Multiple Shoot Formation and Plantlet Regeneration from Cotyledonary Node in Peanut (*Arachis hypogaea* L.)

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Multiple shoots were formed on cotyledonary nodes of seedlings in one Virginia type and two Spanish type peanuts, *A. hypogaea*, on B5 medium supplemented with 6-benzylamino-purine (BAP) at 10 or 50mg/l. The frequency of multiple shoots formation varied among the cultivars tested. Histological observation showed that multiplication of buds was induced by the differentiation of axillary buds from initially emerged buds. Peanut plants were established from multiple shoots after rooting on the medium without growth regulators.

KEY WORDS: *Arachis hypogaea* L., multiple shoot, plant regeneration.

Introduction

Establishment of efficient method for plant regeneration is prerequisite for micropropagation of favorable germplasms and improving crops through unconventional breeding methods such as somaclonal variation, somatic hybridization and genetic transformation. In *Arachis hypogaea*, however, there has been little information on cell and tissue culture techniques such as callus induction and proliferation, control of organogenesis and embryogenesis, and regeneration of intact plants (HAZRA *et al.* 1989, MROGINSKI *et al.* 1981, NARASIMHULU and REDDY 1983, STILL *et al.* 1987).

In the present work, multiple shoot formation from cotyledonary node of cultivated peanuts is reported by using three leading varieties in Japan.

Materials and Methods

Three varieties of *A. hypogaea* L., one Virginia type, Chiba-Handachi and two spanish type, Java No. 13 and Hakuyu 7-3, were used. The seeds which had been stored at 5°C for 5 months after harvesting on 10 Nov. 1988, were surface-sterilized in sodium hypochlorite solution (1% active chlorine) with a drop of Tween 20 for 10 min and rinsed 3 times with sterilized water. These seeds were inoculated on the culture medium after removing seed coats. The culture medium contained the inorganic salts and vitamins of B5 medium (GAMBORG *et al.* 1968), 20g/l sucrose, 2g/l Gellan Gum (Kelco, Division of Merck & Co., Inc.) and BAP at 1, 10, or 50mg/l. The pH of the media was adjusted to 5.8 before autoclaving for 15 min at 121°C. Each culture flask (100ml wide mouth flask) contained one seed and at least 40 replicates were made for each treatment.

The cultures were first incubated at 25°C under dark condition for 7 days, and then transferred to the condition of continuous light of 60 $\mu\text{mol m}^{-2}\text{s}^{-1}$ provided by cool white

fluorescent tubes.

The multiple shoots raised from cotyledonary node were dissected from the explant and transferred onto B5 medium supplemented with zeatin (trans isomer) and gibberellin (GA₃) at 1mg/l respectively for shoot development. When the shoots had tetrafoliate leaves, they were transferred for rooting onto HYPONEX (2m/l, N:P:K=5:10:5, liquid type, HYPONEX Japan Licensee, Murakami Bussan Co., Inc.) medium containing 8g/l agar and no plant growth regulators.

For histological observation, some explants were fixed in FAA (70% ethanol:formalin:glacial acetic acid=90:5:5) after 20 days of culture, and then dehydrated in ethanol series and embedded in paraffin (56-58°C). Embedded tissues were cut into 10µm sections by rotary microtome and stained with 0.5% Toluidine blue O solution.

Results and Discussion

Table 1 summarized frequency of multiple shoot formation from cotyledonary node of mature seeds cultured on the medium supplemented with BAP in different concentrations. Without BAP, normal seedlings with a pair of primary branches from cotyledonary nodes were established after 10 days of culture. On the other hand, the growth of primary branches from cotyledonary node was inhibited on the medium with BAP at 1, 10 and 50mg/l. After 15 days of culture, the basal part of epicotyl enlarged (Fig. 1A) and small shoot-buds appeared at the cotyledonary node (Fig. 1B) on the media containing BAP at 10 or 50mg/l in Chiba-Handachi and Java No. 13, and at 50mg/l in Hakuyu 7-3. Large number of protrusions appeared on the enlarged epicotyl tissue within 25 days after inoculation (Fig. 1C), and subsequently they developed into multiple shoots (Fig. 1D). These shoots gave rise to tetrafoliate leaves 10 days after transfer onto B5 medium containing zeatin and GA₃ at 1mg/l respectively (Fig. 1E).

Shoot formation was also affected by the genotype. Spanish type varieties, Java No. 13 and Hakuyu 7-3, showed higher frequency of multiple shoot formation than Virginia type variety, Chiba-Handachi (Table 1). The same organogenic response had previously been observed in the culture of immature leaves (DAIMON and MII 1989).

