ANTHER CULTURE OF *SORGHUM BICOLOR* (L.) MOENCH

by

FUSHI WEN

B.S., Northwestern University, China, 1982

A MASTER'S THESIS

submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

Genetics

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1988

Approved by:

Major Professor

ACKNOWLEDGMENTS

I wish to thank my advisor Dr. George H. Liang for guidance, many helpful suggestions, even useful criticize, and financial support throughout this research project.

I wish also to thank Drs. E. L. Sorensen and P. P. Wong for the review of my thesis and very useful comments.

Finally, I express deep gratitude to my parents, professor Honghan Wen and Zhiming Zhang, other members of my family, and my friend Zhongguo Xiong and Ailan Guo, for their unwavering support.

Table of contents

Table List

Anther Culture of Sorghum bicolor (L.) Moench

F. WEN AND G. H. LIANG

Department of agronomy, Throckmorton Hall

Kansas State University, Manhattan, Kansas 66506, USA

ABSTRACT

Haploid plants are very useful for breeding new cultivars. Theoretically, haploids can be obtained from microspores when anthers are placed on a specifically formulated environment. Anther culture of sorghum bicolor has been unsuccessful. The objective of this study was designed to develop techniques for haploid induction from anther culture of sorghum bicolor (L.) Moench.

For callus induction, anthers containing microspores at uninucleate stage were sampled. Twenty-nine genotypes were tested on various media and six of them responded. Anthers of Xin-w and TSB produced many more calluses than anthers of other four genotypes did. Twenty-eight media were screened for callus induction and twelve induced calluses. C17-2 and MS-t-z-2 were the most effective media. When anthers of Xin-w were cultured on C17-2 medium, 10.7% anthers produced calluses. Media containing sucrose at 20-60 mg/l level induced significantly higher frequency of callus induction than those containing sucrose at 90 g/l level did.

For callus differentiation, modified MS and SHAP media

were used. MS-d-4 with 2.0 mg/l IAA and 2.5 mg/l kinetin was best medium for callus differentiation. Longer callus induction time increased the frequency of calluses with only roots and frequency of albino plants production, while decreased the frequency of callus differentiation and plants regeneration.

Chromosome counting of regenerated plants showed different numbers: 20, 10, 15, 40, and 60. Despite the absence of haploid plants, it was still possible that some of the diploid plants were haploid in origin in this study.

Additional Index Words: Sorghum Bicolor, Anther culture, Haploid, Microspore.

Introduction

Haploid plants are very useful for breeding new cultivars. Homozygous plants can be obtained by doubling the chromosomes in haploids. This can shorten the time needed to develop new cultivars or inbreds. A comparison between the procedure of haploid breeding and conventional breeding in wheat showed that the former may reduce the time for cultivar development by three to four generations (Hu and Zeng, 1984). Haploids are useful also for mutation research and fundamental genetic studies (Sunderland, 1971; 1977a).

Theoretically, haploids can be obtained from microspores (young pollen grains) when anthers are placed on a specifically formulated nutrient medium under a given

cultural environment. Since Guha and Maheshwari (1964 and 1966) first reported the direct development of haploid embryos from microspores of Datura innoxia by anther culture in vitro, successful anther culture has been reported among many Gramineae species and hybrids (Dunwell, 1985). In subfamily Panicoideae, most studies have been with Zea mays (Research 401 Group,1975; Chang et al., 1981; Genovesi and Collins, 1982; Nitsch et al., 1982; and Ting et al., 1981) with much more limited studies on Sorghum bicolor (Rose, et al., 1986; Smith, personal communication) In Rose et al's work (1986), only four albino plants were regenerated from 1,000 calluses produced from anthers. Anther culture of Sorghum bicolor has been unsuccessful. My study on Sorghum bicolor was designed to develop techniques for haploid induction by using an anther culture technique.

In anther culture, there are several important factors affecting the induction of plants from pollen. The first one is the cultivar. Genetic constitution determines, to a great extent, the induction of pollen plants (Research Group 303, 1977, Wenzel and Uhrig 1981, Foroughi-Wehr et al.,1982) In the study of corn anther culture in one laboratory, 159 cultivars were tested, only nine responded (Miao et al., 1978). The influences of cultivar on anther culture also were reported for wheat, rice (Ouyang, 1986; Chen, 1986), and tobacco (Phillips and Collins, 1977). Thus selection of cultivars is necessary for anther culture study.

The second factor is the composition of medium. Kinds of auxin and cytokinin, and their ratios in media influence anther culture response (Sunderland, 1971 and 1977a; Dunwell, 1985). The level of sucrose in the medium also influences the induction of callus from anthers (Chen, 1986). A study in China on plant induction from maize pollen showed the effect of sucrose concentration on callus induction. High frequency of callus formation was observed in N_6 medium with 12% sucrose (Miao et al., 1978). In Genovesi and Collin's (1982) study on corn anther culture, YP medium was used. They found that the removal of activated charcoal from the medium resulted in a significant reduction of callus or embryoid induction.

The third important factor in anther culture is environmental conditions. Rose et al. (1986) found in sorghum anther culture that incubation temperature had a significant effect on yields of microspore-derived callus. A temperature of 33°C was superior to 25°C, 28°C, or 37°C. Callus induction can be affected also by illumination. In sorghum and maize anther culture, Rose et al. (1986), Ting et al. (1981), and Genovesi and Collins (1982) used constant darkness. However, for callus differentiation, certain photoperiods are needed. In the induction of pollen callus in wheat, a long photoperiod (about 14 h) was much better than a short photoperiod (about 8 h) for plant differentiation (Li, 1978).

