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Heritability and Physiology of Anther Culture and Inhibitor Selections in Wheat

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by

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Heritability and Physiology of Anther Culture
and Inhibitor Selections in Wheat

Gideon W. Schaeffer

Abstract

The anther culture of wheat and other cereals is exceptionally useful for the recovery of haploids, the immediate recovery of homozygous recessives, the recovery of in vitro - induced somaclonal variation, the immediate recovery of segregants of alleles and gametoclonal variation from microspores as well as the recovery of useful tissues for numerous developmental, biochemical and physiological studies. The research on this project was designed to improve the efficiency of anther/microspore culture and plant recovery in wheat, assess the variation recovered from different procedures, as well as assess at the whole plant level, both field and greenhouse grown plants.

The work showed that the Chinese potato medium with 2mg/l 2,4-D is efficient, cold temperature shock improves androgenesis and growth chamber conditions which favor plump growth (non etiolated) plants improve androgenesis. The work proved that there are strong genetic components in androgenesis. Crosses of cultivars with low and high androgenetic potentials produced progeny with intermediate values for both anther culturability and plant regeneration.

Variation recovered was wide-ranging and included altered ploidy levels, chromosome eliminations and chromosomal rearrangements. Cytogenetic variation was readily scored and plants were recovered with numerous morphological characteristics which included:

- a. Awned types from awnletted cultivars
- b. Very tall plants
- c. Plants shorter than parents
- d. Plants with increased tillers
- e. Lower yielding plants (increased infertility) and at the biochemical level
- f. alterations in the electrophoretic banding patterns of seed storage proteins

Progeny of many doubled haploids were grown in the field and reductions in yield were the most prominent changes. Plants with useful characteristics must be crossed for complete evaluation. Backcrosses were beyond the scope of this project.

Anther/microspore culture will become a valuable adjunct for the predictable production of useful variants required for progress in standard plant breeding practices.

Introduction and Experimental purpose

There are numerous advantages in the use of haploids for plant breeding, for biochemical selections and for the study of gene expression during growth and development. Anther/microspore culture techniques are among the most efficient techniques for the recovery of large numbers of haploid. Nonetheless the techniques are not well defined and not sufficiently predictable. Additionally the in vitro environment induces variation and this variation has not been adequately defined particularly when anther-derived tissues are used as starting materials. Variation may be either genetic or epigenetic and techniques need to be developed to distinguish the types of variation and control the variation. Chromosomal characteristics need to be defined and anther - derived plants need to be fully characterized under field conditions. Eventhrough the literature on the merits of tissue and anther culture is voluminous few complete studies have been reported to document precisely the type and stability of in vitro - induced variation.

The purpose of the proposal was to increase the efficiency of haploid production by androgenesis in wheat, to determine the heritability of androgenesis, to evaluate the genetic changes in anther-derived plants to develop improved conditions for the tissue culture and regeneration of plants from tissue culture and to evaluate in vitro derived plants under field environment normally utilized during plant breeding procedures.

Materials and Methods

The methods utilized are described in detail in the attached abstracts of meetings, manuscripts and preprints on:

- a. Genotype - environment interaction in anther culturability
- b. Combining abilities and heritabilities of callus formation and plantlet regeneration in wheat (Triticum aestivum L.) anther cultures.
- c. Assessment of anther - derived and in vitro cultures for the regeneration of doubled haploids of the Centurk, Chris, Downy and Kitt.
- d. Refine portions of the potato medium responsible for the improved culture and regeneration of immature pollen grains.
- e. Define cytological and cytogenetic characteristics of variants recovered from anther culture.
- f. Define precisely the period of maximum microspore reprogramming and development in vitro.

Additionally numerous experiments were designed and done which gave no definitive results. Among them were the in situ addition of numerous hormone combinations to the developing inflorescence. Also, studies were designed to recover cells resistant to analogs of amino acids including lysine plus threonine and elevated levels of proline.

There was steady research progress associated with the BARD project on "Heritability and Physiology of Anther Culture and Inhibition Selections in Wheat" US-234-80. Both Israeli and U.S. cooperations have worked effectively on elements of the project and good progress was made.

