Kasetsart J. (Nat. Sci.) 35 : 361 - 367 (2001)

Micropropagation of Kluai Khai (*Musa acuminata* 'Klaui Khai') Using Sword Suckers and Inflorescences at Various Development Stages

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ABSTRACT

Comparison the use of inflorescence at various stages of development versus meristem of sword suckers of Kluai Khai, as a source of the primary explants for *in vitro* culture was conducted . For shoot initiation, the explants were cultured on semi solid and liquid medium of Murashige and Skoog (1962) supplemented with 5 ppm. of 6-benzylaminopurine (BA). It was found that on liquid medium, the culture produced the first shoot faster than the ones on semi solid medium. The first shoot of suckers occurred on the 30th and 45th days after culturing on liquid medium, and semi solid medium respectively, while those at the 5th, 3thand 1st days of developmental stage occurred on the 52nd, 52nd and 56th days of culturing respectively. After 2 months, the sucker on liquid medium was noticed to give the shoot number of 1.88 shoots per explant, where as on semi solid medium, it yielded 0.6 shoot per explant. The shoot number of inflorescence at the 5th, 3rd and 1st days of developmental stage cultured on liquid medium were 2.08 (the highest), 1.93 and 1 shoots per explant respectively. At this stage, no development of the shoots on semi-solid medium had been found to develop yet. Shoot multiplication was also revealed to be stimulated by transferring explants into semi solid medium, thus yielding such higher shoot number than the others. On the 5th month, the inflorescence at the 5th day of developmental stage gave the highest shoot number of 9.06 shoots per explant which was not significantly different from those of 8.9 shoots per explant at the 3rd day of developmental stage. The shoot number of the others were significantly lower different. 10% off-type shoots, occurred in inflorescence culture. After transplanting plantlets to the nursery, the survival rate was 96 percent.

Key words: banana, Kluai Khai, micropropagation, sword sucker, inflorescence

INTRODUCTION

The most cultured of all plant species just about any part of the plant body taken at any time of development has successfully produced somatic embryo in culture. Normally in banana, sucker has been used as explants for micropropagation as well as growing in the field. Sword sucker is most suitable for propagation because of more food accumulated in rhizome.The media formulated by Murashige and Skooge (1962) provides good regeneration and good multiplication in many kinds of bananas such as: Cavendish, Gros Michel, and many AA Group of bananas (Cronauer and Krikorian, 1984; Keawsompong, 1991; Atthachat, 1992; Ruangueng-Kajornlert, 1993, Tinsirisuk, 1994). The inisotol, thiamine HCl, sucrose and coconut milk are also added to the MS medium and

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good proliferation is obtained (Cronauer and Krikorian, 1985). Sometimes friable calli occur and develop into embryoid thereafter (Fitchet, 1987). Since K. Khai is also in AA Group, the MS semi solid medium with BA should be good for regeneration.

Aside from sucker, the flower bud or inflorescence of banana can be used as an explant as well (Fitchet, 1990). Doreswamy and Sahijram (1989) reported that, disease free planting material could be obtained from micropropagation of terminal male bud of Rasthali banana. MS medium (Murashige and Skooge, 1962) is the one suitable for regeneration. Growth regulator supplemented to MS medium is 2.5-5 ppm BA which is good for Cavendish banana flower bud (Fitchet, 1987; Balakushnamurthy and Sree Rangaswamy, 1988). For ABB Group of banana, the 2, 4-D, 2, 4,5-T or Kinetin are also added to the MS medium and it is found that, the vegetative stage can be induced after cultured 2-3 months (Ling *et al.*, 1990). In 8-10 weeks vegetative stage can be produced in Gros Michel banana flower bud, when cutured in MS medium with BAP and IBA (Murali and Duncan, 1991). Inflorescence of banana is composed of female flower at distal, male flower at the end and hermaphrodite flower at the middle (Simmonds, 1966). Flower or reproductive part, is generally proven to be an excellent source of embryogenic material (Litz and Conover, 1982; Krikorian and Kann, 1981). The age of inflorescence might be a good factor for shoot regeneration of banana.

The purpose of this study was to compare the meristem from sword sucker to various stages of inflorescence in semi solid and liquid MS media on proliferation and multiplication of Kluai Khai.

