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Anther Culture of Wheat (*Triticum aestivum* L.) F_1's and Their Reciprocal Crosses*

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Summary. Anthers from three sets of wheat (*Triticum aestivum* L. em. Thell) F_1's and their reciprocal crosses, made between parental lines differing greatly in their ability to produce microspore derived callus, were cultured on the Chinese potato medium so that we could 1) more clearly define the role of nuclear or cytoplasmic factors within *T. aestivum* in transferring the ability to undergo in vitro androgenesis, and 2) to briefly review the gametic representation and disease screening potential of the resulting polyhaploid wheat plants. The microspore derived calli values from F_1's were slightly less than the midparental value. Statistical analysis indicated that the ability of each F_1 to produce callus either did not significantly differ from that of the respective parental line having the highest androgenic yield or it exceeded its respective parental line having the lowest yield. No differences were noted between the members of each pair of reciprocal crosses. The results indicate that the transfer of in vitro androgenic ability to F_1 hybrids is not dependent upon the maternal cytoplasm source. Polyhaploid plants, carrying the Pm 3a powdery mildew resistance gene, expressed resistance to culture 4a of powdery mildew.

Key words: Polyhaploids – Microspore – *Erysiphe graminis tritici* – in vitro androgenesis

Introduction

Our understanding of in vitro androgenesis has increased greatly since the initial success of Guha and Maheshwari (1964) in obtaining haploid embryos and plantlets from the anthers of *Datura inoxia* Mil. We now know that in many crops certain species or cultivars demonstrate a proclivity to anther culture while others are still very difficult or impossible to culture. For the plant breeder to take full advantage of the potential benefits offered by the products of this doubled haploid system, the factors which limit anther culture to certain species or cultivars must be further understood. The anther culture of wheat has provided some insight into this area.

Chinese (Chu et al. 1973; Ouyang et al. 1973), French (De Buyser and Henry 1979; Picard and De Buyser 1975, 1977; Picard et al. 1978), and American (Schaeffer et al. 1979) researchers have reported variation in the production of microspore derived callus among cultivars and/or hybrids of *Triticum aestivum* L. em. Thell. Heszky and Mesch (1976) successfully cultured only 11 of 66 wheat cultivars and believe that genetic factors determine cultivar response to anther culture media. Although these various studies indicate the importance of plant genotype in mediating in vitro androgenic ability, a formal analysis of any determinant genetic factors is still lacking.

Rives and Picard (1977) have theorized that a recombinational event may result in the development of microspores better suited to in vitro androgenic development. Additionally, Picard et al. (1978) have stated that cytoplasmic influences affect the embryoid production in cytoplasmic male sterile (*T. timopheevi* cytoplasm), nuclearly restored fertile, and normal fertile lines of 'Rex'. Picard and De Buyser (1977) have also reported that increased in vitro androgenic ability is found in wheat doubled haploids when compared to their parental cultivars. This increased in vitro androgenic ability of the doubled haploids was reported as being a heritable character. Thus, preliminary evidence indicates that genetic and cytoplasmic components may be involved in determining the ability to undergo in vitro androgenesis.

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Table 1. Awn type, powdery mildew resistance and in vitro androgenic capacity of the wheat cultivars and F_1 hybrids used as anther donors

Genotypes	Abbreviation	Awn type	Powdery mildew resistance at maturity	In vitro androgenic capacity
Asosan/8* Chancellor	(A)	Tip	Resistant	
Centurk	(C)	Full	Intermediate	Good
Chancellor	(Cc)	Tip	Susceptible	Poor
Kitt	(K)	Full	Susceptible	Good
Potomac	(P)	Tip	–	Poor
F_1 Hybrids				
Asosan/8* Chancellor × Centurk	(A – C)			
Centurk × Asosan/8* Chancellor	(C – A)			
Asosan/8* Chancellor × Kitt	(A – K)			
Kitt × Asosan/8* Chancellor	(K – A)			
Kitt × Potomac	(K – P)			
Potomac × Kitt	(P – K)			

Resistance to *Erysiphe graminis* f. sp. *tritici*, culture 4a. In vitro androgenic capacity as determined by Schaeffer et al. (1979)

In order for anther culture to become a practical technique in plant breeding, a thorough study of these genetic and cytoplasmic components and their ability to be transferred is needed. The objectives of this study were 1.) to determine whether the capacity for in vitro androgenesis can be transferred to F_1 hybrids of wheat when the parents differ vastly in their androgenic ability, 2.) to more clearly define the role of nuclear and/or cytoplasmic factors within *T. aestivum* in the transmission of the ability to undergo in vitro androgenesis, and 3.) to briefly review the gametic representation and disease screening ability of the resulting polyhaploid wheat plants.

