Progress Report on Spring Wheat Cell Culture Efforts

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The many possibilities of improving present crop varieties by various applications of biotechnology including cell and tissue culture with plant regeneration, transfer of specific genes from one crop to another, and cell hybridization are often reported. These new techniques are possible because a plant is made up of millions of cells, each containing the complete genetic information (DNA) necessary to produce a whole plant, and the DNA of all plant species is written in the same language. Therefore, whole plants can be produced from individual cells and genes from one species can function in a different species.

The tissue culture techniques involve encouraging division of plant cells in a test tube or petri dish for several cell generations and then regenerating whole plants from those cells. Cultures are initiated by placing a small piece of a plant part in a medium containing the minerals, vitamins, hormones and sugar necessary for those cells to grow and divide. This medium prevents the cells from forming plant parts such as leaves and roots, and the cells form a callus growth. For reasons not yet clear, as these cells grow and divide, some will undergo genetic change (3). Transferring the callus to a different medium can cause expression of genes necessary to produce a plant (leaves, stem, and roots). These small plants can then be placed in soil and grown normally to maturity. Since a portion of the cells that regenerated whole plants had previously undergone genetic change, those changes may be observed in the regenerated plants. Genetic changes have been observed for a wide array of plant characteristics including maturity, morphology, and resistance to diseases and herbicides (3). These genetic changes during tissue culture could be continual supply of "new" genes to breeding programs.

Other biotechnology techniques involve the transfer of beneficial genes between species that cannot be crossed by sexual means. One procedure requires the transfer of a specific gene(s) to a cultured cell, incorporation of that gene into the genetic information of the cell, and regeneration of a plant from that altered cell. The transferred gene, unfamiliar to that species, may or may not be expressed in the regenerated plant (1). Obviously, this procedure is complex, and, in addition, the researcher must assure that the gene is expressed in the right plant part at the right time during development. The second procedure involves somatic cell hybridization, which requires combining a cultured cell from one species with that of another species and regeneration of a plant from that new combined cell (2). This procedure provides a tool for bypassing the sexual process and "crossing" species which have not been possible to cross before.

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Most of the successful biotechnology techniques have been developed with species such as tobacco or carrot. Most of the cultivated agricultural land is used to grow cereal crops which have not been easy to manipulate with the new biotechnology techniques. However, in recent years some of these tissue culture and plant regeneration procedures have been altered to accommodate the cereal crops. This report presents some preliminary results with wheat plants regenerated from cultured cells.

GENERAL CULTURE PROCEDURES

Any plant part should have the capability of producing regenerable calli, but the greatest success would be expected from parts with meristematic regions, as these cells are already in the dividing process. We have tested meristematic regions of leaves, stems, roots, inflorescences, and embryos from wheat plants using various combinations and concentrations of hormones, vitamins, and minerals in the media. Our greatest success has been with immature embryos using the procedure that follows.

Immature seeds are harvested from wheat plants when the embryos are about 1 mm in length. The embryos (scutellum and main axis) are placed on a solid agar medium (4) containing the herbicide 2,4-D. The cultured embryos are placed under low light conditions (16 hour photoperiod per 24 hours day) at 25 C. Undifferentiated cells are visible at the edges and surface of the scutellum within about one week. After four weeks the growing calli are transferred to a maintenance medium which is similar to the initiation medium except the 2,4-D concentration is reduced. Lowering the 2,4-D concentration allows for more rapid callus growth. The calli are transferred to fresh medium at four-week intervals. These scutellar-derived calli are yellow to yellow-white and many genotypes begin developing green areas or leaf-like structures about three to four weeks after initiation.

Shoots are regenerated by placing portions of greening calli on shoot initiation medium which is similar to the preceding medium, except the 2,4-D concentration is reduced again. This lower 2,4-D level promotes shoot development but partially inhibits root development. After about three weeks, the shoots are separated and transferred to media without 2,4-D to stimulate root development which takes another three to four weeks. The plants are then removed from sterile conditions for the first time, transplanted into small pots containing vermiculite, and grown under humid, medium light conditions. After two to three weeks, the plants are transplanted into soil and grown to maturity in the greenhouse.