Results and Discussion

Highlights of the results include:

- A. Recovery of doubled Haploids. Hundreds (approximately 500) of haploids were doubled and seed has been generated from test nurseries for evaluations (publication 7, 8, and 9).
- B. Variations in field progeny. As part of a series of experiments to determine if doubled haploids from anther culture accurately represent the genetic potential within an F_1 , anthers from F_1 plants of Kitt x Chris and Chris x Kitt were anther cultured. 6.7% of the anther from Kitt x Chris formed callus and 8.4% of the anthers from Chris x Kitt formed callus which indicated that when crossing two good cultivars for androgenesis, the F_1 was also good for androgenesis. Reciprocal effects were inconsequential as was expected from our earlier work. Doubled haploid plants are currently being regenerated from these calli. Concurrent with the anther culture of this cross and its reciprocal, a single seed descent (SSD) population of Kitt x Chris was started. The doubled haploid plants and the SSD plants were compared in the field. In 1983 experiments 75 doubled haploids of Kitt and 50 single seed descent derived lines of Kitt were compared as were, in a separate experiment, 25 doubled haploids of Chris and 25 single seed descent derived lines of Chris. In these experiments, heading date, plant height seed weight, kernels/spike, spikes M^2 , and yield were measured. These variables will be statistically analyzed in 1984. If yield reductions are found in these experiments, the components of yield measurements should determine how yield was affected. These experiments will be repeated in 1984. In cooperation with Dr. John Schmidt of the University of Nebraska, double haploids of Centurk were planted in Nebraska where Centurk was developed. This experiment is to measure agronomic variation in winter wheat. (See publications 6, 7, 8, 9, 12, and 13).

To understand the genetic basis for reduced yield in some of the wheat doubled haploids, crosses were made between low, medium, and high yielding doubled haploids and with single seed descent derived Kitt lines. The F_1 's of these crosses were grown in Aberdeen, Idaho in 1983 to increase seed for a 1984 F_2 yield trial.

- C. Changes in seed yield. Additionally seed of doubled haploid lines of Centurk, Chris, Downy and Kitt have been collected. In the case of Kitt, 23 unique doubled haploids now have sufficient seed to be field tested for agronomic traits including yield in 1982 and 1983. The test is to determine if deleterious variation is induced by wheat anther culture. Single seed descent populations to measure the natural variation within the cultivars Chris and Kitt were started and used in these comparisons of variation in 1983. The seed of Centurk, Chris and Downy was used for similar experiments measuring variation. Evaluations showed some lines to be stable and good yielding. Other lines produced individual off plants within plots such as very tall plants. (See publications 8, 9, and 11).

- D. Cytogenetic Variation: Part of the variability induced by in vitro procedures is due to chromosomal variability. Cytological experiments are nearly complete showing the effects of time in culture and colchicine treatments upon ploidy levels or chromosome loss or gain (aneuploids or trisomics). The bulk of the data for the cultivar 'Centurk' has been summarized and documented in the publication by Kudirka, et al. (See publications # 4, 5 and 10)

Evidence from the Israeli cooperators suggests that there exist specific genetic components conferring stability and instability. Progeny from anther-derived plants of a synthetic wheat showed a wide range of variability including lines that were either stable or unstable in the expression of morphological characteristics. The work is now being summarized for publication by Dr. Feldman/Galun.

- E. Improvements in Anther Culturability. The average yield of double - haploids was reduced by 16% although some lines were equal to the control. (See publication # 3)

The current studies examined frequencies of callus initiation and plant regeneration in anther cultures of three wheat cultivars and randomly selected dihaploid lines previously derived from each cultivar via anther culture to determine the heritability of the androgenic response. Environmental effects were generally nonsignificant. However, genotype significantly affected the parameters measured and significant differences were observed between cultivars and their respective dihaploid lines. Further, very significant line x environment interactions were observed. For example, while the cultivar 'Centurk' produced calli five fold more frequently than a dihaploid of 'Centurk' when grown in the field, the same dihaploid line significantly outproduced the cultivar when grown under growth chamber conditions. It is demonstrated that it is possible to select from a population of dihaploid lines for increased androgenic frequencies. It is unlikely that the improvement in anther culturability can be attributed to selective pressure imposed by the culture process. (See publications 1, 2, 4, 6, 8, and 11).

