รายงานผลการวิจัย
มหาวิทยาลัยแม่โจ้

เรื่อง การเพาะเลี้ยงอับละอองเรณูถั่วลิสง

ANTHER CULTURE OF PEANUT (*Arachis hypogaea* L.)

ได้รับงบประมาณ ประจำปี 2541

จำนวน 314,500 บาท

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วันที่ 30 เมษายน 2543

คำนิยม

คณะผู้วิจัยขอขอบคุณคณะกรรมการวิจัย และสำนักวิจัยและส่งเสริมวิชาการการเกษตร มหาวิทยาลัยแม่โจ้ จังหวัดเชียงใหม่ ที่ได้ให้การสนับสนุนทุนโครงการวิจัยประจำปี 2541 ทำให้โครงการวิจัยนี้สำเร็จลุล่วงตามเป้าหมาย

คณะผู้วิจัย

สารบัญ

สารบัญตาราง

การเพาะเลี้ยงอับละอองเรณูถั่วลิสง

ANTHER CULTURE OF PEANUT (*Arachis hypogaea* L.)

ศิริพร พงศ์ศุภสมิทธิ์ สมจิตต์ กิจรุ่งเรือง ชลิต พงศ์ศุภสมิทธิ์

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บทคัดย่อ

ทำการเพาะเลี้ยงอับละอองเรณู ที่มีการแบ่งเซลของละอองเรณูในระยะ uninucleate ของถั่วลิสงจำนวน 6 พันธุ์ในอาหารวุ้นที่แตกต่างกัน 4 สูตร พบว่าอาหารทั้ง 4 สูตรสามารถชักนำให้เกิดแคลลัสได้ไม่แตกต่างกัน และพันธุ์ขอนแก่น 60-4 มีการตอบสนองได้ดีกว่าอีก 5 พันธุ์ เมื่อทำการย้ายแคลลัสของทั้ง 6 พันธุ์ไปเพาะเลี้ยงในอาหารวุ้นชุดที่สองที่แตกต่างกันจำนวน 5 สูตรเพื่อชักนำให้เกิดยอดพบว่า อาหารสูตร N_6 + 2 mg L^{-1} IAA + 2 mg L^{-1} Kinetin + 2 mg L^{-1} GA_3 สามารถชักนำแคลลัสของถั่วลิสงจำนวน 3 พันธุ์ให้เกิดยอดได้ 20 เปอร์เซ็นต์

ABSTRACT

Two media experiments were conducted to induce shoot regeneration from the cultured anthers of the seven peanut cultivars. In the first experiment, the excised anthers at the uninucleate microspore stage were cultured on four different agar media. It was found that all four media were equal effective in inducing callus formation of all seven cultivars. While the cv. Konkan 60-4 was found to be more responsive than the other six cultivars. The analyses of variances for response of anther 30 days after cultured indicated the main effects

while the main effects of medium (A) were not significant. The analyses of variances for number of days to anther callusing and callus growth weight 30 days after cultured indicated the main effects of medium (A) and cultivar (B) were significant ($P \leq 0.01$) but the interaction effects (A x B) were not significant. While the main effects of medium (A) and cultivar (B) and interaction effects (A x B) for callus growth weight were not significant ($P > 0.05$). In the second experiment, the anther–derived calli of the seven cultivars were transferred to five different regeneration media. Twenty percents of the regenerated shoots were obtained from the anther-derived calli of the three cultivars cultured on N_6 + 2 mg L^{-} IAA + 2 mg L^{-} Kinetin + 2 mg L^{-} GA_3 medium.

Key words: anther culture, peanut, *Arachis*, media, uninucleate microspore

INTRODUCTION

The development of haploids and doubled haploid plants *via* anther culture, microspore, ovule or ovary culture has a number of practical advantages for plant improvement. These haploid or doubled haploid plants have been used in varietal development, genetic, mutation, genetic engineering, biochemical and physiological studies (Ferrie *et. al.*, 1995). Doubled haploid plants can be produced through spontaneous chromosome doubling *in vitro* (Chame *et. al.*, 1988; Chen and Beversdorf, 1992) or by colchicine treatment of the microspores, embryo (Loh and Ingram, 1983) or plants (Siebel and Pauls, 1989; Wong, 1989). Spontaneous chromosome doubling rate varies depending on species and genotype (Beversdorf *et. al.*, 1987).