Materials and Methods

Five common wheat cultivars were evaluated for their capacity to undergo in vitro androgenesis, (formation of embryoids and/or calli by the in vitro development of microspores). In addition, crosses and their reciprocals were made among four of these cultivars and the resulting F_1 hybrids were tested for their in vitro androgenic capacity. The parents differed widely in awn type, powdery mildew resistance, and capacity for in vitro androgenesis (Schaeffer et al. 1979). A description of the parents and a list of the hybrids are found in Table 1.

The five cultivars and the six F_1 hybrids were grown in a greenhouse environment. Anthers from each of the 11 sources were aseptically excised and cultured similarly to the methods described by Schaeffer et al. (1979). Anthers, containing microspores in the mid to late uninucleate stages, were cultured in tubes on the Chinese (Anonymous 1976) potato (*Solanum tuberosum* L., cv. 'Kennebec') medium. Initially, the tubes containing the anthers were placed in darkness at 4 °C for 64 h and then kept at 26 °C for an additional 5 d before exposure to constant cool white fluorescent light (1.5 uE m^{-2} sec^{-1}). After 6 to 7 weeks, anthers containing macroscopic embryoids and callus were transferred to a shoot regeneration medium (Schaeffer et al. 1979). When the resulting plantlets had grown to a height of 3 cm, they were placed in flasks containing a liquid root regeneration medium (Schaeffer et al. 1979). Regenerated plants were potted in Jiffy Mix[1] and kept under conditions of high humidity and continuous low light (6 uE m^{-2} sec^{-1}) for 5 d. The plants were then repotted in sterilized soil and grown in the greenhouse.

For the statistical analysis of in vitro androgenesis, the experiment was considered a completely randomized design, having 11 anther sources (5 cultivar parents and 6 hybrids) and 10 replications. The replications were the spike values for in vitro androgenesis (percentage of successfully cultured anthers per spike). Hence, from within each genotype the anthers from 10 spikes were cultured. Each spike had a minimum of 30 cultured anthers. The data were transformed by using the arcsine square root transformation for all statistical analyses.

The hexaploid cultivars, 'Asosan/8* Chancellor', and 'Kitt', and all the polyhaploid plants obtained from the F_1 hybrids made between these lines were evaluated for disease resistance to powdery mildew caused by *Erysiphe graminis* DC. ex Merat f. sp. *tritici* em. Marchal, culture 4a. Asosan/8* Chancellor is a near isogenic line of 'Chancellor', differing in that it contains the dominant gene *Pm 3a* (Briggle 1969), which confers resistance to culture 4a. Kitt is susceptible to this culture.

Adult plants were inoculated with fungal conidiospores by uniformly shaking plants heavily infected with culture 4a approximately 25 cm above the individuals to be evaluated. Controlled environmental conditions were sustained during the disease testing period by maintaining the plants in a growth chamber (12 h of fluorescent light, 250 uE m^{-2} sec^{-1}, at 20 °C). On the ninth day after inoculation, the plants were rated for disease expression.

The ploidy levels of 24 anther-derived plants were determined by making root tip squashes. The roots were fixed in Carnoy's solution and stored in 70% ethyl alcohol. The root tips were later hydrolyzed in 0.1 N HCl at 45 °C for 8 min and then squashed in acetocarmine.

1 Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA, and does not imply its approval to the exclusion of other products that may also be suitable

Results

Causal Factors of In Vitro Androgenesis

With the exception of Chancellor, in vitro androgenic development was induced in varying degrees from all cultivars and hybrids (Table 2). Highly significant differences for in vitro androgenesis were found among the anther sources. Centurk and Kitt, selected for good androgenic ability (Schaeffer et al. 1979), were not significantly different in their in vitro androgenic capacity. Potomac, Chancellor, and its near isogenic line Asosan/8* Chancellor, all of which were selected for their poor androgenic ability (Schaeffer et al. 1979), were not significantly different from each other. The two good lines, Centurk and Kitt, had significantly better androgenic ability than the three poorer lines, Asosan/8* Chancellor, Chancellor, and Potomac.

