GENERATION OF MAIZE HAPLOIDS VIA ANTHER CULTURE

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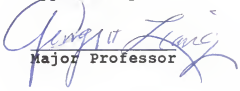

Major Professor

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Part I

Effects of Hormones and Genotypes on Callus Induction and Haploid Generation from Maize Anther Culture

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ABSTRACT

Fifty genotypes and nine growth regulators were used in two separate experiments (I and II) to investigate genotypic and hormonal effects on haploid production via anther culture. Hormonal effects were not significant in either experiment. Highest callus-induction frequencies in both experiments, however, occurred on YP basal medium plus 2,4-D at 2.0 mgL^{-1} and kinetin at 1.5 mgL^{-1} indicating that this treatment was more effective than others. Effects of genotypes on callus and embryoid induction were significant. The most responsive genotypes in experiment I were Yuanwu x 592 and K727 x K305 which produced 18.30% and 6.67% calli, respectively, with their appropriate media. The most responsive entry in experiment II was Pool 29, a CIMMYT source, which produced 15.0% calli on appropriate medium and an average of 10.0% calli across 10 media. A total of 23 plantlets was regenerated from this study. Most of them developed embryogenically.

INTRODUCTION

Haploid generation of maize via anther culture as an

alternative approach to inbred line development has attracted the interest of many researchers and much progress has been made. Callus-induction frequency is a major obstacle to the practical application of haploid generation but it has been increased greatly since the beginning of anther culture. Researchers at Academia Sinica (Beijing) increased the callus-induction frequency from 0.52% to 1.03-1.09% by increasing sucrose concentration from 6% to 12-24%.[6] Ku et al.[4] obtained 13.1% of callus-induction frequency with the addition of 0.1 mgL^{-1} TIBA to YP medium. Callus-induction frequency was increased to 17.0% using more responsive genotypes and Zhen-15 medium.[11] A. D. Genovesi and G. B. Collins[3] obtained 18.3% of callus initiation with modified YP medium and cold-pretreatment. Recently, 49.2% callus induction was achieved by Kuo Chung-shen et al. using a particular genotype (personal communication). It is believed that genotype and culture medium are important factors determining the induction frequency in maize anther culture.[3,4,6]

Some researchers found that callus induction was insensitive to hormonal stimulation. They believed that endogenous hormones of maize anthers meet the requirements of callus and embryoid initiation, and that the addition of exogenous hormones was unnecessary.[1,4,12] Intensive studies about the effects of various growth regulators, however, have not been conducted.

The objectives of this study were to explore the effects of various growth regulators and genotypes on callus and embryoid initiation, to test the culturabilities of some collected germplasms, and to develop a specific procedure for producing haploids via anther culture in maize.

MATERIALS and METHODS

Materials used in experiments I and II included 40 U.S. germplasms and 10 exotic collections from China, Morocco, and CIMMYT (Table 1). Same hormone treatments were used in both experiments, but different genotypes were used in an attempt to test a wider range of materials. Experiment I was planted on May 11, 1989 and experiment II was planted on June 10, 1989 at the Ashland Agronomy Farm (Riley County, Kansas).

YP basal medium[3], which was developed for maize anther culture by Chinese scientists, containing 15% sucrose was used. Nine growth regulators were added to the medium separately and 10 treatments were formed:

(1) YP + 2,4-D 2.0 mgL^{-1}

(2) YP + 2,4-D 2.0 mgL^{-1} + KT 1.5 mgL^{-1}

(3) YP + 2,4-D 2.0 mgL^{-1} + 6-BA 1.5 mgL^{-1}

(4) YP + 2,4-D 2.0 mgL^{-1} + 2-ip 1.5 mgL^{-1}

(5) YP + 2,4-D 2.0 mgL^{-1} + zeatin 1.5 mgL^{-1}

(6) YP + dicamba 3.5 mgL^{-1} + KT 1.5 mgL^{-1}

(7)	YP + NAA	3.5 mgL^{-1} + KT	1.5 mgL^{-1}
(8)	YP + pCPA	3.5 mgL^{-1} + KT	1.5 mgL^{-1}
(9)	YP + IBA	3.5 mgL^{-1} + KT	1.5 mgL^{-1}
(10)	YP + 2,4-D	1.0 mgL^{-1} + dicamba	2.0 mgL^{-1}
	+ KT	1.5 mgL^{-1}	