Three breeding methods were compared in rice, anther culture, bulk method, and pedigree method. Among the three methods, anther culture was ideal for developing homozygous lines in the shortest time, i.e., in only four generations (Hu and Zeng, 1983). The doubled haploids produced from the F_1 by anther culture increase selection efficiency, especially when dominance variation is significant. In conventional breeding, early generation lines exhibit variation due to both additive and dominance effects whereas doubled haploid lines derived by anther culture of an F1 will show only additive variance; therefore, high

heritability can expected due to the elimination of dominance effects. Shen *et. al.*(1983) estimated that about 150 pollen plants derived from F_1 anthers instead of 4000-5000 F_2 plants would be sufficient for selection for desirable genotypes. By selection of doubled haploid plants from either an F1 hybrid or a promising segregant, desirable recombinants can be selected and no segregation in successive generations is expected.

Anther culture can save time, labor and money in achieving specific goals in plant breeding. A cost /benefit analysis in rice breeding made by Sanint *et. al.* (1992) indicated that anther culture could reduce the cost by 60,000 to 200,000 US$ per cultivar depending upon the genotype/ecosystem targeted.

The induction of haploid embryos from male or female gametophytic tissues/cells is still a poorly understood process even though embryos can be readily induced in some species, notably the *Brassicas*. The response is largely genotype independent and there are many species such as the legumes, which are recalcitrant. Even within responsive genotypes, there may be differences between individual plants, particularly for self-incompatible species (Farrie *et. al.*, 1995).

Production of haploids of *Arachis hypogaea L.* (2n=4x=40) would allow peanut breeders to release lines more quickly and screen for resistance to diseases more efficiently. The *Arachis hypogaea L.* has been reported to be one of the recalcitrant species in obtaining haploid plants from anther culture (Wilcox *et. al.*, 1991). The only successful production of haploids of *A. hypogaea* L was reported by Bajaj *et. al.* (1980, 1981), however, the frequency at which haploids were obtained was not given in that report nor have any follow–up studies been reported (Wilcox *et. al.* ,1991). The effects of stage of microspore development and culturing media on androgenic response in Virginia-type peanut (*A. hypogaea* L.), PI 109839, were studied (Wilcox *et. al.*, 1991). The early uni-nucleate microspore stage was identified as producing the highest anther response rating and the best medium tested was N_6 basal medium with 1 mg L^{-} NAA, 0.1 mg L^{-} BA, 5.5% sucrose, and 3.5 g L^{-} glutamine. However, no regenerated plants were obtained from the experiment (Wilcox *et. al.*, 1991).

Therefore, it appears that the efficient regeneration procedures are still not available for use with peanut anther culture. Many factors influence androgenesis, including plant genotype, physiological state of the parent plant, pollen stage, pretreatment of flower buds,

and culture medium (Maheshwari *et.al.*,1983). The current study was aimed to test the effects of the culturing media on anther callusing and shoot regeneration.

MATERIALS AND METHODS

Seeds of seven peanut cultivars; Tainan 9, Konkan- 60-1, Konkan 60-2, Konkan 60-3, Konkan 60-4, Konkan 4 and MK 72-2, were grown in the 12 inches under the field condition during April–August 2000 at Department of Agronomy, Maejo University. Twenty-five young flower buds from each cultivar, 2-4 mm long, were collected from the mother plants during the first two weeks of flowering. Five flower buds from each cultivar were fixed in acetic acid: alcohol (1:3 v/v) and studied after staining with the Modified Feulgen for the anther development stage. Another twenty flower buds from each cultivar were surface sterilized with 5 % of sodium hypochlorite for 3 minutes and rinsed with sterilized distilled water for three times. Two media experiments were conducted. In the first experiment, four media, MS + 4 mg L^{-} IAA + 2 mg L^{-} Kinetin , MS + 1 mg L^{-} NAA + 2 mg L^{-} BAP , N_6 + 4 mg L^{-} IAA + 2 mg L^{-} Kinetin , N_6 + 1 mg L^{-} NAA + 0.1 mg L^{-} BAP + 5.5% sucrose + 3.5 g/L^{-} L-glutamine, were tested. The media were solidified with 8gL^{-}. The anthers were then excised from the flower buds after surface sterilized and each oblong anther was placed on each media in a completely randomized design with 25 replications. Cultures were incubated at 25$\pm$2 °C with 16-h photoperiod.