Cooperative efforts in assessing the anther culturability of Israeli crosses sent to me by Dr. Galun also progressed well. Twelve interspecies crosses with genotypes for high protein and drought resistant characteristics have been anther cultured. Progeny from anther culture of wide crosses produced segregant for future stability and instability.

- F. Response to hormones. One of the goals was to improve the haploid yield from anther cultures by growth factor pretreatments. Over 20 hormone and inhibitor treatments have been tested by infusing compounds through the flag leaf at meiosis. Anther cultures were then established of the pretreated plants. The variability encountered was larger than expected but there are compounds, both promoters and inhibitors, that can be used to improve anther culturability. These will be tested under conditions where components of variability can be reduced. Compounds of interest include colchicine, azauracil, ethrel, proline, gibberelic acid, among others. (See publications # 9 and 10).
- G. Genetics and physiology of Androgenesis. Work on genetics and physiology of androgenesis has been completed, (publications 12, 13, 14, 15, and 16) with the exception of a Ph.D. thesis, on the physiology of androgenesis. The fractionation of the potato medium to classes of compounds based on size show that the fraction containing the small molecular unit fraction is more androgenetically promotive than the larger molecular weight fraction. (See publication 18). Additional work on the genetics of androgenesis was done with Maryland cultivars by the Ph.D. candidate. This work will be summarized later. These works described in previous publications and in attached drafts have established significant improvement in androgenesis by genetic manipulations, by cold treatments, by fractionations of the potato medium, by hormone pretreatment and by improved growth conditions of the source plant. Temperature is particularly critical.
- H. Description of Cooperation. The cooperation in this project has been wide ranging among the USDA researchers at Beltsville (Schaeffer, Baenziger, Kudirka, Lazar), the Weizmann Institute (E. Galun and M. Feldman), the University of Maryland (Dr. David Sammons and Joy Marburger (Ph.D. student), Dr. Wesenberg, USDA, in charge of the wheat nurseries in Idaho, University of Nebraska (J. Schmidt) and others. Wheat crosses were made and grown at Beltsville, the Weizmann Institute, and University of Maryland and Idaho and have been anther cultured here at Beltsville. Anther derived plants were regenerated and returned to their sources. Evaluations of these crosses were for biochemical, cytological and agronomic properties induced by anther culture, the genetics of androgenesis and the nature and sources of genetic instabilities. The cooperation has been excellent due to the substantial contribution of both U. S. and Israeli researchers making contributions much beyond the scope of the original proposal.

For example, Dr. Galun has the important collaboration of Dr. Feldman in cytogenetics and they are examining an unstable variant recovered from the anther culture of our cultivar, Downy. The awned variant was derived from an 'awnletted' (greatly reduced awns) cultivar. This variant has intriguing cytogenetic characteristics and has important genetic implications for the derivation of variants from tissue culture. Also U. S. scientists have cultured Israeli cultivars mentioned above and obtained haploids and seed from one doubled haploid. This material has important implications for the introgression of 'useful genetic' material into important cultivars.

Cooperative efforts in assessing the anther culturability of Israeli crosses sent to me by Dr. Galun also progressed well. Twelve interspecies crosses with genotypes for high protein and drought tolerance characteristics have been anther cultured. Progeny from anther culture of wide crosses produced segregants for future stability and instability.

- I. Benefits to Agriculture. The benefits to agriculture are direct in the sense that the anther culture procedures are intermediate between the molecular aspects of biotechnology and the procedures used by plant breeders.

Anther culture reduces the time frame for cultivar development and increases the opportunity to broaden the range of useful variation within a cultivar.

Additionally the in vitro techniques contribute directly to the scientific information base whereby plants can be modified in a specific and predictable manner.

The information developed has advanced significantly the application of In Vitro techniques, specifically tissue and anther culture to a very important crop species. I anticipate private and public breeders will soon use the techniques, established in part by work associated with the project to systematically use doubled haploids in plant breeding and gene transfer concepts. The work contributes directly to the improvement of crop plants.

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