MATERIALS AND METHODS

Experiment 1 Proliferation

Sword suckers and inflorescences were used as explants for the experiment. Leaf sheaths and bracts were removed to 2X2 mm. size. The explants were sterilized 2 times in 10% chlorox for 15 min., then were rinsed in sterile distilled water 3 times. They were longitudinally cut into 4 pieces, every piece with apical meristem.

The explants from sword suckers, and 1, 3, 5 days old inflorescences, were cultured in liquid and semi solid MS (1962) supplemented with 5 ppm BA and pH at 5.8. The 4X2 factorial in CRD was designed with 4 treatments of explant, 20 replications and 2 factors. All cultures were maintained at 25-28°C with 1,500-2,000 Lux of light and 80-85%RH. The liquid medium treatments were shaken in the shaker at 100-120 cycle/min.

Observation was made on plant proliferation and number of shoots regenerated.

Experiment 2 Shoot multiplication

The shoots produced in experiment 1 were cultured in semi solid MS (1962) medium with 5 ppm BA. CRD was designed with 8 treatments and 20 replications. There were 4 treatments from liquid medium and 4 treatments from semi solid medium. All were incubated in 25-28°C with 1,500-2,000 Lux of light and at 80-85%RH.

Observation was made on number of shoots produced.

RESULTS AND DISCUSSIONS

Experiment 1

After culturing the explants of sword suckers and inflorescences of different ages on semi solid and liquid MS medium supplemented with 5 ppm BA for 2 months, it was found that the explants of suckers cultured on liquid medium had the shortest shoot developing time of 30 days. While the explants of 5, 3 and 1 days old of inflorescences cultured on liquid medium produced the first shoots at 52^{nd}, 52^{nd} and 56^{th} days respectively, those of 5, 3 and 1 days old inflorescences, and sword sucker on semi solid medium were found to have them at 130^{th}, 135^{th},$142^{th}$and 45^{th} days respectively (Table1).

The explants of suckers and inflorescences

of various ages on liquid medium were also noticed to grow and develop into shoots faster than those on semi solid medium. The explants of 5, 3 and 1 days old inflorescences and suckers cultured on liquid MS medium had the average shoot number of 2.08, 1.93, 1 and 1.88 respectively (Table 2, and Figure 1), whereas those of suckers and inflorescences on semi solid media developed into shoots in the 2nd and 4th months respectively (Figure 2).

Culturing of the explants in liquid medium being shaken enabled the entire tissue to obtain nutrient resulting in fast growth as well as reduced the concentration of phenolic compound, the toxic substance, released by the explants. In addition, shaking of liquid medium also provided more aeration, thus increasing O_2 (Thamasiri, 1999) which in turn enhanced metabolism of the explants of suckers and inflorescences. Such explants in liquid

Table 1 Proliferation of various explants in liquid and semi solid MS medium.

Explants	Proliferation time (days)	
	Liquid MS	Semi solid MS
Sword sucker	30	45
1 day old inflorescence	56	142
3 day old inflorescence	52	135
5 day old inflorescence	52	130

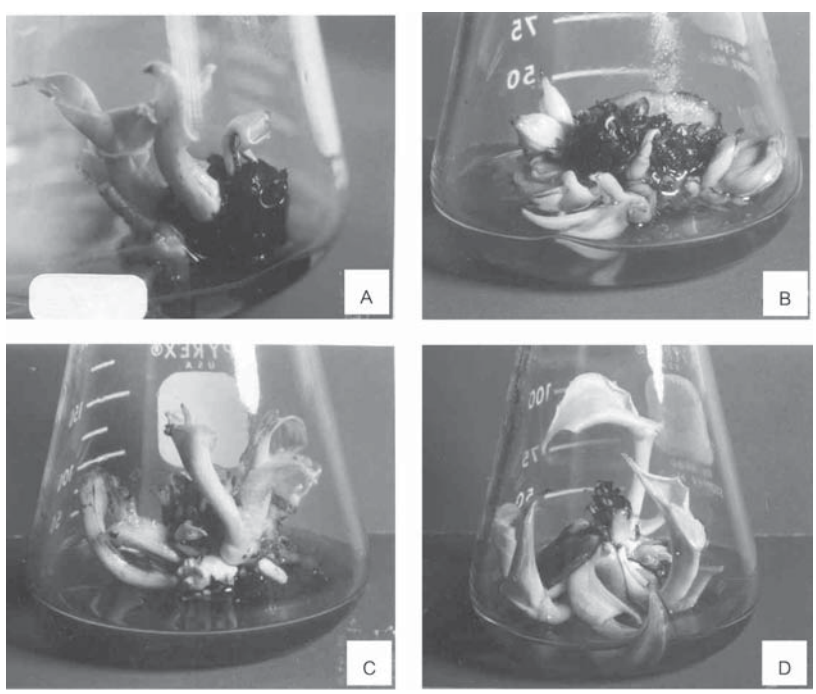

Figure 1 Number of shoots from sucker and various ages of inflorescences on MS liqiuid medium.
A. sucker after 30 days
B. 1 day old inflorescence after 60 days
C. 3 day old inflorescence after 60 days
C. 5 day old inflorescence after 60 days