In addition, the appropriate developmental stage of the microspores determines, to a certain extent, the success of anther culture (Hu and Zeng, 1984). Although tetrads and mature pollen can develop into pollen plants (Bouharmont, 1977; Kameya and Hinata, 1970), the yield of plants from pollen is dependent on their developmental stage. For example,the callus induction frequency was the highest with wheat anthers containing mid- or late-uninucleate microspores (He and Ouyang, 1980). Similar results were obtained from many other species (Hu and Zeng, 1984).

I studied the relative importance of cultivar, media, culture conditions, pretreatment, and the interactions among them in sorghum anther culture.

Materials and Methods

Twenty-nine cultivars were used in this study. They were Xin-W, TSB (selected from a trisomic progeny of Tx403), DDY Soonermilo, TX2779, Brauley, Spur Federal, C401, Tlgm (selected from a trisomic progeny of Tx403), Sart, Rio, Early Sumac, TX2771, Orange, Honey, Sugar Drip, AT3197,Martin B, Redlan B, KS34A, KS35A,KS36A, KS37A, KS38A, KS39A, Martin A, Redlan A, S. almum (PI 302249) (2n=40), S. versicolor (2n=10), KS34xSal6 (sorghum bicolor x S. halepense hybrids), and Jn3-11 6750-1-8xSal2 (sorghum bicolor x S. halepense hybrids). Major cultivars tested were Xin-W, Tx401, Double dwarf yellow soonermilo (DDYS), TX2779,

Brauley, Spur Federal, and C401.

Twenty eight kinds of media were selected for callus induction (MS-t, MS-t-z, MS-t-z-2, MS-t-z-3, MS-t-z-4, C17, C17-2, C21, C22-1, C22-2, C22-3, C23, 85D3, 85D12, 85D13, 85D14, 85D15, 85D16, 85D17, N_6, N_6-2, N_6-K, J1, J2, J3, P1, P2, and YP), which mainly differed in the components of major, minor elements and hormones. The basic components of the media were those used in wheat and corn anther culture. Four of the most effective media among the 28 that we screened are presented in Table 1. Hormonal differences among major induction media are listed in Table 2.

Inflorescences were collected from various plants grown in a greenhouse. As a rule, the anthers contain microspores at the uninucleate stage when the emerging base of the flag leaf measures about 2.5 cm from the first leaf or when the lateral expansion of the panicle just caused the leaf sheath to split open. Owing to variations among cultivars, plants, and seasons, the rule mentioned above was not always a reliable indicator of development stages of microspores. Thus anthers with microspores at the uninucleate stage were selected by observation of stained microspores under a light microscope. Panicles were surface sterilized with 60% ethanol for 3 minutes. Anthers were dissected out from floral buds and placed directly onto media (about 20 anthers on every petri dish). The dishes were sealed with parafilm and kept in darkness at 28 ± 1°C and 33 ± 1°C, respectively

on response of anthers in vitro, inflorescences were collected randomly over the course of the growing season.

After calluses developed to 3 mm in diameter, they were transferred to modified MS media containing IAA and kinetin at different ratios,and SHAP media with and without hormones for redifferentiation. The influence of callus age on callus differentiation was evaluated. For the further growth of regenerated plants, they were transferred to either vermiculite or culture solution. The key for successful transformation was temperature. The best temperature for the survival of plants was 16 ± 1°C. After a good root system developed, the plants were transferred to pots and maintained in a greenhouse.

The technique used to count chromosomes was described by Hoang and Liang (1987). Regenerated seedlings were grown in vermiculite and root tips were collected and fixed in 3 ethanol : 1 propionic acid solution with 1% $FeCl_3$ as an additive. They were stained in 1% acetocarmine for 0.5 to 1 h., then immersed into 4% pectinase solution for 15 to 20 h at 24 ± 1°C. Then squashes were made and chromosomes were counted.

Results and Discussion

CALLUS INDUCTION

a. Effect of media on callus induction

Among 28 media tested with anthers of various

cultivars, only 12 produced calluses or embryoids. They were MS-t, MS-t-z, MS-t-z-2, MS-t-z-3, MS-t-z-4, C17, C17-2, C22, 85D3, 85D3-2, N_6, and N_6-2. Media C21, C22-2, C22-3, C23, 85D12, 85D13, 85D14, 85D15, 85D16, 85D17, N_6-K, J1, J2, J3, P1, P2, and YP did not induce any callus.

Media which were effective in inducing calluses were evaluated further across three cultivars, Xin-W, TSB, and Spur Federal (Tables 3 and 4). The most effective media were C17-2 and MS-t-z-2. They elicited callus formation of 6.2% and 5.6%, respectively, across three cultivars (Table 4). Media from MS-t-z and C series apparently have higher callus-inducing capacity than those from N_6 and 85D series for the cultivars tested in this study.

The differences of hormone composition of several major media were listed in Table 2. In addition to the listed differences, there are some other differences among them, such as the NH_4^+ to NO_3^- ratio. MS-t series had a ratio of 0.87; C series had a ratio of 0.21; N_6 and 85D3 series have the same ratio of 0.16. The minor elements were also different in kinds and concentrations among these media. MS-t and C series contained Cu, Co, and Mo elements while N_6 and 85D3 series did not. Mn and Zn concentrations in MS-t and C series is much higher than that in N_6 and 85D3 series. Zn was involved in the biosynthesis of the plant auxin (IAA) (Skoog, 1940).