In the second media experiment, five shoot regeneration media were tested. The media consisted of MS + 1 mg L^{-} NAA + 2 mg L^{-} BAP, MS + 1 mg L^{-} NAA + 2 mg L^{-} BAP + 2 mg $L^{-}GA_3$, N_6 + 1 mg L^{-} NAA + 2 mg L^{-} BAP, N_6 + 2 mg L^{-} IAA + 2 mg L^{-} Kinetin + 2 mg L^{-} GA_3 and N_6 + 2 mg L^{-} IAA + 2 mg L^{-}Kinetin + 2 mg L^{-} BAP. After 30 days, the anther-derived calli from each cultivar from the first media experiment were transferred to five different shoot regeneration media in a completely randomized design with 3-12 replications depending on the number of the anther derived calli from each cultivar. Cultures were incubated at 25$\pm$ 2 ° C with 16-h photoperiod.

Analyses of variances were conducted for response of anther 30 days after culture, number of days to anther callusing, callus growth weight 30 days after culture and days to first shoot regenerated from callus after transferred to the regeneration media. Duncan Multiple Range tests were used for mean separation ($P \leq 0.05$).

For the statistical analysis, the following rating scales were used for the response of cultured anthers:

Cultured anther callusing = 1

Cultured anther not callusing = 0

RESULTS

The microscopic studies of the excised anthers from the young flower buds, 2-4 mm long, of all seven cultivars in the present study showed the microspore development at the uninucleate stage (Fig 1).

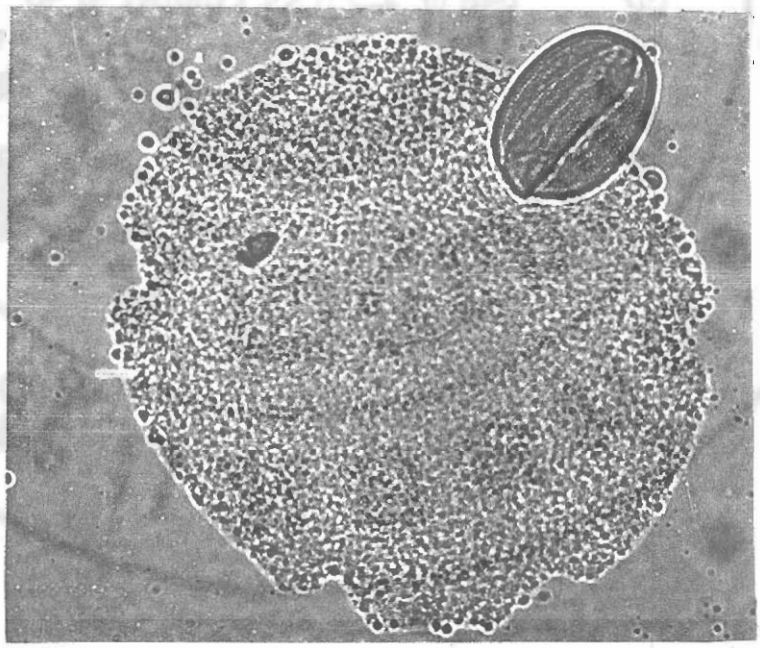

Fig. 1 Pollen grain (microspore) at the uninucleate stage.

The excised anthers of all seven cultivars cultured at the uninucleate stage on four different media formed callusing with the percentages of anther callusing ranging from 12.0% to 60.0% (Table 1). The *cv.* MK 72-2 cultured on N_6 + 1 mg L^{-} NAA + 0.1 mg L^{-} BAP + 5.5% sucrose + 3.5 g/L^{-} L-glutamine gave the lowest percentage of anther callusing (12%) while the *cv.* Konkan 60-2 cultured on N_6 + 4 mg L^{-} IAA + 2 mg L^{-} Kinetin gave the highest percentage of anther callusing (60%) (Table 1).