RESULTS

The potential for biotechnological applications in crop improvement programs requires identifying genotypes that allow cell/tissue culture with predictable plant regeneration. About 100 genotypes of spring wheat (*Triticum aestivum* L. and *T. turgidum* var. durum) have been examined for potential use in tissue culture studies. The results of one experiment utilizing immature embryos from 15 hard red spring wheat genotypes are presented in Table 1. The influence of genotype on callus initiation was evident ranging from Era being the least responsive (only 14 percent of the embryos developing calli) to several genotypes being very responsive (over 70 percent of the embryos developing calli). The embryos that did not develop calli either germinated on the initiation medium or developed few callus cells with no potential for plant regeneration. The genotype also influenced the quality of the calli that developed, especially the texture, growth rate, and color.

The ability to regenerate plants is an important characteristic which also was influenced by genotype (Table 1). In this experiment, all calli were placed on regeneration medium following two months on maintenance medium. The number of regenerated plants ranged from 0 for Era to 58 for Angus. One-third of the genotypes developed calli that regenerated 20 or more plants. The performance of Angus in this study and others (unpublished) approached the reported performance of ND7532, a hard red winter wheat genotype that has become the standard for winter wheat tissue culture systems (5).

For additional studies, we selected Angus, Chris, and Coteau as genotypes with different genetic backgrounds and responses to the culture process. These three genotypes differed for several plant characteristics including plant height, awns, spike morphology, maturity, and color. These characteristics have been reported to be altered by the tissue culture process in other crops (5).

Table 1. Screen of 15 hard red spring genotypes for potential in the tissue culture process as determined by frequency of callus development and plant regeneration.

Genotype	Percentage* of embryos developing calli	Total number of regenerated plants
Angus	86	58
Butte	74	23
Chris	76	36
Coteau	62	22
Era	14	0
Glenlea	58	2
Justin	90	16
Lee	66	4
Len	84	9
Marquis	66	2
Olaf	48	15
Protor	70	40
Rival	68	3
Selkirk	72	8
Waldron	68	12

*Percentage of 50 immature embryos used for initiating calli of each genotype.

Cultures were developed from these three cultivars, and plants were regenerated over a several month period to determine the types of genetic change that occurred during the culture process. As expected, the Angus cultures produced more regenerated plants than the Chris or Coteau cultures (Table 2). These regenerated plants were evaluated for several characteristics including plant height; flag leaf length, width, and area; spike length and number of kernels per spike. Certain plants were obviously different from the parental genotype for each of the measured characteristics. For example, plants were identified that were taller or shorter and exhibited longer or shorter spikes than the parent (Table 2). However, plants shorter in height and spike length were observed more frequently than plants which exceeded the parents.

Table 2. Number of regenerated plants from three genotypes and the proportion of those plants exhibiting variability for two plant characters.

Parent genotype	Total Number of regenerated plants	No. of plants different* from parent			
		Plant height		Spike length	
		Higher	Lower	Higher	Lower
Angus	535	17	52	3	235
Chris	196	1	113	9	62
Coteau	157	2	42	8	25

*Different by more than two standard deviation units from the mean of the parent genotype.

Plants inferior to the parent for a characteristic are more frequent than superior plants, since changes detrimental to growth are more likely to occur than beneficial changes. The following analogy explains this principle. Plant growth has been visualized as a wooden barrel made up of staves with various lengths. The maximum amount of water the barrel could hold would be determined by the shortest stave (the most limiting factor). To increase the capacity of the barrel, it would be necessary to increase the height of the shortest stave. To decrease the capacity, the height of any stave could be decreased as long as it was decreased to a height shorter than the shortest stave. Analagously, to increase plant growth, a characteristic that limits growth would have to be altered, while to decrease growth, any growth characteristic could be altered if the decrease is great enough. Therefore, when examining genetic change, most changes with an effect will tend to decrease plant growth. However, a few changes almost certainly will act to increase plant growth. That is, a limiting characteristic will be positively affected.

The results in Table 2 agree with these expectations in that most changes decreased plant height and spike length but few changes increased the characters. We are assuming that plant height changes are an indication of changes in plant vigor, not semidwarf genes. Angus, Coteau, and Chris are considered a semidwarf, medium height, and tall cultivar, respectively. Therefore, perhaps it is not surprising that Angus cultures produced a higher proportion of taller plants and a smaller proportion of shorter plants compared to the two taller genotypes.