Genotypes and media were considered as two factors. A factorial experiment design with two replications was used. Due to contamination, only one replication was used to analyze data. Tassels were pretreated at 4°C for 7 days prior to anther inoculation. Florets with anthers containing microspores at the uninucleate stage were collected and sterilized by submerging them in 20% commercial bleach solution for 10 min and then washed 4 times with sterile distilled water. Anthers were then plated on the medium in 100 x 15 mm disposable petri dishes containing 25 ml of medium each. Petri dishes were kept in a darkened incubation chamber at 28°C until calli or embryoids formed. They were then transferred to a chamber under 12-h light at 28°C.

Callus induction frequency was measured as the percentage of anthers forming one or more calli and embryoids. Percentages were converted to arcsin for statistical analysis. Genotypes and media that produced calli and embryoids were used for data analysis. Comparisons among mean induction frequencies were made using Duncan's New Multiple Range Test.[7]

As embryoids or calli reached 2 mm in diameter, they were transferred to the regeneration medium: YP + KT (2.0 mgL^{-1}) + CH (500 mgL^{-1}) + sucrose (50 gL^{-1}). They were later transferred to hormone-free YP medium when roots and shoots were formed.

Regenerated seedlings were grown in vermiculite. They were transferred to pots in a greenhouse at 20 to 28°C when 2 to 4 leaves and 2 to 3 roots appeared. Root tips were taken at the time of transplantation, pretreated in distilled water at 1°C for 24 h, and fixed in Carnoy B fixing solution (6 parts 95% ethanol : 3 parts chloroform : 1 part acetic acid) at 4°C until slide preparation. Enzyme treatment of root tips and slide preparation were carried out as reported by Song et al.[10]

RESULTS

Effects of Genotypes

Among the 25 genotypes tested in experiment I, only 13 produced calli and embryoids. Induction frequencies ranged from 1.7 to 18.3% in their most responsive media and mean callus-induction frequencies across 10 media varied from 0.2% to 6.0% (Table 2).

There was a significant genotypic effect on callus and embryoid initiation (Table 3). Genotypic differences were evaluated from mean induction frequency across all media.

Comparisons based on mean callus-induction frequency indicated that Yuanwu x 592, a Chinese source, was the most responsive and produced an average of 6.0% calli. Genotypes K727 x K305 produced an average of 3.5% calli and was significantly higher than others (Table 2).

Similar results were obtained from experiment II. Thirteen genotypes among 30 materials tested produced calli and embryoids. Callus-induction frequencies ranged from 1.7 to 15.0% in their most responsive media. The average callus-induction frequency across 10 media ranged from 0.2 to 10.0% (Table 4).

Genotypic effects on callus induction were significant (Table 5). CIMMYT Pool 29 was the most responsive with callus-induction frequency of 15% on the medium: YP + 2,4-D (2.0 mgL^{-1}) + kinetin (1.5 mgL^{-1}) and an average induction frequency across the 10 media of 10% (Table 4). Pool 29 consistently produced calli and embryoids on every medium.

Effect of Medium

Effects of medium were nonsignificant in both Exp. I and Exp. II. This indicates that growth regulators and their combinations at concentrations tested were not significantly different in promoting callus induction. Results from experiment I and II, however, revealed that all high induction frequencies occurred on medium YP + 2,4-D (2.0 mgL^{-1}) + kinetin (1.5 mgL^{-1}). This treatment appeared to be

more effective for callus induction than others.