medium, therefore, had greater development than the one cultured on semi solid medium alone. The continuous growth of the explants transferred to be cultured on semi solid medium agreed with the experimental result by Cronauer and Krikorian (1981) who studied the explants of Dwarf Cavendish inflorescences on liquid medium.

Experiment 2

After culturing, plantlets produced from suckers and inflorescences of different ages from experiment 1 on semi solid MS medium supplemented with 5 ppm BA for 5 months, it was found that all explants of suckers and inflorescences could produce shoots. The number of shoots from explants of suckers and inflorescences of various ages produced each month significantly differed from one another and had increasing shoot multiplication. The number of shoots from the explants of sucker and various ages inflorescences cultured on liquid medium were also noticed to be higher than those that did not go through culturing on liquid medium.

At the 5th month, the experiment revealed 5

Table 2 Number of shoots from various explants in liquid and semi solid medium 2 months after culturing.

Explants	Number of shoot	
	Liquid MS	Semi solid MS
Sword sucker	1.88$^{a1/}$	0.6^{c}
1 day old inflorescence	1.00^{b}	0^{c}
3 day old inflorescence	1.93^{a}	0^{c}
5 day old inflorescence	2.08^{a}	0^{c}

CV = 49.6

1/ The figures on the same row with the same letter showed no significant differences at 99% by DMRT.

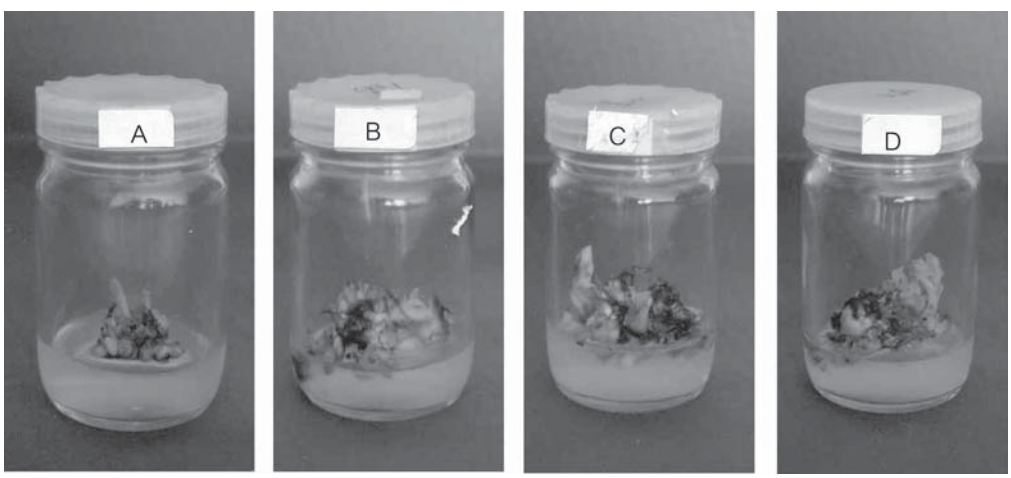

Figure 2 Number of shoots from suckers and various age of inflorescences on semi solid medium.
A. sucker after 30 days
B. 1 day old inflorescence after 90 days
C. 3 day old inflorescence after 90 days
D. 5 day old inflorescence after 90 days

days old explants of inflorescences on liquid medium to give the highest number of shoots. There were no significant differences between the number of shoots of 3 days old (8.9 shoots) and 5 days old (9.06 shoots) on liquid medium. It was also found that the 5 days old explants of inflorescences cultured on the semi solid medium produced the average number of shoot equally 8.74 shoots/explant. Those on liquid medium were not significantly different from the shoot number of 3 days old explants of inflorescences (7.99 shoots/explants) that did not go through culturing on liquid medium. For suckers and one day old inflorescences not cultured on liquid medium, the average number of shoot were significantly lower than the explants mentioned above with 7.14 and 7.17 shoots respectively (Table 3).