Table 1 Response of excised anthers of seven cultivars cultured at the uninucleate pollen stage on four different media

Factor		No. of cultured anthers			No. of callusing	Percentage of callusing
A (Medium)	B (Cultivar)	Total (1)	Contaminated (2)	Clean(3)	anthers (4)	anthers (5) = (4)/(3) x100 (%)
A1 = MS + IAA(4mg/L) + Kinetin(2 mg/l)	B1= Tainan 9	25	2	23	5	21.7
	B2 = Konkan 60-1	25	1	24	11	45.8
	B3 = Konkan 60-2	25	5	20	3	15.0
	B4 = Konkan 60-3	25	4	21	4	19.0
	B5 = Konkan 60-4	25	1	24	14	58.3
	B6 = Konkan 4	25	2	23	13	56.5
	B7 = MK 72-2	25	1	24	3	12.5
A2 = MS + NAA(1mg/l) + BAP (2mg/l)	B1= Tainan 9	25	0	25	12	48.0
	B2 = Konkan 60-1	25	2	23	8	34.8
	B3 = Konkan 60-2	25	4	21	7	33.3
	B4 = Konkan 60-3	25	1	24	4	16.7
	B5 = Konkan 60-4	25	0	25	12	48.0
	B6 = Konkan 4	25	0	25	7	28.0
	B7 = MK 72-2	25	1	24	5	20.8

A3 = N6 + IAA(4mg/l) + Kinetin(2 mg/l)	B1= Tainan 9	25	0	25	9	36.0
	B2 = Konkan 60-1	25	3	22	9	40.9
	B3 = Konkan 60-2	25	5	20	12	60.0
	B4 = Konkan 60-3	25	0	25	8	32.0
A3 = N6 + IAA(4mg/l) + Kinetin(2 mg/l)	B5 = Konkan 60-4	25	1	24	14	58.3
	B6 = Konkan 4	25	2	23	11	47.8
	B7 = MK 72-2	25	3	22	7	31.8
A4 = N6 + NAA(1mg/l) + BAP(2 mg/l) + 5.5 % sucrose + L-glutamine (3.5 g/L)	B1= Tainan 9	25	2	23	5	21.7
	B2 = Konkan 60-1	25	6	19	6	31.6
	B3 = Konkan 60-2	25	4	21	11	52.4
	B4 = Konkan 60-3	25	0	25	11	44.0
	B5 = Konkan 60-4	25	0	25	6	24.0
	B6 = Konkan 4	25	6	19	8	42.1
	B7 = MK 72-2	25	0	25	3	12.0
Total		700	56	644	228	Mean = 35.4

The analyses of variances for response of anther 30 days after cultured indicated the main effects of cultivar(B) and the interaction effects of medium x cultivar (AxB) were significant ($P \leq 0.01$) while the main effects of medium (A) were not significant (Table 2). The mean for response of anther 30 days after culture for Konkan 60-4 was 0.46 which was significantly higher than that for MK 72-2(0.22) but not significantly higher than those for Konkan 4 (0.42), Konkan 60-3(0.37), Konkan 60-2 (0.37), Konkan 60-1 (0.33) and Tainan 9 (0.32) (Table 3). The mean for response of anther 30 days after cultured for Konkan 60-3 cultured on MS + 4 mg L^{-} IAA + 2 mg L^{-} Kinetin (0.56) and Konkan 60-4 cultured on N_6 + 4 mg L^{-} IAA + 2 mg L^{-} Kinetin (0.56) were significantly higher than those for Tainan 9 (0.20) and Mk72-2 (0.16) cultured on N_6 + 1 mg L^{-} NAA + 0.1 mg L^{-} BAP + 5.5% sucrose + 3.5 g/L^{-} L-glutamine and Konkan 60-4 cultured on MS + 1 mg L^{-} NAA + 2 mg L^{-} BAP (0.12) but not significantly higher than those for the others (Table 3).

The analyses of variances for number of days to anther callusing and callus growth weight 30 days after cultured indicated the main effects of medium (A) and cultivar (B) were significant ($P \leq 0.01$) but the interaction effects (A x B) were not significant. While the main effects of medium (A) and cultivar (B) and the interaction effects (A x B) for callus growth weight were not significant ($P > 0.05$)(Table 2). The mean for number of days to anther callusing for N_6 + 4 mg L^{-} IAA + 2 mg L^{-} Kinetin (8.8 days) was significantly faster than that for N_6 + 1 mg L^{-} NAA + 0.1 mg L^{-} BAP + 5.5% sucrose + 3.5 g/L^{-} L-glutamine (11.1days) but not significantly faster than those for MS + 4 mg L^{-} IAA + 2 mg L^{-} Kinetin (9.6 days) and MS + 1 mg L^{-} NAA + 2 mg L^{-} BAP A(9.7 days). The mean for number of days to anther callusing for Konkan 60-4 (8.1days) was significantly faster than those for MK 72-2(10.5 days), Konkan 60-1 (10.5 days), Tainan 9 (11.3 days) and Konkan10-3 (11.7 days) but not significantly faster than that for Konkan 4 (9.4 days) (Table 3).