Multiple shoot formation by application of cytokinin had been reported in some other legumes, such as *Glycine max* (CHENG *et al.* 1980, SAKA *et al.* 1980, WRIGHT *et al.* 1986), *Vigna radiata* (MATHEWS 1987), *Phaseolus vulgaris* (McCLEAN and GRAFTON 1989), and *Vicia faba* (SELVA *et al.* 1989). In the present experiment, BAP at 1, 10 and 50mg/l inhibited the growth of primary branches and induced lots of protrusions which later developed into numerous buds. These buds were newly formed at the cotyledonary node (Figs. 1A and 1B). Histological observation indicated that the buds were successively formed at the axillary region of initially formed buds (Fig. 1F). These results indicate that cotyledonary node region of *A. hypogaea* has high morphogenetic potential which could be activated by *in vitro* BAP application.

Root formation from the shoots was rather difficult and less than 20% of shoots produced roots on B5 medium containing no plant growth regulators or NAA 1mg/l. Rhizogenesis was improved on HYPONEX medium in which 40% of shoots produced roots 10 days after trans-

Table 1. Formation of multiple shoots on cotyledonary nodes of mature seeds in *A. hypogaea* cultured on B5 medium containing three levels of BAP for 40 days

Cultivars	BAP concentration (mg/l)	% of seedlings showing multiple shoot formation ¹⁾
Chiba-Handachi	0	0
	1	0
	10	20
	50	40
Java No. 13	0	0
	1	0
	10	30
	50	80
Hakuyu 7-3	0	0
	1	0
	10	0
	50	80

¹⁾ Data were obtained from each group consisting of at least 40 cultures.

fer. Shoots rooted are now growing in pots filled with soil containing small amounts of fertilizer under greenhouse conditions (Fig. 1H). However, the frequency of root formation was still low and remained to be considerable obstacle for propagation. Similar problems in root formation have been reported in *in vitro* regenerated shoots of *A. hypogaea* (KARTHA *et al.* 1981, MROGINSKI *et al.* 1981), *A. paraguariensis* (STILL *et al.* 1987) and the other legumes (TAJI and WILLIAMS 1987, WRIGHT *et al.* 1987).

In conclusion, efficiency of multiple shoot formation in mature seed of *A. hypogaea* established in the present experiment is satisfactory high but still unsatisfactory for micropropagation and for improving peanut through the selection of somaclonal variation due to the low efficiency of root formation. Further studies for improving rhizogenesis are now in progress.

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Fig. 1 A-H. Multiple shoot formation on cotyledonary node in *A. hypogaea*, cv. Java No. 13, cultured on B5 medium supplemented with BAP at 50 mg/l. A: Enlargement of basal part of epicotyl (a pair of cotyledons were removed to exhibit enlarged site). B: Emergence of shoot-buds at cotyledonary node. C: Formation of large numbers of protrusions. D: Formation of multiple shoots from protrusions. E: Development of tetrafoliate leaves in multiple shoot. F: Continual multiplication of buds in cotyledonary node. G: Rooting of shoot on the HYPONEX medium without growth regulators. H: Regeneration of plant from multiple shoot.

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ラッカセイにおける子葉節からのマルチプルシュートの誘導

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ラッカセイ (*Arachis* 属) において、細胞育種的な技術を用いて育種素材を作出するための基礎となる、器官分化の制御に関する知見を得るために、完熟種子の子葉節からのマルチプルシュートの誘導条件について検討した。

1988年11月に採種後、5℃で5カ月間貯蔵した栽培種 *A. hypogaea* (品種：千葉半立、ジャワ13号、白油7-3) の種子を1%次亜塩素酸ナトリウム溶液(含 Tween20)に10分間浸漬して表面殺菌し、滅菌水で3回洗浄後、種皮を取り除き、ベンジルアミノプリン(BAP)を0, 1, 10, 50 mg/l 添加したB5培地(しょ糖20 g/l, ジェランガム2 g/l)に置床した。各品種ともにBAP添加区において、培養開始後15日目頃から上胚軸の基部が肥大し始め(Fig. 1A), 25日目には10 mg/l および50 mg/l 添加区において、肥大した部位に小さな突起が生じた(Fig. 1B, C)。これらの突起は40日目には多くのシュート(マルチプルシュート)に発育した(Fig. 1D)。マルチプルシュートの形成率は、BAP 50 mg/l 添加区において高く、千葉半立で40%、ジャワ13号と白油7-3でそれぞれ80%であった(Table 1)。組織学的な観察から、これらのシュートは、培養初期に発育した子葉節分枝に新たに形成された腋芽が発育したものであることが示された(Fig. 1F)。

形成されたシュートをジベレリンとゼアチンをそれぞれ1 mg/l 添加したB5培地に移植すると、10日目には正常な複葉を展開した(Fig. 1E)。これらの発育したシュートを植物ホルモン無添加あるいはNAA 1mg/l を添加したB5培地に移植したところ、発根率は20%と低かったが、植物ホルモン無添加のハイポネックス培地に移植すると、移植後10日目には約40%のシュートが発根した(Fig. 1G)。培地中に十分な根が生長し、3~4葉が発育した幼植物体を瓶から取り出し市販の培土に移植して順化したところ、ほとんどの個体が活着した(Fig. 1H)。現在、温室内で生育中である。

以上のように、本研究では、代表的な栽培種において子葉節から高頻度でマルチプルシュートを誘導することができた。