Among media that induced calluses from anthers and

those did not, it is difficult to pin point a simple cause of callus induction because the media formulations are so different. When the media were examined series by series, the significant differences between these callus-inducing and non-inducing media are the kinds and concentration of auxin and cytokinin used in the media. For example, in C series where C17 contained 2,4-D (2 mg/l) and others contained other auxins such as C22 contained IBA (2 mg/l), C18 with dicamba (2 mg/l), C19 had 2 mg/l pCPA (p-chlorophenoxyacetic acid), C20 contained 2 mg/l PIC (picloram), and C21 contained 2,4,5-T (1 mg/l). C23 did not contain any auxin but zeatin (0.22 mg/l), a cytokinin. Among these media, except for C17 which induced 1.8% anthers to form callus and a few calluses were initiated from anthers on the C22 medium, all others failed to induce callus from sorghum anthers. The major difference between the C series and the 85D series was the types of auxins and cytokinin contained except for 85D3. Most media in the 85D series, such as 85D12 (NAA 0.75 mg/l, kinetin 1.5 mg/l), 85D13 (NAA 0.5 mg/l, kinetin 1.5 mg/l), 85D14 (NAA 0.5 mg/l, IAA 0.5 mg/l, kinetin 1.5 mg/l), 85D15 (IAA 0.5 mg/l, IBA 0.5 mg/l, kinetin 1.5 mg/l), 85D16 (NAA 0.75 mg/l, N^6,2-isopententenyl adenine 1.5 mg/l), and 85D17 (NAA 0.75 mg/l, N^6-benzyleadenine 1.5 mg/l) did not induce anthers to form callus. NAA, IAA, and IBA were not as effective as 2,4-D in inducing callus. The concentration of NAA,IAA,and IBA

used were lower also than that of 2,4-D in the C17 series. This could be one reason why the 85D series except 85D3 did not induce callus. The influence of hormones on anther culture not only depends on the kinds, ratio, and concentrations of different hormones, but also on the composition and concentrations of major salt, sugar, and other components in the media. In this experiment, when NAA combined with major elements of 85D the series , no response was observed in sorghum anthers . When NAA was combined with major components of N_6 to form a new medium (85D3), relatively good results were obtained. The same phenomenon was reported in anther culture of other crops (Chen,1986).

Besides the differences in auxin and cytokinin, differences in major inorganic salt components, vitamins, and proteins could be important also between the callus-inducing and the non-inducing media.

Concentration of sucrose influenced callus induction in this study (Table 5, 6). Three levels of sugar concentration (20, 60, and 90 g/l) were tested to determine if the concentration of sucrose affects callus induction rate. In the MS-t-z and C series, which differed only in the concentration of sucrose, 20 and 60 g/l of sucrose gave a very high frequency (7.7% and 6.9%) of callus induction while sucrose at 90 g/l yielded a very disappointing result (1% callus). The result of this study indicated that

higher sucrose concentration (90 g/l) inhibited callus formation. No significant difference in callus induction between media containing 2% and 6% sucrose was observed. A similar observation was reported also in rice anther culture (Chen, 1986). Sucrose usually is added into the medium as a carbon source and as an osmoticum. It was suggested that sugar concentration influences the speed and quantity of inorganic salts and exogenous hormones absorbed by plant cells, leading to a series of secondary effects (Chen, 1986).

b. Effect of cultivar and hybrids on callus induction

Over a period of 18 months, 21,340 anthers of various cultivars including inbred, hybrid, and a few wild species were cultured. Many cultivars were tested on various media, but only a few responded. they were Xin-W, TSB , DDY soonermilo, TX2779, Brauley, and Spur Federal.

Genotypes which did not respond or gave a very low response include:C401, Tlgm, Sart, Rio, Early Sumac, TX2771, Orange, Honey, Sugar Drip, AT3197,Martin B, Redlan B, KS34A, KS35A,KS36A, KS37A, KS38A, KS39A, Martin A, Redlan A, S. almum (PI 302249) (2n=40), S. versicolor (2n=10), KS34xSal6, and Jn3-11 6750-1-8xSal2.

Among the cultivars and hybrids which had a response, significant differences ($p<0.01$) were observed. The genotypic effect was evaluated across five media. Statistical analysis indicated that the anthers of Xin-W and

TSB cultured on media MS-t-z-2, C17-2, C17, and 85D3-2 produced many more calluses than the anthers of DDY Soonermilo, TX2779, Brauley, and Spur Federal cultured on the same media (Table 7). The mean response of Xin-W and TSB anthers were 8.9 and 5.1%, respectively, when anthers were cultured on MS-t-z-2 medium; 10.7 and 6.6%, respectively, when anthers were cultured on C17-2 medium. (Table 8) Although Xin-W was significantly better than other cultivars in callus induction, when anthers of Xin-W were cultured on 85D3-2 medium, the response was less than that for TSB anthers cultured on MS-t-z-2 and C17-2 (Table 8). This indicated that the interaction of variety and media was another important factor involved in anther culture.

In this experiment, anthers from only 6 of 29 cultivars developed calluses. In corn anther culture study, it was reported that among 159 cultivars tested, only 9 responded (Miao et al., 1978). The difference of cultural ability of various cultivars was considered by some scientists as an impassable barrier using anther culture for crop improvement (Chen, 1986). Much work is needed to reveal the genetic control of the regeneration process.

c. Influence of temperature on callus induction

Statistical analysis showed that anthers of Xin-W cultured on MS-t, MS-t-z and MS-t-z-2 media under 33^{o}C gave significantly higher response than at 28^{o}C (Tables 9 and 10). This is parallel to the result from Rose et al. (1986).