Direct Initiation of Embryoids

Embryoids were initiated directly from anther culture in some cases. Those embryoids were smooth on the surface and globular in shape (Figure 1), whereas calli generally were rough on the surface and irregular in shape (Figure 2). Among 95 calli or embryoids induced from experiment I, 65% (62 of 95) were embryoids while in experiment II, 73% (65 of 89) were embryoids.

Generation of Haploid Plantlets

Twenty days after embryoids were transferred to the regeneration medium, roots and/or shoots began their initiation (Figure 3). Some embryoids developed roots but no shoots (Figure 4) whereas others developed shoots with no roots (Figure 5). Twenty three plantlets were developed, 15 from Exp. I and eight from Exp. II. Fourteen plantlets were from genotype Yuanwu x 592, one from K727 x K305, six from Pool 29, and two from NDD 26.

DISCUSSION

The lack of medium effects on callus induction suggests two possibilities. One is that maize anthers are insensitive to hormonal stimulation as reported previously.[1,4,11] We cannot confirm this conclusion from our results because a hormone-free medium was not included as a check. The

nonsignificant difference between medium containing YP plus auxin and YP plus auxin and cytokinin, however, may indicate that cytokinins are not essential for callus induction. A second possibility is that growth regulators at the concentrations we tested did not permit expression of hormonal differences in callus induction. Further studies should be conducted to investigate the effects of various hormones at different concentrations on callus initiation.

Genotype has been considered to be a critical factor in anther culture of maize.[5,8,9] Nearly half of the genotypes we tested failed to develop calli and embryoids. Of the materials which responded positively, most had very low callus-induction frequency. CIMMYT Pool 29, however, was consistently responsive on every media tested. Moreover, Pool 29 is a heterogeneous population. If selection for high-culturable individuals within the population is made, the frequency of callus induction from those plants may be increased. Further efforts should be made in two directions. One is to investigate a wide range of germplasms and select those that are able to produce callus at high frequency. Then, transfer of the high culturability to other germplasms through crosses and backcrosses would broaden the application of the anther culture technique (Guo personal communication 1989)[2]. The other is to develop an anther culture procedure applicable to a wide range of genotypes.

Once a high callus induction frequency is achieved through a technique that is applicable to a wide spectrum of genotypes, the practical application of anther culture may then become a reality in maize breeding.

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Table 1. Germplasm sources used in anther culture.

No.	Name				No.	Name	
Central US Germplasm					Northern US Germplasm		
01	Va35w	x	Va35	*	21	NDD5 (A639 x A641) x ND301	*
02	WF9	x	T226W		22	NDD12 (NDD474 x ND468) x ND250	*
03	A619wBc	x	A632ht	*	23	NDD24 (ND252 x ND245) x ND259	*
04	K301	x	H28	*	24	NDD26 (A665 x CM105) x ND82-26	*
05	K55	x	K302		25	NDD30 (A665 x CM105) x ND82-29	*
06	K55	x	A90	*	26	NDD803 (CM105 x ND300)	*
07	Te	x	M2	*	27	NDF811 (ND256 x A554)	
08	B68	x	K302	*	28	NDC861 (ND247 x ND474)	*
09	(H28	x	Z9ch) x H28		29	ND249 x ND252	
10	GH45	x	SD10		30	ND259 x ND245	
11	(SD10	x	H28) x SD10*		31	K730	
12	K303	x	C166	*	32	DC-405	**
13	K201G	x	WF9syn4	**	33	K127	
14	IS70	x	IS82		34	CM105 x A554	
15	K731	x	K306	*	35	K2204	
16	K722A	x	K304	*	36	R x 337	*
17	K301	x	K303		37	R x 406	
18	K809	x	A619w	*	38	(CM105 x A641) (FR22)	
19	K727	x	K305	**	39	B73 x Mo17ht	
20	K64	x	K302		40	TF5 x 78	
Chinese Germplasm					Germplasm from Morocco		
41	A2-1 line			*	46	DRA-400	
42	A2-2 line				47	TX-21	
43	Guihua No. 1				48	CVB-39-3B	
44	592 x Yuanwu				49	CVM-147-1B	*
45	Yuanwu x 592			**	CIMMYT Germplasm		
					50	Pool 29	**

* Materials having low response
** Materials having high response
\# The rest without mark having no response

Table 2. Genotypic effects on callus and embryoid initiation (Exp. I).