Table 2 ANOV of response of excised anthers 30 days after cultured , number of days to anther callusing and callus growth weight 30 days after cultured on 4 different media of 7 peanut cultivars

Source	DF	Response of anthers 30 days after cultured			No. of days to anther callusing (days)			Callus growth weight 30 days after cultured (gm)		
		SS	MS	F value	SS	MS	F value	SS	MS	F value
A (Medium)	3	1.044	0.348	1.62ns	174.49	58.16	6.32**	0.246	0.082	2.3 ns
B (Cultivar)	6	4.149	0.691	3.22**	411.39	68.57	7.45**	0.440	0.073	2.06 ns
A*B	18	7.806	0.434	2.02**	193.18	10.73	1.17ns	0.982	0.055	1.54 ns

** Significantly different at P≤ 0.01, n s = Not significant (P>0.05)

Table 3 Mean of response of excised anthers 30 days after cultured, number of days to anther callusing and callus growth weight 30 days after cultured on 4 different media of 7 peanut cultivars

Factor		Response of excised anthers	No. of days to anther	Callus growth weight 30
A (Medium)	B (Cultivar)	30 days after cultured #	callusing (days) #	days after cultured(gm)
A1=MS + IAA(4mg/L) + Kinetin(2 mg/l)	-	0.30	9.6 b	0.35
A2 = MS + NAA(1mg/l) + BAP (2mg/l)	-	0.33	9.7 b	0.38
A 3=N6 + IAA(4mg/l) + Kinetin(2 mg/l)	-	0.41	8.8 b	0.45
A4=N6 + NAA(1mg/l) + BAP(2 mg/l)	-	0.33	11.1 a	0.39
-	B1= Tainan 9	0.32 abc	11.3 a	0.33
-	B2 = Konkan 60-1	0.33 abc	10.5 ab	0.43
-	B3 = Konkan 60-2	0.37 ab	8.2 c	0.43
-	B4 = Konkan 60-3	0.37 ab	11.7 a	0.41
-	B5 = Konkan 60-4	0.46 a	8.1 c	0.45
-	B6 = Konkan 4	0.42 a	9.4 bc	0.37
-	B7 = MK 72-2	0.22 c	10.5 ab	0.34
A1 = MS + IAA(4mg/L) + Kinetin(2 mg/l)	B1= Tainan 9	0.20 bcd	11.8	0.25
	B2 = Konkan 60-1	0.40 abcd	10.3	0.37
	B3 = Konkan 60-2	0.12 d	8.0	0.28
	B4 = Konkan 60-3	0.16 cd	11.3	0.35
	B5 = Konkan 60-4	0.56 a	8.1	0.43
	B6 = Konkan 4	0.52 ab	9.2	0.32
	B7 = MK 72-2	0.16 cd	11.5	0.25
A2 = MS + NAA(1mg/l) + BAP (2mg/l)	B1= Tainan 9	0.52 ab	11.2	0.32
	B2 = Konkan 60-1	0.32 abcd	10.8	0.51
	B3 = Konkan 60-2	0.40 abcd	8.1	0.38
	B4 = Konkan 60-3	0.12 d	12.5	0.53
	B5 = Konkan 60-4	0.44 abcd	7.7	0.32
	B6 = Konkan 4	0.28 abcd	8.9	0.44
	B7 = MK 72-2	0.24 abcd	10.0	0.30
A3 =	B1= Tainan 9	0.36 abcd	9.8	0.37
	B2 = Konkan 60-1	0.36 abcd	11.0	0.41

