM) or kinetin (10 μM) to find suitable hormonal conditions for induction of multiple shoot formation from the shoot meristems (**Table 1**). When 30 shoot meristem explants were cultured on

Table 1. Effects of NAA and cytokinin combinations on shoot formation from shoot meristems of lemon grass.

NAA (μM)	Cytokinin (μM)		Number of meristems cultured	Number of meristems regenerating shoots		
				Single shoot	Multiple shoot	Total
0	BA[1]	10	30	2	3	5(17)[2]
0.1		10	30	2	14	16(53)
1.0		10	30	3	2	5(17)
0	K[1]	10	30	3	1	4(13)
0.1		10	30	2	5	7(23)
1.0		10	30	3	3	6(20)

1) BA=Benzyladenine; K=Kinetin.
2) % of the meristem explants regenerating shoots.

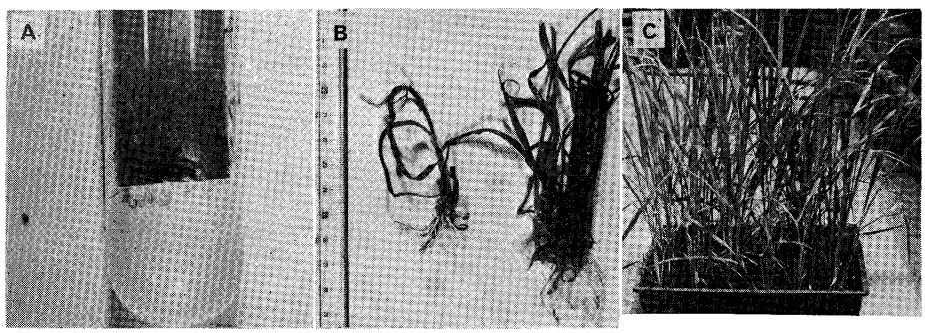

Fig. 1. (A) Multiple shoots regenerated from shoot meristems cultured for one month on basal medium supplemented with 0.1 μM NAA and 10 μM BA. (B) Rooted shoots two months after inoculation of shoots on basal medium containing 1.0 μM NAA. (C) Potted plantlets acclimatized for two months in the greenhouse.

medium containing 0.1 μM NAA and 10 μM BA, 14 explants developed multiple shoots (**Fig. 1 A**) and two explants developed single shoots within one month. This hormonal combination was the most productive of multiple shoots and used throughout the remainder of these experiments. The multiple shoots were divided into five to six pieces, each of which developed multiple shoots again within four weeks when cultured on the same medium. The capability to develop multiple shoots has remained high for at least six months after shoot tip culture initiation.

Rooting of shoots

Various auxins (0.1 and 1.0 μM) were evaluated for their ability to stimulate root formation from

Table 2. Effects of auxins on rooting of the shoots.[1]

Auxin	Conc. (μM)	No. of shoots cultured	No. of rooted shoots	No. of roots per shoot
None	—	9	5	17.0±9.1[2]
NAA	0.1	9	6	10.2±1.8
	1.0	9	9	9.4±2.0
IAA	0.1	10	7	9.1±2.4
	1.0	10	9	8.3±2.5
IBA	0.1	10	6	4.7±1.4
	1.0	10	7	6.1±2.3

1) Assessment was made two months after the initiation of root induction.
2) mean±standard error.

shoots. As shown in **Table 2**, shoots rooted effectively when 1 μM NAA or IAA was added to the LS basal medium (**Fig. 1 B**). Indole-3-butyric acid (IBA) was less effective for rooting when compared with NAA or IAA. When NAA was added to the culture medium, root formation occurred within four weeks, however, it took six to eight weeks for rooting of the shoots when other auxins were used. Considering these factors we chose 1.0 μM NAA as the growth regulator for the rooting medium.

Transplantation to soil

Plantlets cultured on a medium containing 1 μM NAA for about one month were transferred to potting mixtures (volcanic ash soil-compost, 1 : 1). They were cultivated in the greenhouse with thin transparent plastic coverings for one week and without coverings for another seven weeks (**Fig. 1 C**). More than 90% of the rooted plantlets survived after transplantation to soil and grew to maturity when transferred to the field.

Characteristics of the *in vitro* propagated plants such as chromosome numbers, isozyme banding patterns and essential oil compositions are now under investigation.

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≪和文要約≫

茎頂培養法によるレモングラス（*Cymbopogon citraus* STAPF）のクローン増殖法

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組織培養法によるレモングラス（*Cymbopogon citratus*）の迅速なクローン繁殖について報告する．レモングラスの茎頂を摘出し，0.1 μM の NAA と 10 μM の BA を含む LS 培地を用いて約1カ月間培養することにより，multiple shoot を得た．これを分割し，同じ培地に移植して培養することにより容易に再び multiple shoot を発生した．また，この shoot を分割後，1 μM の NAA を含む基本培地に移植し約1カ月間培養することにより，容易に発根させることが可能である．再分化した幼植物は土壌に移植し，1～2カ月間温室内で馴化後，圃場に定植することにより生育させることができた．