Entry	Induction freq. on appropriate medium %	Mean induction frequency (%)	arcsin	class*
Yuanwu x 592	18.3	6.0	14.2	a
K727 x K305	6.7	3.5	10.8	ab
K201G x WF9syn	10.0	1.8	7.8	b
K809 x A619w	3.3	1.2	6.2	bc
K722A x K304	5.0	0.8	5.2	cd
K731 x K306	3.3	0.7	4.7	cd
K301 x H28	1.7	0.3	3.3	d
K55 x A90	3.3	0.3	3.3	d
Te x M2	1.7	0.3	3.3	d
B68 x K302	1.7	0.3	3.3	d
Va35w x Va35	1.7	0.2	2.3	d
A619WBC x A632ht	1.7	0.2	2.3	d
K303 x C166	1.7	0.2	2.3	d

* Means without the same letter are significantly different at P=0.01 based on Duncan's New Multiple Range Test

Table 3. AOV for the genotypic and medium effects on callus and embryoid initiation (Exp. I).

Source	df	MS
Genotype	12	136.75**
Medium	9	7.43
Error	108	18.46
Total	129	

** Significantly different at P=0.01

Table 4. Genotypic effects on callus and embryoid initiation (Exp. II).

Entry	Induction freq. on appropriate medium %	Mean induction frequency (%)	arcsin	class*
Pool 29	15.0	10.0	18.5	a
DC-405	1.7	0.7	4.7	b
NDD12	3.3	0.5	4.1	b
NDD24	1.7	0.5	4.1	b
NDD30	1.7	0.5	4.1	b
NDD803	1.7	0.5	4.1	b
NDD861	1.7	0.5	4.1	b
A2-1 line	1.7	0.5	4.1	b
NDD26	1.7	0.3	3.3	b
Yuanwu x 592	1.7	0.3	3.3	b
NDD5	1.7	0.2	2.3	b
R x 337	1.7	0.2	2.3	b
CVM-147-1B	1.7	0.2	2.3	b

* Means without same letter are significant differently at P=0.01 based on Duncan's New Multiple Range Test

Table 5. AOV for the genotypic and medium effects on callus and embryoid initiation (Exp. II).

Source	df	MS
Genotype	12	215.64**
Medium	9	8.80
Error	108	10.53
Total	129	

** Significantly different at P=0.01

Figure 1. Two embryoids initiated directly from a maize anther.

Figure 2. A callus initiated from a maize anther.

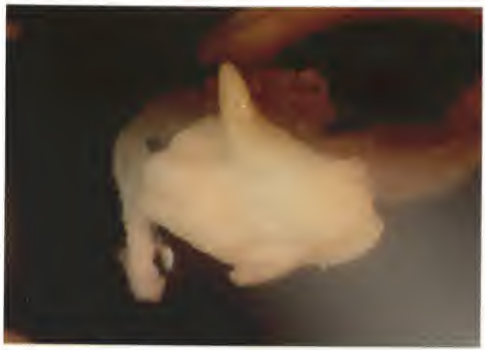

Figure 3. Formation of a root and a shoot from an embryoid of maize.

Figure 4. Root initiation and elongation from a maize anther.

Figure 5. Shoot formation from an embryoid in maize anther culture.

Figure 6. A cluster of shoots developed from maize anther culture.