CALLUS REDIFFERENTIATION

a. Redifferentiation medium

To induce callus differentiation, MS medium was used with different concentrations of IAA and kinetin (Table 11). MS-d-9 (3.5 mg/l IAA, 2.5 mg/l kinetin) and MS-d-7 (3.5 mg/l IAA, 2.5 mg/l zeatin) gave the highest frequency of callus redifferentiation. However, among the differentiated callus, a high percentage showed only roots. MS-d-1 (2 mg/l IAA, 2 mg kinetin), MS-d-6 (2 mg/l IAA, 2.5 mg kinetin) gave a relatively low frequency of callus redifferentiation. A high frequency of regeneration was obtained from the calluses transferred to MS-d-4 and MS-d-9 media, respectively. IAA stimulated the formation of roots, while kinetin increased shoot induction. The optimum concentrations of IAA and kinetin and their ratio should be studied further to determine optimum amounts for plant regeneration from calluses. Generally, calluses started to undergo redifferentiation one to three weeks after they were transferred to the redifferentiation medium. For normal differentiation of calluses, shoots formed first (Fig. 1 and 2). If roots initiated first, it was difficult to obtain plants.

b. Induction time

Data indicated that the induction time (from anther inoculation to callus transferring) strongly affected callus differentiation (Table 12). As the induction time increased,

the number of differentiated calluses and plants derived from calluses decreased, and calluses with only roots and albino plants increased (Figs. 3 and 4). The longer (more than 50 days) the calluses remained on induction media, the weaker ability of callus to differentiate.

Although a high percentage of plants was obtained from calluses in redifferentiation media, contamination of redifferentiated calluses occurred. A reliable method to transfer regenerated plants to vermiculite is also needed. Only six plants developed to maturity in a greenhouse. The critical factor for successfully transferring plants from redifferentiation media to pots containing vermiculite or to a culture solution was the temperature after transplanting. Low temperature (16 ± 1°C) was necessary for plant development after transfer. Liquid MS-t medium (MS-t-z medium without agar, sucrose, 2,4-D, zeatin and kinetin) was used for culturing regenerated plants. New roots began to grow about one week after transplanting. Some plants with a very weak root system or even with no roots developed a root system (Fig. 5). About 80% of plants cultured in the MS-t-z solution initiated new roots. After a good root system developed , the plants could be transferred to pots and maintained in the greenhouse at 25-28°C. Use of a culture solution also provides the convenience of taking root tips for chromosome counts.

c. Occurrence of albino plants

Callus induction time strongly affected the frequency of albino plants (Table 12). Temperature was another factor that affects the production of albino plants. All the albino plants in this experiment came from anthers cultured at 33°C. No albino plants were produced from anthers cultured at 28°C. It is possible that the critical temperature for induction of albino plants varies with different crops. A study on wheat anther culture showed that when inoculation temperature was higher than 30°C most calluses differentiated into albino plants (Chen, 1986). Other data (Dunwell, 1978) showed that an increase of temperature from 24°C to 26°C produced approximately twice as many albino plants with a further increase up to 100% at 35°C. Possible origins of albinos are:

1) Inability of proplastids to develop into chloroplasts during gametophytic development (Huang, 1986; Dunwell, 1978)

2) The culture procedure induces a genetic change in embryogenic pollen and the cytoplasmic reorganization lead to permanent destruction of chloroplasts.

3) Albino plants are the results of chloroplast DNA deficiency during the culture process (Day & Ellis, 1984).

4) Unsuitable culture conditions for chloroplasts to develop normally.

The albinism seems to be partly genetic and partly environmental in origin. Data from this experiment support

such a contention. The fact that a long callus-induction time induced more albino plants may be explained as follows:

Segregation of altered chloroplast genomes produced before or during the callus stage would be expected in the following cellular division (Birkey, 1978). According to Day and Ellis (1984),there may be selective forces ensuring the preferential amplification and therefore survival of particular chloroplast genomes during this phase. This implies that the altered ctDNA molecules present in albino pollen plants represent a subset of a larger spectrum of altered ctDNA molecules associated with anther culture. So, the longer the duration of callus culture, the more such subset of altered ctDNA could be produced and more albino plants could be initiated.

Based on Huang's (1986) hypothesis that a transitional phase occurs in the development of microspores. Pollen plants would comprise mainly green plants if multiple nucleate pollen grains (MPG) originate from microspores which have not passed through the transitional phase. In contrast, if MPGs originate from microspores which have passed the transitional phase, the regenerated pollen plants would comprise mainly albinos. It is a conjecture that the high culture temperature in this experiment enhanced the rate of microspore development. Thus more microspores could pass the transitional phase before they developed to the

MPGs stage despite excision of microspores before the transitional phase. Thus high temperature induces more albino plants. The temperature at which transition is enhanced seems to be crop dependent, because different crops have different critical albino-plant induction temperatures.