N6 + IAA(4mg/l) + Kinetin (2 mg/l)	B3 = Konkan 60-2	0.48 abc	6.3	0.42
	B4 = Konkan 60-3	0.32 abcd	12.6	0.33
A3 =	B5 = Konkan 60-4	0.56 a	7.4	0.62
A 3 = N6 + IAA(4mg/l) + Kinetin (2 mg/l)	B6 = Konkan 4	0.44 abcd	7.6	0.47
	B7 = MK 72-2	0.32 abcd	9.1	0.40
	B1 = Tainan 9	0.20 bcd	13.4	0.33
	B2 = Konkan 60-1	0.24 abcd	9.7	0.44
A4 =	B3 = Konkan 60-2	0.48 abc	10.3	0.47
N6 + NAA(1mg/l) + BAP (2 mg/l) +	B4 = Konkan 60-3	0.48 abc	11.0	0.44
5.5 % sucrose + L-glutamine(3.5g/L)	B5 = Konkan 60-4	0.28 abcd	10.3	0.34
	B6 = Konkan 4	0.44 abcd	11.8	0.30
	B7 = MK 72-2	0.16 cd	13.0	0.31

Duncan 's Multiple Range Tests were used for mean separation at $P \leq 0.05$

Analyses of variances for days to first shoot regenerated from callus after transferred to the shoot regeneration media could not be conducted because only three out of 35 treatment combinations regenerated shoots and only one replication per treatment combination regenerated shoot.

Twenty percents of anther-derived calli regenerated shoot after transferred to the second media were observed from three cultivars, Tainan 9, Konkan 60-1, Konkan 60-2, culturing on N_6 + 2 mg L^{-} IAA + 2 mg L^{-} Kinetin + 2 mg L^{-} GA_3 medium(Table 4) (Fig.2). Days to first shoot regenerated from anther-derived callus ranged from 37-72 days. The anther-derived callus from Konkan 60-2 regenerated shoot within 37 days while the calli from Tainan 9 and Konkan 60-1 regenerated shoot after transferred to the second media within 66 and 72 days respectively (Table 4).

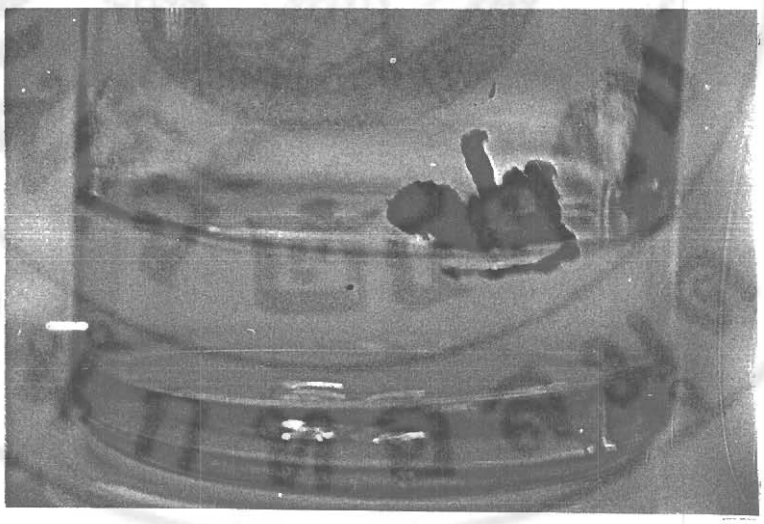

Fig. 2 Regenerated shoot from anther-derived callus.

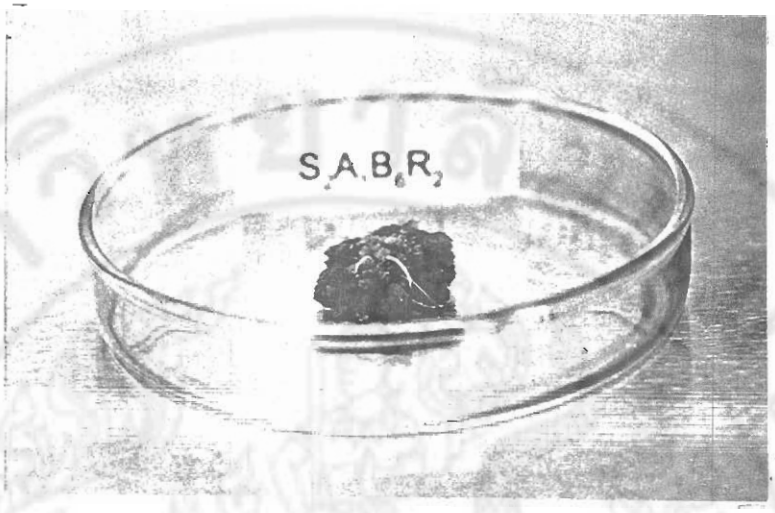

Fig. 3 Roots produced from anther-derived callus.