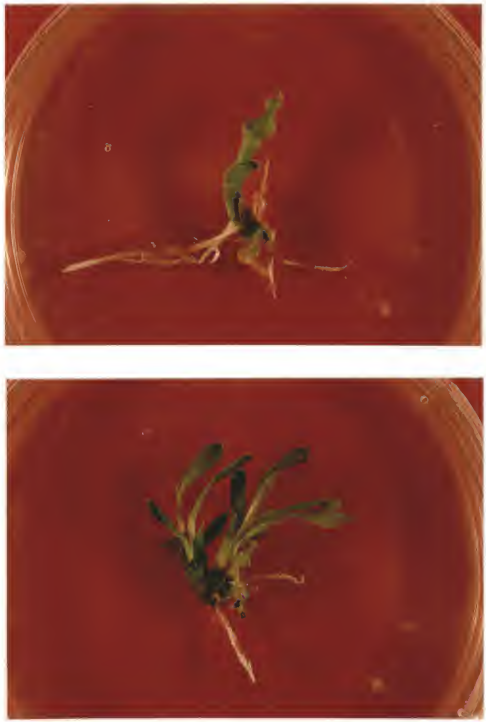

Figure 7. Development of plantlets from the regeneration medium in maize anther culture.

Figure 8. A maize plantlet in hormone-free YP medium.

Figure 9. A maize plantlet growing in vermiculite.

Part II

Direct Generation of Maize Haploids via Anther Culture

Direct Generation of Maize Haploids via Anther Culture

ABSTRACT

Anther culture of maize usually follows an indirect or two-step procedure involving callus induction and differentiation on at least two different media. Development of a direct or one-step procedure by which plantlets can be regenerated directly on one medium seems desirable, if acceptable frequencies of plant regeneration can be obtained. In this experiment YP and N6, as basal media, were each supplemented with one or two hormones to evaluate their effects on direct generation of haploids in corn. Nineteen genotypes, including six single-cross hybrids, 11 inbred lines and two wild species, were used as plant materials. Anthers containing microspores primarily at the uninucleate stage were plated on the media. Calli or embryoids were induced from most media. Plantlets were directly regenerated from hybrid (K809 x A619w) on N6 basal medium plus 2,4-D (2.0 mgL^{-1}) and kinetin (1.5 mgL^{-1}). Regeneration frequency (percentage of anthers producing plantlets) was 4%. Chromosome counts on one plantlet showed that the regenerated plantlet was a true haploid.

INTRODUCTION

Production of maize haploids through anther culture is considered a useful alternative approach in the development of pure lines of maize. Haploid production could shorten development time to produce homozygous diploid lines. Since the first corn plant was induced from anther culture in 1975 [5], much progress has been made. A number of pure lines have been developed, and many hybrids of those lines have shown desirable prospects.[6]

A two-step procedure is generally used in maize anther culture, i.e., callus induction and differentiation are carried out in separate operations using at least two different media, induction and regeneration media. We were attempting to develop a one-step procedure by which plantlets can be regenerated directly from plated anthers on one medium. If acceptable frequencies of plant regeneration could be obtained, time, space, and materials will be saved.

Technically, it is possible to generate plantlets with a one-step approach. There were successful examples using a one-step approach in wheat (Triticum aestivum L.) [7] and tobacco (Nicotiana tabacum L.) [8]. It has been reported that calli and embryoids of maize can be induced in media without auxins and cytokinins because maize anthers are less sensitive to hormones than those of other plant

species.[1,5,9] Chen reported that some plantlets were generated in the induction medium directly.[1] So anther culture with a one-step procedure could be successful.

The objective of this study was to develop a reliable procedure for direct (one-step procedure) generation of maize haploids using various hormone combinations and concentrations.

MATERIALS and METHODS

Plant materials included six hybrids (Zea mays ssp. mays), 11 inbred lines (Zea mays ssp. mays), and Teosinte (Zea mays ssp. Mexicana), and a hybrid between Teosinte and Zea mays ssp. mays.