d. Chromosome numbers in the regenerated plants

Chromosome counts from root tips of regenerated plants showed different numbers. Most of them were 20. Cells with chromosome numbers of 10 (Fig. 6), 15, and 17 also were observed. However, pollen mother cells showed only 20 chromosomes (Fig. 7). The majority of root tips from calluses, which had been treated with 37°C temperature for seven days, had 40 chromosomes. Other chromosome numbers of 10, 44 (Fig. 8), and 60 were also observed. Chromosome pairing was observed in some root tip cells derived from anthers-callus (Fig. 9). Instability of chromosome number and structure is a characteristic of cultured cells of both animal and plant tissues (Bayliss 1973, Sunderland 1977b, and Constantin, 1981). Some diploid plants in this experiment were generated from the filament of the anthers. Anther wall may be another origin of diploid cells. On the other hand, when anthers without filament were cultured, they still formed callus (callus was not derived from the position where the filament was located); anther walls (anthers with pollen removed) were cultured, but they did not form callus. So the source of diploid plants regenerated

from anther calluses is unknown. Despite the absence of haploid plants in this series of experiments, it is still possible that some of the diploid plants were haploid in origin, because:

1) Spontaneous chromosome doubling is one possible source of diploid cells in the regenerated plants. Genetic studies (Ouyang et al., 1973) on wheat anther culture provide ample evidence that hexaploid plants can be derived from microspores. That indicated some of the mechanisms by which doubling of a genome may occur. Both 10 and 20 chromosomes were observed in cells from the same root tip of regenerated plants.

2) Some main somatic sources, such as anther wall and filament, of diploid cells in the regenerated plants could be ruled out. Tapetum cells are binucleate and terminally differentiated (Bennett et al., 1973) and anther wall was observed to senesce in tissue culture. Most success in initiating callus has been from tissues that are young and actively dividing, such as the basal leaf meristem, young roots, and immature embryos (Wernicke and Milovits, 1984). No callus was initiated from the anther wall in this experiment. For these reasons, it seems unlikely that anther calluses were derived from the cells of the anther wall. Efforts were also made to remove the filaments completely and anthers without filaments were still able to form callus (Fig. 10).

3) A few albino plants were obtained in this series of experiments. A study on barley anther culture indicated that albinos are common in pollen plants whereas plants developed from normal zygotic embryos are exclusively green (Huang,1986). In somatic cell cultures, such as leaf-disk culture of African violet (Saintpaulia ionantha) and embryo culture of wheat (Triticum aestivum), albinos never appear (Liang et al., 1988). The mechanism for albino development is not clear, but to date all the explanation of it has emphasized the state of plastids, chloroplasts, and mitochondria in the pollen or unpollinated ovary, rather than somatic cells. Therefore, occurrence of albinos may be an indication of microspore plants.

Because the regenerated plants came from anthers of inbreds, it is very difficult to distinguish spontaneous doubled dihaploids from somatic-cell-derived plants. In the future, more hybrids will be used. To facilitate the identification of haploid plants, more cytological observations such as the development of cultured microspore and callus cells should be made. In the light of rice anther culture, where only 50-60% of the plants regenerated from anther culture were haploids, a large number of regenerated plants may have to be examined to determine if haploid plants may be obtained from sorghum anther culture.

REFERENCES

Bayliss M. W. 1973. Origin of chromosome number variation in culture plant cells. Nature 246:529-530.

Bennett, M. D., M. K. Rao, J. B. Smith, and M. W. Bayliss. 1973. Cell development in anthers, the ovule and the young seed of Triticum aestivum L. var. Chinese Spring. Phil. Tran. R. Soc. Lond. B 266:39-81.

Birkey, C. W. 1978. Transmission genetics of mitochondria and chloroplasts. Ann. Rev. Genet. 12:471-512.

Bouharmont, J. 1977. Cytology of microspores and calli after anther culture in Hordeum vulgare. Caryologia 30:351-360. Chang, Y., M. Yu, and W. Z. Zheng. 1981. Improved anther culture of maize (Zea mays). Pl. Sci. Lett., 23:139-145.

Chen, Y. 1986. Anther and pollen culture of rice. p. 1-25. In: H. Hu, and H. Yang (ed.) Haploid of Higher Plants in Vitro. Springer-Verlag, New York.

Constantin, M. J. 1981. Chromosome instability in cell and tissue cultures and regenerated plants. Envir. Exp. Bot. 21:359-368.

Day, A., and T. H. N. Ellis. 1984. Chloroplast DNA deletion associated with wheat plants regenerated from pollen: possible basis for maternal inheritance of chloroplasts. Cell 39:359-368.

Dunwell, J. M. 1978. Division and differentiation in

cultured pollen. p.103-112. In: T. A. Thorpe (ed.) Frontiers of Plant Tissue Culture. Intern'l Assoc. Plant Tiss. Cult.. Calgary. Canada. Dunwell, J. M. 1985. Anther and ovary culture. p.1-44. In: S. W. J. Bright and M. G. J. Jones (ed.) Advances in Agricultural Biotechnology: Cereal Tissue and Cell Culture. Dordrecht:Martinus Nijhoff/Dr.W.Junk.

Foroughi-Wehr B, W. Friedt, and G. Wenzel. 1982. On the genetic improvement of androgenetic haploid formation in Hordeum vulgare L. Theor. Appl. Genet. 62:233-239.

Genovesi, A. D., and G. B. Collins. 1982. In vitro Production of haploid plants of corn via anther culture. Crop Sci. 22:1137-1144.

Guha, S. and Maheshwari, S. C. 1964. In vitro production of embryos from anthers of Datura. Nature 204: 497.

Guha, S., and Maheshwari, S. C. 1966. Cell division and differentiation of embryos in the pollen grains of Datura in vitro. Nature 221:97-98.

He, D. G., and J. W. Ouyang. 1980. Effect of stages of anther development on anther culture of wheat (Triticum aestivum L.). p.74. In Annual Report of the Institute of Genetics, Academia Sinica.