After the explants were transferred to semi solid MS supplemented with 5 ppm BA, the continuous increasing of shoots into the 5th month was revealed. Aside from these, 10% of abnormal shoots produced from explants of inflorescences were also found, such as, the water swollen shoots, curly leaves, abnormally long petioles, etc. (Figure 3). The result were similar to those of the result of Silayoi (1995) who report that a great variation of abnormalities as, dwarf plants or tapered leaves, occurred from banana flower bud propagation. However, such abnormalities were not encountered when the plantlets were transferred to grow in the greenhouse.

Comparing of the suckers and different ages explants of inflorescences induced to produce shoots in liquid MS medium, then transrfered to the previous formula of semi solid medium found the explants of older inflorescences to produce more shoots than

Figure 3 Abnormal shoots from inflorescence explants after cultured 7 months.

Table 3 Number of shoots produced per explant .

Explants	1st month	2nd month	3rd month	4th month	5th month
Liquid medium					
Sword sucker	1.68$^{c1/}$	2.89^{b}	5.48^{c}	6.5^{de}	8.28^{bc}
1 day old inflorescence	1.91^{bc}	2.57^{c}	6.43^{b}	7.24^{c}	7.90^{c}
3 day old inflorescence	2.31^{ab}	2.85^{b}	6.8^{b}	8.50^{a}	8.90^{a}
5 day old inflorescence	2.39^{a}	3.28^{a}	8.4^{a}	8.75^{a}	9.06^{a}
Semi solid medium					
Sword sucker	2.00^{abc}	3.16^{a}	4.08^{d}	6.03^{e}	7.14^{d}
1 day old inflorescence	0.85^{d}	1.25^{e}	2.54^{f}	6.29^{e}	7.17^{d}
3 day old inflorescence	1.20^{d}	1.80^{d}	2.84^{f}	6.91^{cd}	7.99^{c}
5 day old inflorescence	1.80^{c}	2.70^{bc}	3.24^{e}	7.93^{b}	8.74^{ab}
C.V. (%)	34.93	14.50	12.75	11.27	10.60

1/ The figures on the same row with the same letters showed no significances difference at 99% by DMRT.

those of young inflorescences. The results agreed with those of culturing sucker and various ages inflorescences on the same semi solid formula. The explants of inflorescence composing of flower buds readily to develop into flowers (reproductive stage) were also noticed to be able to change into vegetative stage and produced a great deal of shoots. The pattern of development depended on kind and concentration of growth regulators as well as plant genetics (Bakry *et al*., 1985). In addition, most culturing medium had high amount of N, compared to C, thus producing vegetative growth instead of flowering stage which was hard and slow (Tachapinyawat, 1993).

One day old inflorescences of banana would have only female flowers readily to develop into fruits while the older ones would have both male and female flowers. Therefore, the result of the experiment finding the old inflorescences to produce more number of shoots than the young ones indicated that the female flowers about to develop into fruits could never change into vegetative stage due to the occurence of one step further development into fruit (Figure 4). When the old inflorescences had more male than female flowers, they would then have a better chance to develop into plants than the young ones. This suggested the use of male flower inflorescences or male flower bud in aseptic culture in the future experiment.

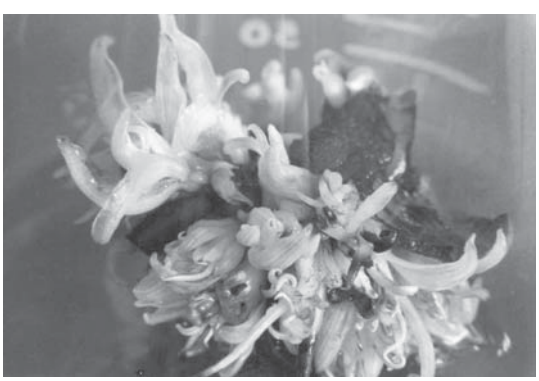

Figure 4 Development of female flower *in vitro*.

CONCLUSION

1 Sword sucker enables to regenerate shoot faster than inflorescence

2 5 days old inflorescence of K.Khai which is normally cut off (or the so called male bud), is good explant for tissue culture

3 Proliferation should be induced in liquid medium before transferred to semi solid medium since a greater number of shoots were produced in liquid medium than in semi solid one.

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Received date : 25/10/01
Accepted date : 06/12/01