K731	x K306	K731	K306	Teosinte x Maize-2
K722A	x K304	K301	K303	Teosinte
K301	x K303	A619w	K727	
K809	x A619w	K64	K302	
K727	x K305	K304	K809	
K64	x K302	K305		

The YP and N6 basal media were used, and various growth regulators were added. Twenty treatments were formed as follows:

(1) N6 + kinetin 1.5 mgL^{-1}

(2) N6 + 2,4-D 2.0 mgL^{-1} + kinetin 1.5 mgL^{-1}

(3) N6 + NAA 2.0 mgL^{-1} + kinetin 1.5 mgL^{-1}

(4) N6 + IAA 2.0 mgL^{-1} + kinetin 1.5 mgL^{-1}

(5) N6 + IBA 2.0 mgL^{-1} + kinetin 1.5 mgL^{-1}

(6) N6 + dicamba 2.0 mgL^{-1} + kinetin 1.5 mgL^{-1}

(7) N6 + pCPA 2.0 mgL^{-1} + kinetin 1.5 mgL^{-1}

(8) N6 + NAA 2.0 mgL^{-1} + 6-BA 1.5 mgL^{-1}

(9) N6 + NAA 2.0 mgL^{-1} + 2-ip 1.5 mgL^{-1}

(10) N6 + NAA 2.0 mgL^{-1} + hypoxantine 1.0 mgL^{-1}

(11) YP + kinetin 1.5 mgL^{-1}

(12) YP + 2,4-D 2.0 mgL^{-1} + kinetin 1.5 mgL^{-1}

(13) YP + NAA 2.0 mgL^{-1} + kinetin 1.5 mgL^{-1}

(14) YP + IAA 2.0 mgL^{-1} + kinetin 1.5 mgL^{-1}

(15) YP + IBA 2.0 mgL^{-1} + kinetin 1.5 mgL^{-1}

(16) YP + dicamba 2.0 mgL^{-1} + kinetin 1.5 mgL^{-1}

(17) YP + pCPA 2.0 mgL^{-1} + kinetin 1.5 mgL^{-1}

(18) YP + NAA 2.0 mgL^{-1} + 6-BA 1.5 mgL^{-1}

(19) YP + NAA 2.0 mgL^{-1} + 2-ip 1.5 mgL^{-1}

(20) YP + NAA 2.0 mgL^{-1} + hypoxantine 1.0 mgL^{-1}

The plant materials were planted at the Ashland Agronomy Farm (Riley County, Kansas) June 7, 1988. Entries were planted in single rows 36 cm between hills within the row and 72 cm between rows. Irrigation and fertilizer applications etc, were made to provide good environmental conditions for plant growth.

Florets with anthers containing microspores at the uninucleate stage were collected prior to anthesis. Florets

were sterilized by submerging them in 20% commercial bleach solution for 10 min and then washed 4 times with sterile distilled water. Anthers were then plated on 25 ml of medium in 100 x 15 mm disposable petri dishes. The petri dishes were kept in an unlighted incubation chamber at 28°C until there were signs of callus or embryoid formation, they were then transferred to a chamber under 12-h light at 28°C. When roots and shoots formed, they were transferred to hormone-free N6 basal medium.

Regenerated seedlings were grown in vermiculite. When 2 to 4 leaves and 2 to 3 roots appeared, seedlings were transferred to pots in a greenhouse at 20 to 28°C. Root tips were taken at the time of transplantation and pretreated in distilled water at 1 °C for 24 h and fixed in Carnoy B fixing solution (6 parts 95% ethanol : 3 parts chloroform : 1 part acetic acid) at 4°C until slide preparation. Enzyme treatment of root tips and slide preparation were carried out according to the procedure reported by Song et al.[10]

RESULTS

About 30 days after placing anthers on the medium, initiation of calli and embryoids began (Figure 1) and continued for another 30 days. The majority of responding anthers produced embryoids (Figure 2).

Initiation of calli and embryoids depended upon genotypes. Among 19 genotypes tested, 8 showed callus and embryoid initiation (Table 1). The induction frequencies of calli and embryoids ranged from 2.2 to 11.1 per 100 anthers plated. The highest frequency was 11.1% found for genotype K727 x K305. YP medium was more effective than N6 medium in callus and embryoid induction. Eighty percent (53 of 66) of the calli and embryoids were induced on YP medium.

Differentiation of embryoids was observed about 15 days after their initiation. Most had root-like structures but no shoots (Figure 3). Some developed shoots but no roots (Figure 4). Other embryoids developed no further and later died.