Hoang, T, and G. H. Liang. 1987. An improved technique for cytological observations and occurrence of polysomaticism in sorghum root tips. Jour. Hered. 78:51-53.

Hu, H., and J. Z. Zeng. 1984. Development of new varieties via anther culture. p.65-90. In: Ammirato et al. (ed.) Handbook of Plant Cell Culture. Vol. 3. MacMillan Publishing Co., New York, Collire MacMillan, Landon.

Huang, B. 1986. Ultrastructural aspects of pollen embryogenesis in Hordeum, Triticum, and Paeonia. p.91-117. In: H. Hu, and H. Yang (ed.) Haploid of higher plants in vitro. Springer-Verlag, New York.

Kameya, T., and K. Hinata. 1970. Induction of haploid plants from pollen grains of Brassica. Jpn. J. Breed. 20:82-87.

Li, C. L. 1978. Effects of temperature and illumination on induction of pollen plants in wheat. p.290. In: H. Hu et al. (ed.) Proc. Symp. Anther Culture. Science Press, Beijing, China.

Liang, G. H, A. Xu, and T. Hoang. 1987. Direct generation of wheat haploids via anther culture. Crop Sci. 27:336-339.

Liang, G. H., J. H. Qi, and D. Hassawi. 1988. A direct generation system for wheat haploid production. In: Y. P. S. Bajaj (ed.) Biotech. Agr. Forestry. Springer-Verlag. (In Press).

Miao, S. H., C. S. Kuo, Y. L. Kwei, A. T. Sun, S. Y. Ku, W. L. Liu, Y. Y. Wang, M. L. Chen, M. K. Wu, and L. Hang. 1978. Induction of pollen plants of maize and observations on their progeny. In Proc. Symp. Plant

Tissue Culture, 25-30 May 1978. Science Press. Beijing, China.

Nitsch, C., S. Andersen, M. Godard, M. G. Neuffer, and W. F. Sheridan. 1982. Production of haploid plants of Zea mays and Pennisetum through androgenesis. p.69-91. In: E. D. Earie and Y. Demarly (ed.) Variability in Plants regenerated from tissue culture. Praeger Press, New York.

Ouyang, J. 1986. Induction of pollen plants in Triticum aestivum. p.26-41. In: H. Hu, and H. Yang (ed.) Haploids of Higher Plants in Vitro. Springer-Verlag, New York.

Ouyang, T. W., H, Hu, C. C. Chuang, and C. C. Tseng. 1973. Induction of pollen plants from anthers of Triticum aestivum L. cultured in in vitro. Sci. Sin. 16:79-95.

Phillips, G. C. and G. B. Collins. 1977. The influence of genotype and environment of haploid plant production from anther cultures of Nicotiana tobacum. Tob. Sci. 21:112-116.

Research Group 303, Institute of Genetics, Academia Sinica. 1977. Studies on the inducing factors and genetic expressions of Triticum aestivum pollen plants. p.65-81. In: Institute of Botany, Academia Sinica (ed.) Collection of informations on haploid breeding, vol.3. Science Press, Beijing, China.

Research Group 401. 1975. Primary study on induction of

pollen plants of Zea mays. Acta Genet. Sin. 2:138-143.

Rose, J. B., J. M. Dunwell, and N. Sunderland. 1986. Anther culture of Sorghum bicolor (L.) Moench. I. Effect of panicle pretreatment, anther incubation temperature and 2,4-D concentration. Plant Cell Tiss. Org. Cult. 6:23-31.

Smith, R. 1985. Personal communication from Texas A&M Univ.

Skoog, F, 1940. Relationships between zinc and auxin in the growth of higher plants. Am. J. Bot. 27:939.

Sunderland, N. 1971. Anther culture: a progress report. Sci. Prog. Oxf. 59: 527-549.

Sunderland, N. 1977a. Pollen and anther culture. p.223-268. In: H. E. Street (ed.) Plant Tissue and Cell Culture. Blackwell Scient. Publ., Oxford, U. K.

Sunderland, N. 1977b. Nuclear cytology. p.177-205. In: H. E. Street (ed.) Plant tissue and cell culture. Univ. Calif. Press, Berkely.

Ting, Y. C., M. Yu, and W. Z. Zheng. 1981. Improved anther culture of maize (Zea mays). Plant Sci Lett. 23:139-145.

Wernicke, W., and L. Milovits. 1984. Developmental gradients in wheat leaves - response of leaf segments in different genotypes cultured in vitro. J. Plant Physiol. 115:49-58.

Wenzel, G, and H. Uhrig. 1981 Breeding for nematode and virus resistance in potato via anther culture. Theor.

Appl. Genet. 59:333-340.