The two F_1 hybrids, A-C and C-A, were not significantly different from each other in their in vitro androgenic capacity. Both crosses did not perform as well as their good parent, Centurk, but each hybrid was significantly better than the poor parent Asosan/8* Chancellor. The second set of reciprocal crosses, K-P and P-K, were not significantly different from each other, and no significant differences in androgenic capacity were noted between K-P and P-K and either parent cultivar. The third set of reciprocal crosses, A-K and K-A, were not significantly different from each other or from their good parent, Kitt. Both crosses were significantly better than their poor parent, Asosan/8* Chancellor. Lastly, all hybrids were found not to be significantly different from each other in androgenic capacity. Additionally, when all hybrids having a good anther culture parent as the maternal parent (C-A, K-A, K-P)

Table 2. In vitro androgenic capacity of the five wheat cultivars and six F_1 hybrids

Genotypes	Number of cultured anthers	Number of androgenic anthers	Genotype means (spike value %)*
Asosan/8* Chancellor	720	2	0.25 D
Centurk	729	61	8.07 A
Chancellor	738	0	0.00 D
Kitt	492	29	5.39 AB
Potomac	729	5	0.67 CD
Asosan/8* Chancellor × Centurk	517	20	3.60 B
Centurk × Asosan/8* Chancellor	592	20	3.31 B
Asosan/8* Chancellor × Kitt	600	17	2.79 BC
Kitt × Asosan/8* Chancellor	705	10	1.55 BC
Kitt × Potomac	509	17	2.86 BC
Potomac × Kitt	489	13	2.87 BC
Total	6820	194	

* Based on data from 10 spikes. Treatment means not followed by the same letter are significantly different at the 0.05 level of probability as determined by a Duncan's multiple range test

Table 3. Summary by genotype of regenerated green and albino plants*

Genotypes	Number clusters of calli undergoing differentiation	Number of green plantlets	Number of albino plantlets	Total
Asosan/8* Chancellor	0	0	0	0
Centurk	14 (22%)	3	32	35
Chancellor	–	–	–	–
Kitt	13 (41%)	46	11	57
Potomac	1 (17%)	3	0	3
Asosan/8* Chancellor × Centurk	7 (21%)	2	7	9
Centurk × Asosan/8* Chancellor	5 (24%)	3	13	16
Asosan/8* Chancellor × Kitt	7 (33%)	13	0	13
Kitt × Asosan/8* Chancellor	4 (24%)	15	0	15
Kitt × Potomac	13 (62%)	15	4	19
Potomac × Kitt	6 (29%)	15	0	15
Total		115	67	182

* Includes additional data not encompassed in statistical analysis. Data in parenthesis indicate percent of calli that differentiated

were statistically analyzed by using a single degree of freedom comparison to all hybrids having a poor anther culture parent for the maternal parent (A-C, A-K, P-K), no significant differences between the two group means (2.6 and 3.1, respectively) were found.

Plantlet Formation

Plantlet formation was observed approximately 10 days after the transfer of the calli to the shoot regeneration medium. Shoot differentiation occurred 30% of the time with one to three plantlets often being produced by a single anther's cluster of calli (Fig. 1), although as many as 13 were observed. The initial shoots were often distorted, with a flat and twisted leaf blade, but later developing shoots appeared normal in structure. No shoot development was ever observed when root formation occurred first. From a total of 182 plantlets, 121 individuals developed roots. Of the 24 plantlets checked for ploidy level, 21 were polyhaploid (n=3x=21) and 3 were hexaploid.

Most clusters of calli from a single anther produced either green or albino plantlets exclusively, but six groups produced both types of plantlets (Fig. 2). Cultivar differences in green and albino plantlet formation were also noted (Table 3). Centurk and the hybrids, A-C and C-A, were particularly prone to albino production. Many green plantlets stopped chlorophyll synthesis during the root development phase or died soon after potting, which left a total of 15 hybrid-derived plants for further analysis.