Two directly regenerated plantlets (Figure 5) came from hybrid K809 x A619w about one month after embryoid initiation and occurred on N6 basal medium plus 2,4-D ($2.0\ mgL^{-1}$) and kinetin ($1.5\ mgL^{-1}$). The plantlets were transferred to N6 basal medium and placed under 12-h light at $28^{o}C$. When 3-5 roots appeared, they were transferred to vermiculite and later transplanted into pots in a greenhouse (Figure 6). Chromosome counts of a plantlet showed that it was a haploid (Figure 7). Following chromosomal doubling by using colchicine (0.05%), the female and male organs were developed normally but no seed was set because that the flowering of female and male did not occur

at the same time.

DISCUSSION

The results of this study showed that direct generation of haploids via anther culture is possible even though the regeneration frequency was low. Two phenomena occurred in maize anther culture support this possibility. One is that direct initiation of embryoids in most cases as we found in this study and reported by Kuo et al.[6] Those somatic embryoids, as zygotic embryos, have bipolar structures with root and shoot meristems. It will be easier to generate plantlets from those embryoids than from calli as reported by Cheng et al.[2] that the formation of plantlets derived from embryoids was fivefold to that derived from callus. Another one is that many embryoids developed roots while in few cases developed only shoots. If the ratio of auxin and cytokinin was adjusted to a proper level, shoots and roots could be regenerated simultaneously because the initiation of shoots and roots is affected by hormone balance [4].

Same as in two-step procedure, plantlet generation through one-step procedure is also genotype dependent. The success of this technique requires a search for more responsive genotypes. It would be reasonable to develop a one-step procedure by using materials having high responsive

abilities tested with two-step procedure.

Different operations between one-step and two-step procedures appeared to make no difference in the manner of plantlet generation. In most cases plantlets were regenerated by embryogenesis. In this study, two plantlets were developed from somatic embryoids and also in the previous research we found that most plantlets were generated from embryoids too. This may indicate that the entire path of plantlet development can be achieved by a one-step procedure.

Further efforts should be made to improve the regeneration frequency by exploring other factors affecting the mode of direct haploid generation, such as genotype, temperature, other hormone concentrations and their combinations, and so on. We believe that the frequency of direct regeneration of maize haploids will be increased by using improved techniques.

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Table 1. Differential response of genotypes on YP and N_6 media.

Entry	Induction frequency	
	YP	N6
K727 x K305	11.1	10.0
K809 x A619w	6.3	3.3
K722A x K304	4.4	0
K731 x K306	2.2	0
A619w	4.4	0
K306	2.2	0
K301	2.2	0
K303	2.2	0

Figure 1. Callus initiation from a maize anther.

Figure 2. Initiation of embryoids from a maize anther.

Figure 3. Initiation and development of roots from embryoids of maize.

Figure 4. A shoot formation from a maize embryoid.

Figure 5. Direct generation of two maize plantlets from N6 + 2,4-D (2.0 mgL^{-1}) + kinetin (1.5 mgL^{-1}) + sucrose (10%).

Figure 6. A maize haploid plantlet developed from anther culture.

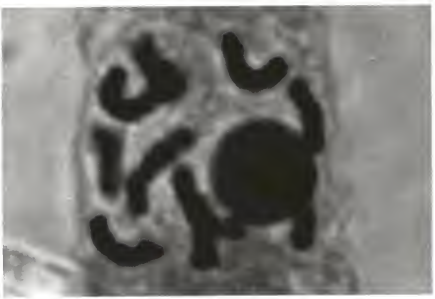

Figure 7. Ten chromosomes are shown in a root-tip cell from a haploid plantlet.