Table 1. Composition of callus induction media

Composition	MS-t-z-2	C17-2	85D3-2	N6-2
	mg l^{-1}			
KNO_3	1,900	1,400	2,830	2,830
KH_2PO_4	170	400	400	400
NH_4NO_3	1,650	300	--	--
$(NH_4)_2SO_4$	--	--	463	463
$CaCl_2.2H_2O$	440	150	166	--
$MgSO_4.7H_2O$	370	150	185	185
(or $MgSO_4$)	180.6	73.2	90.3	90.3
Na_2-EDTA	37.3	37.3	37.3	37.3
$FeSO_4.7H_2O$	27.8	27.8	27.8	27.8
$MnSO_4.4H_2O$	22.3	11.2	4.4	4.4
(or $MnSO_4.H_2O$)	17.1	8.6	3.4	3.4
$ZnSO_4.7H_2O$	8.6	8.6	1.5	1.5
H_3BO_3	6.2	6.2	1.6	1.6
KI	0.83	0.83	0.8	0.8
$CuSO_4.5H_2O$	0.025	0.025	--	--
$CoCl_2.6H_2O$	0.05	0.025	--	--
$NaMoO_4.2H_2O$	0.25	--	--	--
Glycine	2	2	2	2
Thiamine.HCl	0.1	1	1	1
Pyridoxine.HCl	0.5	0.5	0.5	0.5
Nicotinic acid	0.5	0.5	0.5	0.5
Myo-inositol	100	--	--	--
Casein Hydrolysate	--	--	500	--
Biotin	--	1.5	--	--
2,4-D	3	2	--	2
NAA	--	--	1	--
Kinetin	0.3	1	1.5	1
Zeatin	2.2	--	--	--
Sucrose	20,000	20,000	30,000	30,000
Difco agar	5,000	7,000	5,000	10,000
pH	5.8	5.8	5.8	5.8

Table 2. Hormone and sucrose differences among 12 callus induction media.

Media	2,4-D	Kinetin	Zeatin	Sucrose
	mg l^{-1}			
MS-t	2.5	0.05	---	20
MS-t-z	2.5	0.05	2.2	20
MS-t-z-2	3.0	0.30	2.2	20
MS-t-z-3	3.0	0.30	2.2	60
MS-t-z-4	3.0	0.30	2.2	90
C17	2.0	1.00	---	90
C17-2	2.0	1.00	---	20
N6	2.0	0.50	---	90
N6-2	2.0	0.50	---	20
85D3	1.0(NAA)	1.50	---	60
85D3-2	1.0(NAA)	1.50	---	30
C22	2.0(IBA)	1.00	---	--

Table 3. Effect of 8 media on callus induction of 3 cultivars.

Media	Cultivar		
	Xin-w	TSB	Spur Federal
	No. of calli*		
MS-t-z-2	8.9 ± 1.8	5.1 ± 1.8	1.9 ± 2.1
MS-t-z	4.6 ± 2.1	5.6 ± 3.7	0
C17-2	10.7 ± 2.1	6.6 ± 2.1	1.3 ± 2.1
C17	4.0 ± 1.8	0	0
N6-2	2.0 ± 2.1	2.1 ± 2.6	0
N6	0	3.6 ± 2.6	0
85D3-2	0.4 ± 2.1	--	0
85D3	1.4 ± 2.6	0	0

* Mean values and standard errors for number of calli induced from 100 anthers

Table 4. Effect of media on callus induction and embryoid formation for 3 cultivars.

Media	Frequency of calli induction
	—— %* ——
C17-2	6.2 a**
MS-t-z-2	5.6 ab
MS-t-z	3.4 bc
C17	1.8 bc
N_6-2	1.7 c
N_6	1.0 c
$85D_3$-2	0.7 c
$85D_3$	0.2 c

* Percentage of anthers producing callus or embryoid

** Means within a column of each medium with the same letter are not significantly different ($P < 0.05$) according to LSD test.

Table 5. Effect of sucrose on callus induction from anthers of Xin-W cultivar on 2 media.

Sucrose	Media	
	MS-t-z	C17
— g l^{-1} —	No. of calli*	
20	8.5 ± 2.4	3.2 ± 2.7
60	7.6 ± 3.5	---
90	0.3 ± 2.4	0.6 ± 2.7

* Mean values and standard errors for number of calli induced from 100 anthers.

Table 6. Effect of sucrose concentration on callus induction and embryoid formation.

Sucrose	Frequency of calli induction
— g l^{-1} —	—— %* ——
60	7.7 a**
20	6.9 a
90	1.0 b

* Percentage of anthers producing callus or embryoid

** Means within a column with the same letter are not significantly different ($P < 0.05$) according to LSD test.

Table 7. Cultivar response to sorghum anther culture on 5 media.

Cultivar	Frequency of calli induction
	—— %* ——
Xi-W	6.1 a**
TSB	4.1 ab
DDY sooner milo	2.3 b
TX2776	1.5 b
Brawley	0.7 b
Spur Federal	0.7 b

* Percentage of anthers producing callus or embryoid.

** Means within a column of each cultivar with the same letter are not significantly different ($P < 0.05$) according to LSD test.

Table 8. Effect of Genotypes on callus induction on 4 media.

Genotypes	Media			
	MS-t-z-2	C17-2	C17	85D3-2
	No. of calli*			
Xin-W	8.9 ± 2.0	10.7 ± 2.3	4.0 ± 2.0	0.4 ± 2.3
TSB	5.1 ± 2.0	6.6 ± 2.3	0	--
DDY sooner milo	1.9 ± 2.3	5.0 ± 2.3	0	0
TX2779	0	2.4 ± 2.3	0	1.1 ± 3.9
Brawley	2.1	0	0	0
Spur Federal	1.9 ± 2.3	1.3 ± 2.3	0	0

* Mean values and standard errors for number of calli induced from 100 anthers.

Table 9. Effect of temperature on callus induction from Xin-W anthers on 3 media.

Temperature (°C)	Media		
	MS-t	MS-t-z	MS-t-z-2
	No. of calli*		
28	0	2.5 ± 0.6	2.2 ± 0.6
33	4.3 ± 0.6	5.8 ± 0.7	8.1 ± 0.6

* Mean values and standard errors for number of calli induced from 100 anthers.

Table 10. Effect of temperature on callus induction and embryoid formation.