Roots (Fig.3) were produced from the anther-derived calli after transferred to the second media from four treatment combinations with the percentages raging from 10% to 42.85%. The calli from Konkan 60-3 cultured on MS + 1 mg L^{-} NAA + 2 mg L^{-} BAP and Konkan 4 cultured on MS + 1 mg L^{-} NAA + 2 mg L^{-} BAP + 2 mg $L^{-}GA_3$ gave the highest percentage of calli produced roots (42.85%). While 12.5 % and 10 % were observed from the Konkan 60-4 calli cultured on two different induction media, N_6 + 1 mg L^{-} NAA + 2 mg L^{-} BAP and MS + 1 mg L^{-} NAA + 2 mg L^{-} BAP + 2 mg $L^{-}GA_3$, respectively (Table 4). Days to first root regenerated from the anther-derived calli after transferred to the second media ranged from 48-70 days (Table 4).

Table 4 Regeneration ability of anther derived calli of 7 peanut cultivars after transferred to five different shoot induction media

Factor		Total number	Number of calli	Percentage of calli	Days to first shoot	No. of regenerated	Number of calli	Pecentage of calli	Days to first root	No. of roots
S (Medium)	B (Cultivar)	of cultured calli	regenerated shoot	regenerated shoot(%)	regenerated from callus	shoots per callus	regenerated root	regenerated root(%)	regenerated from callus	per callus
S1 = MS + NAA(1mg/L) + BA(2 mg/l)	B1= Tainan 9	7	-	-	-	-	-	-	-	-
	B2 = Konkan 60-1	8	-	-	-	-	-	-	-	-
	B3 = Konkan 60-2	9	-	-	-	-	-	-	-	-
	B4 = Konkan 60-3	7	-	-	-	-	3	42.85	57	2
	B5 = Konkan 60-4	12	-	-	-	-	-	-	-	-
	B6 = Konkan 4	7	-	-	-	-	-	-	-	-
	B7 = MK 72-2	4	-	-	-	-	-	-	-	-
S2 = MS + NAA(1mg/l) + BA (2mg/l)+GA(2mg/l)	B1= Tainan 9	7	-	-	-	-	-	-	-	-
	B2 = Konkan 60-1	7	-	-	-	-	-	-	-	-
	B3 = Konkan 60-2	9	-	-	-	-	-	-	-	-
	B4 = Konkan 60-3	6	-	-	-	-	-	-	-	-
	B5 = Konkan 60-4	10	-	-	-	-	1	10	48	3
	B6 = Konkan 4	7	-	-	-	-	3	42.85	50	5
	B7 = MK 72-2	5	-	-	-	-	-	-	-	-
S3 = N6 + NAA(1mg/l) + BA(2 mg/l)	B1= Tainan 9	6	-	-	-	-	-	-	-	-
	B2 = Konkan 60-1	8	-	-	-	-	-	-	-	-
	B3 = Konkan 60-2	5	-	-	-	-	-	-	-	-

S3 = N6 + NAA(1mg/l) + BA(2 mg/l)	B4 = Konkan 60-3	5	-	-	-	-	-	-	-	-
	B5 = Konkan 60-4	8	-	-	-	-	1	12.5	70	2
	B6 = Konkan 4	7	-	-	-	-	-	-	-	-
	B7 = MK 72-2	5	-	-	-	-	-	-	-	-
S4 = N6 + IAA(2mg/l) + Kinetin(2 mg/l)+GA(2mg/l)	B1= Tainan 9	5	1	20	66	1	-	-	-	-
	B2 = Konkan 60-1	5	1	20	72	1	-	-	-	-
	B3 = Konkan 60-2	5	1	20	37	1	-	-	-	-
	B4 = Konkan 60-3	3	-	-	-	-	-	-	-	-
	B5 = Konkan 60-4	8	-	-	-	-	-	-	-	-
	B6 = Konkan 4	6	-	-	-	-	-	-	-	-
	B7 = MK 72-2	1	-	-	-	-	-	-	-	-
S5 = N6 + IAA(2mg/l) + Kinetin(2 mg/l)+BA(2mg/l)	B1= Tainan 9	6	-	-	-	-	-	-	-	-
	B2 = Konkan 60-1	6	-	-	-	-	-	-	-	-
	B3 = Konkan 60-2	5	-	-	-	-	-	-	-	-
	B4 = Konkan 60-3	5	-	-	-	-	-	-	-	-
	B5 = Konkan 60-4	8	-	-	-	-	-	-	-	-
	B6 = Konkan 4	7	-	-	-	-	-	-	-	-
	B7 = MK 72-2	3	-	-	-	-	-	-	-	-
Total		222	3	1.35	-	-	8	3.6	-	12