Gemetic Representation

To determine whether multiple plantlet formation from within a single anther's cluster of calli occurred by clonal propagation or by the development of individual microspores, the polyhaploid plants were observed for the independent segregation (1 : 1) of awn type and disease resistance to powdery mildew. The clusters from three anthers each produced three progeny in which all of the plants survived to maturity. In each case, only fully awned plants were observed. Alternatively, both resistant and susceptible plants were derived from each cluster. When considering all of the plants produced in this study, an awn type ratio of (2 : 13) was noted. Among the polyhaploid plants which had 'Asosan/8* Chancellor' in their pedigree, a disease expression ratio of (7 : 6, resistant : susceptible) was observed.

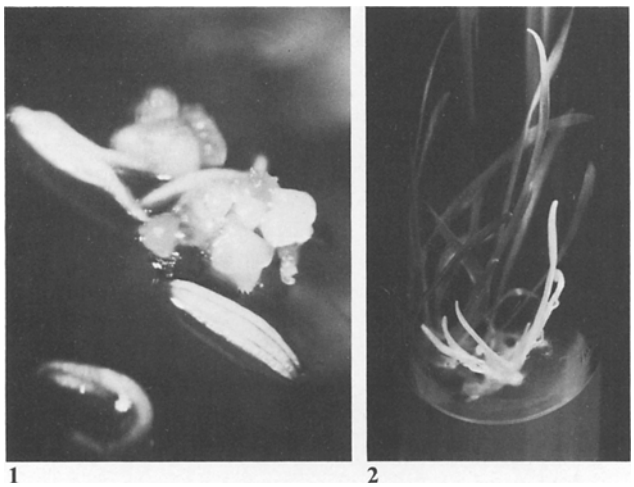

Fig. 1. Cluster of calli from a single anther

Fig. 2. Green and albino wheat plantlets produced from a single cluster of calli

Discussion

We must recognize that variable factors such as the physiological state of the plant, media constitution, developmental stage of the microspore and environmental conditions during the culturing process all play important roles in the success of the in vitro development of the microspore. Yet under the uniform conditions used in this experiment, four of the F_1 hybrids, (A-C, C-A, A-K, K-A), were significantly better for androgenic capacity than their common poor parent, Asosan/8* Chancellor. At the same time, the performances of A-K, K-A, K-P, and P-K were not statistically different from their good parents, respectively. Clearly, this indicates that in vitro androgenic capacity can be transferred from a cultivar with good androgenic capacity to its F_1 hybrids. In other words, wheat F_1 hybrids, having one parent with good androgenic capacity and one parent with very poor androgenic capactiy, will be culturable in an in vitro system.

Examination of the means of the six F_1 hybrids indicated that the transfer of androgenic capacity did not occur in a cytoplasmically inherited manner. No significant differences were found between the two hybrids in any of the three sets of reciprocal crosses. Additionally, the comparison of all hybrids, grouped by the in vitro androgenic ability of their maternal parent, also indicated no significant reciprocal differences existed. Thus, no evidence of cytoplasmic inheritance within the *T. aestivum* cytoplasm was indicated in any of the analyses of specific cross data or group comparison data. Because androgenesis is a transferable character and does not appear to be carried in the cytoplasm, the data suggest that the capacity for in vitro androgenesis is nuclear in origin. This, of course, does not exclude nuclear-cytoplasmic interaction.

The conclusion that in vitro androgenic capacity appears to be a nuclearly transferable character is important to plant breeders, as the utilization of anther culture in doubled haploid breeding may result in a saving

of time and money in some winter wheat programs. The benefits of the haploid system need not be limited to particular cultivars, but can be extended to their hybrids and potentially to other cultivars. This information may be of direct value in wheat breeding programs because cultivars with good androgenic capacity, such as 'Centurk' (hard red winter wheat) and 'Kitt' (hard red spring wheat) are representative of widely grown and economically important types of wheat in the United States.

In three instances, polyhaploid plants segregated for disease resistance to powdery mildew, which indicated the differentiation of more than one microspore from within a cluster of calli. The results also demonstrated that a polyhaploid wheat plant, carrying the Pm 3a mildew resistance gene in the hemizygous condition, can exhibit a resistant disease reaction comparable to its hexaploid counterpart. Although further experimentation is required, this study provides preliminary evidence that disease screening of wheat polyhaploids for powdery mildew resistance may be possible.

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