APPENDIX

ABBREVIATIONS

2,4-D : 2,4-dichlorophenoxyacetic acid

KT : kinetin

6-BA : 6-benzyladenine

2-ip : 6[γ,γ-dimethylallylamino]-purine

NAA : α-naphthalene acetic acid

IBA : indole-3-butyric acid

IAA : indole-3-acetic acid

pCPA : p-chlorophenoxyacetic acid

TIBA : 2,4,5-triiodobenzoic acid

CH : casein hydrolysate

YP : Yu-pei medium (corn tissue culture medium)

Composition of Yu-pei (YP) medium

Composition	Concentration (mg/L)
Major nutrients	
NH_4NO_3	165.00 (2.06 mM)
KNO_3	2500.00 (24.73 mM)
$CaCl_2$ $2H_2O$	176.00 (1.20 mM)
$MgSO_4$	181.00 (1.50 mM)
KH_2PO_4	510.00 (3.75 mM)
Minor nutrients	
KI	0.80 (4.82 μM)
H_3BO_3	1.60 (25.89 μM)
$MnSO_4$ H_2O	3.33 (19.70 μM)
$ZnSO_4$ $7H_2O$	1.50 (5.22 μM)
Chelated iron	
Na_2 EDTA	37.30 (0.10 mM)
$FeSO_4$ $7H_2O$	27.80 (0.10 mM)
Vitamins	
Nicotinic acid	1.30 (10.56 μM)
Thiamine-HCl	0.25 (0.74 μM)
Pyridoxine-HCl	0.25 (9.77 μM)
Glycine	7.70 (0.10 mM)
Ca-Pantothenate	0.25 (0.52 μM)
2,4,5-triiodobenzoic acid(TIBA)	0.10 (0.20 μM)
Casein hydrolysate	500.00
Activated Charcoal	5000.00
Sucrose	150000.00
Agar	8000.00
pH	5.8-6.0

N_6 Medium

Composition	Concentration (mg/L)
KNO_3	2830
$(NH_4)_2SO_4$	463
$MgSO_4 \cdot 7H_2O$	185
$(MgSO_4)$	92.5
$CaCl_2 \cdot 2H_2O$	440
KH_2PO_4	400
$FeSO_4 \cdot 7H_2O$	27.8
Na_2-EDTA	37.3
$MnSO_4 \cdot 4H_2O$	4.4
$ZnSO_4 \cdot 7H_2O$	1.5
H_3BO_3	1.6
KI	0.8
Glycine	2.0
Thiamine-HCl	1.0
Pyridoxine-HCl	0.5
Nicotinic acid	0.5
2,4-D	2.0
Kinetin	0.5
Sucrose	9-10%
Agar	1%
pH	5.8

GENERATION OF MAIZE HAPLOIDS VIA ANTHER CULTURE

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Anther culture of maize is relatively routine, however, low induction frequency of callus and complicated procedure are still obstacles for its practical application. The objectives, in the first experiment, was to develop a one-step procedure by which plantlets could be directly regenerated, and in the second experiment, were to detect the effects of genotypes and hormones on culturability of maize anther, and to test the responsive abilities of some collected germplasms.

In the first experiment, two plantlets were regenerated directly from genotype K809 x A619w on N6 basal medium plus 2,4-D (2.0 mgL^{-1}) and kinetin (1.5 mgL^{-1}). The chromosome counts on one regenerated plantlet showed that it was a haploid. Even though the frequency was low, one-step procedure seems feasible. The preliminary results showed that the same as in two-step procedure genotypes and media are important factors for haploid generation in one-step procedure.

Genotypes were important on callus and embryoid induction but the effects of various growth regulators on callus initiation were not detected in the second experiment. Among 50 genotypes tested in two separate tests, half of them was able to produce calli and embryoids. The most responsive materials were Yuanwu x 592, K727 x K305, and pool 29 which produced 18.3%, 6.7%, and 15.0% calli,

respectively, on their appropriate media. Pool 29 was a promising genetic source for anther culture. It produced calli and embryoids on every media tested. The callus induction frequency can be increased for this genetic source, if selection is made for high-culturable individuals within this heterogeneous population. Twenty-three plantlets were regenerated. Most of them were initiated from the most responsive materials, Yuanwu x 592 and pool 29.