Other regenerated plants exhibited differences for color, spike morphology (awns, branched spikes, compact spikes),

and branched stems (two spikes per stem). Most regenerated plants that exhibited an apparent change showed alterations in only one or two characteristics. This is advantageous since a plant with beneficial change is not likely to have a deleterious change also. Although some of the apparent changes identified in this study could be termed beneficial, they are present in existing germplasm available to the wheat breeder. Nevertheless, the identified morphological changes suggest that beneficial changes not present in existing germplasm also may occur. Progeny of these regenerated plants are being evaluated for the same characters to determine the nature of the changes and for other characteristics to identify additional variation.

The characteristics measured in the preceding study cannot be selected at the cell level, because one cannot predict if a specific cell or region of cells will organize and produce a plant with a certain height, flag leaf, etc. This approach, then, requires that many regenerated plants be evaluated for a specific characteristic. The procedure can be more efficient if the characteristic of interest is expressed in the callus cells. Selection for specific genetic changes at the cellular level requires the addition of a selection agent to the media. Selection agents include chemicals, environments or organisms that only allow the cells of interest to live and grow.

Herbicides are a selection agent we have used to identify herbicide resistant cells. Several million cells can be grown in a few petri dishes or flasks and the odds are reasonable that a few cells will undergo genetic change, allowing the cells to avoid injury from a specific herbicide. The herbicides do not cause the changes leading to resistance, but the procedure merely allows identification of cells in which the desired genetic change has occurred. The procedure used for wheat involved placing a population of wheat cells on the maintenance medium with a herbicide that kills wheat plants. On this medium, most cells senesce and die whereas cells with a genetic change providing resistance will continue to grow. These growing cells can be removed from the herbicide medium and plants regenerated from them. These plants may provide the basis for a herbicide resistant variety.

We have attempted this procedure with four herbicides utilizing Angus and Chris cultures. Several cell regions were identified that grew in the presence of each herbicide, and plants were regenerated from some of these cells (Table 3). Herbicide resistance is complex, and genetic changes providing resistance at the cell level will not necessarily provide whole plant resistance. None of the plants regenerated from cells growing in the presence of trifluralin or atrazine exhibited whole plant resistance. Perhaps this technique is not suitable for selecting plants with resistance to these two herbicides, but more plants would have to be evaluated to make that conclusion. A few plants with apparent resistance to fluazifop (Fusilade) and sethoxydim (Poast) were identified. These plants are being further evaluated. Fusilade and Poast are postemergence herbicides used to control annual and perennial grasses in broadleaf crops. If the apparent resistance obtained through tissue culture is real and stable over generations, these genetic changes could be beneficial for wheat production because they would allow additional choices for controlling grassy weeds. However, much research would be necessary before the usefulness of the resistance could be realized. For instance, the genetic change(s) allowing for herbicide resistance could be associated with a reduced plant growth efficiency or with problems in controlling volunteer grain. In addition, the incorporation of that resistance into tomorrow's cultivars would take plant breeding time and effort.

Table 3. Number of plants regenerated from cells that survived the presence of a herbicide in the growing medium.

Parent genotype	Herbicide			
	Fluazifop*	Sethoxydim*	Trifluralin*	Atrazine
	Number of regenerated plants			
Angus	106	331	48	57
Chris	38	136	—	46

*Trade name of Fluazifop, Sethoxydim, and Trifluralin are Fusilade, Poast, and Treflan, respectively.

SUMMARY

The new techniques of biotechnology offer many exciting possibilities with potential application to crop production. Although these techniques of cell and tissue culture, gene transfer, and cell hybridization have been developed with species other than the important agricultural cereal crops, the culture techniques have been altered in recent years to accommodate these crops. Further research is necessary before the techniques of gene transfer and cell hybridization can be applied to cereal crop improvement. This necessary research not only involves the development of techniques, but also the identification of important genes to be transferred.

Two tissue culture procedures have been used with wheat in our laboratory with some success. Immature embryos have been the explant of choice allowing for long-term cultures that provide for plant regeneration. One procedure attempts to make use of general genetic changes occurring during the culture process. Regenerated wheat plants have been identified with an apparent change in many plant characters including plant height, flag leaf size, spike length, and kernels per spike. The second procedure selects specific genetic changes involving plant characters that can be identified at the cell level, such as herbicide resistance. Using this procedure, regenerated plants have been identified with apparent resistance to fluazifop and sethoxydim. These genetic changes evident in some regenerated plants have potential usefulness to the wheat breeder if they are stable and provide greater variation than presently exists for a specific trait. If certain of the apparent changes observed in these studies can be shown to be real and stable, they will have potential usefulness.

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