DISCUSSION

The anthers at the uninucleate stage of microspores from all seven cultivars were used in the present study. Since the developmental stage of microspores at the time of culture has been cited as an important consideration in successful anther culture (Maheshwari *et al.*, 1983). He and Quyang 1984) found that in some genotypes of wheat, haploid callus and subsequently haploid green plantlets could be obtained from anthers at various stages from as early as meiosis stage to as late as binucleate stage. But the highest peaks of callus plantlet induction frequency occurred at mid- or late uninucleate stage in most all genotypes.The excised anthers of *Arachis hypogaea* and *A. villosa* at three stages of development, the uninucleate, pollen undergoing mitosis and binucleate stage, were cultured and the first mitosis stage was found to be more responsive than the other two stages (Bajaj *et. al.*, 1981.). While the early uninucleate stage was reported to be the most responsive stage of microspore development at which to initiate cultures and was significantly better than PMC, mid and late uninucleate stages.in peanut anther culture (Wilcox *et. al.*, 1991).

From the first media experiment, it was found that all four media were equal effective in inducing callus formation of all cultivars used in the present study while the *cv.* Konkan 60-4 was found to be more responsive than the other six cultivars. This could be due to genotypic differences of the seven cultivars. Different response in plant genotypes in anther culture has been observed in many crop species such as peanut (Baja *et.al.*, 1981), rice (Chen and Lin, 1976), wheat (Hu, 1981), corn (Afele and Kannenberg,1990). Oono (1975) reported variation in callus formation capability among the rice lines tested from 0.5-8%, and regeneration of albino and green plants was also genotype independent. Significant differences in callus formation using various varieties or crosses were also reported in wheat anther culture (Hu, 1997).

The interaction effects of medium x cutivar (genotype) were found to be highly significant for the response of anthers and number of days to anther callusing. Similar finding was observed by Quimio and Zapata(1990) who concludedthat the effects of genotype and genotype x medium interactions were significant from a diallel analysis of rice anther culturability and green plant regeneration.

In the second media experiment, the regenerated shoots obtained from the three cultivars, Tainan 9, Konkan 60-1, Konkan 60-2, culturing on the N_6 + 2 mg L^{-} IAA + 2 mg L^{-} Kinetin + 2 mg L^{-} GA_3 medium indicated some effectiveness of the medium in inducing shoot regeneration from the anther-derived calli. While the other calli continued proliferation. This could be due to genotypic differences of the seven cultivars. The genotype of donor plants was found to affect the regeneration frequency (Hu, 1997). Since haploid embryo induction through zygotic and somatic embryogenesis has been reported to involve growth regulators (Liu *et. al.*, 1993). In most cases, specific inductive conditions are required for embryogenesis and they lead to change in endogenous growth regulators in the microspores. Culture conditions may themselves elicit endogenous growth regulators in responsive microspores. Recent studies have highlighted the importance of polar auxin transport in the development of zygotic embryo (Liu *et. al.*, 1993). Since the development of microspore-derived embryos closely patterns that of the zygote, a similar role for auxin is quiet likely. In some systems, specific mRNAs and proteins related to embryogenesis have been identified. Some embryoid abundant genes were shown to be temporally expressed during induction of pollen embryogenesis in wheat (Reynolds and Kitto, 1992). Although it was demonstrated that non-embryogenic microspores, or those held under non-inductive conditions did not exhibit such gene expression, it remains to be determined whether this is generally true or other systems (Ferrie *et. al.*, 1995). From the present study, we suggest that the higher concentrations of Kinetin (cytokinin) (> 2 mg L^{-1} should be determined to increase the shoot regeneration frequency of this recalcitrant crop.

The results from the present study may be most valuable in providing a protocol for callus formation and shoot regeneration by which 20% of the regenerated shoots can be obtained in peanut anther culture.

ACKNOWLEDGEMENT

This research was financial supported by the Nation Research Council.

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