Temperature	Frequency of calli induction
— °C—	—— %* ——
33	6.1 a**
28	1.6 b

* Percentage of anthers producing callus or embryoid.

** Means within a column of each temperature with the same letter are not significantly different ($P < 0.05$) according to LSD test.

Table 11. Effect of medium on callus redifferentiation.

Regeneration media	Hormones		No. of Calli	Type of differentiation			
	IAA	Kinetin		calli	roots	shoots	plants
	— mg l^{-1} —				% *		
MS-d-1	2.0	2.0	37	62	30	0	32
MS-d-4	2.0	2.5	20	90	0	30	60
MS-d-6	2.5	2.5	99	70	27	10	30
MS-d-9	3.5	2.5	9	100	44	0	56
MS-d-7	3.5	2.5**	13	100	77	0	23
MS-d-10	3.0	2.5	23	74	88	0	12
MS-d-11	0.5	1.5	67	63	81	10	
	0.5NAA						
MS-d-12	0.5	0.5	79	85	67	10	18
MS-d-13	0	0.5	69	74	88	4	8
SHAP			15	80	40	0	40

* Percentage of the total transfered calli.
** Zeatin replaced kinetin.

Table 12. Effect of callus induction time on callus differentiation.

Induction time*	No. of calli	Type of differentiation				
days		Differentiated calli	roots	shoots (%)**	plants	albinos
30	7	100	0	0	100	0
40	7	100	0	14	86	0
60	13	92	33	17	50	17
70	15	100	38	13	50	25
90	29	100	67	0	33	0
100	23	95	79	0	21	50
120	10	83	80	0	0	0
130	10	80	70	0	10	100

* Days from anther inoculated to callus transferred.
** Percentage was calculated against number of calli transferred.

Fig. 1. Shoots initiated from Xin-W callus on MS-d-12 redifferentiation medium.

Fig. 2. Plants derived from Xin-W anther calli on MS-d-12 medium. The normal way for callus redifferentiation was shoots initiated first then roots.

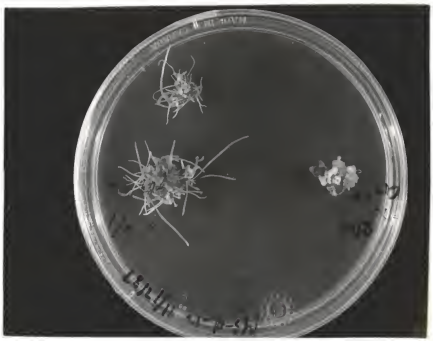

Fig. 3. Photograph showing the effect of callus induction time on root production. The callus on the left was transferred to differentiation medium MS-d-12 after about 90 days in induction medium C17-2, while the calluses on the right were transferred to MS-d-12 after about 150 days in C17-2.

Fig. 4. An albino plant produced from Xin-W anther culture.

Fig. 5. Photograph showing a plant without root developed a root system after it was transferred from redifferentiation medium to liquid MS-t medium.

Fig. 6. A root-tip cell of regenerated Xin-W plant with haploid chromosome number (2n=10).

Fig. 7. A pollen mother cell of regenerated Xin-W plant with somatic chromosome number (2n=20).

Fig. 8. A root tip cell with 44 chromosomes of a regenerated Xin-W plant from anther culture.

Fig. 9. Chromosome pairing in some root tip cells in plants derived from Xin-W anther calluses.

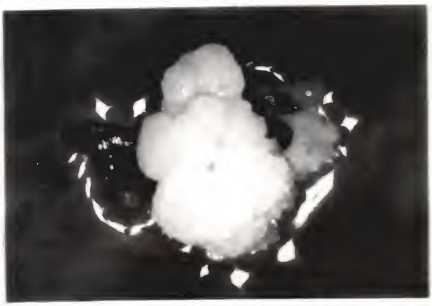

Fig. 10. The anther with filament removed were still able to form callus (callus was not derived from the position where the filament was located).

ANTHER CULTURE OF SORGHUM BICOLOR (L.) MOENCH

by

FUSHI WEN

B.S., Northwestern University, China, 1982

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Genetics

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1988

ABSTRACT

Haploid plants are very useful for breeding new cultivars. Theoretically, haploids can be obtained from microspores when anthers are placed on a specifically formulated environment. Anther culture of sorghum bicolor has been unsuccessful. The objective of this study was designed to develop techniques for haploid induction from anther culture of sorghum bicolor (L.) Moench.

For callus induction, anthers containing microspores at uninucleate stage were sampled. Twenty-nine genotypes were tested on various media and six of them responded. Anthers of Xin-w and TSB produced many more calluses than anthers of other four genotypes did. Twenty-eight media were screened for callus induction and twelve induced calluses. C17-2 and MS-t-z-2 were the most effective media. When anthers of Xin-w were cultured on C17-2 medium, 10.7% anthers produced calluses. Media containing sucrose at 20-60 mg/l level induced significantly higher frequency of callus induction than those containing sucrose at 90 g/l level did.

For callus differentiation, modified MS and SHAP media were used. MS-d-4 with 2.0 mg/l IAA and 2.5 mg/l kinetin was best medium for callus differentiation. Longer callus induction time increased the frequency of calluses with only roots and frequency of albino plants production, while decreased the frequency of callus differentiation and plants regeneration.

Chromosome counting of regenerated plants showed

different numbers: 20, 10, 15, 40, and 60. Despite the absence of haploid plants, it was still possible that some of the diploid plants were haploid in origin in this study.