Biotechnology and Improved Drought Tolerance of Crops

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Crops absorb water from the soil, carbon dioxide from the air, and light energy from the sun to produce carbohydrates through the process of photosynthesis. These carbohydrates are the basic source of plant material as they are used to produce all the necessary organic components. The rate of carbohydrate production and plant growth is influenced by many environmental variables, including light intensity and duration, carbon dioxide concentration of the air, air temperature, and the amount of available water in the soil. If available water is limiting, its effects usually are greater than the other environmental variables due to the many functions of water in plants. These functions include cell turgor, substrate for photosynthesis, plant cooling, media for biochemical processes, and transport of inorganic and organic components. Crops require large quantities of water. For example, about 2.4 million pounds of water per acre (10 inches of rainfall) are required to produce a 50 bushel per acre wheat crop. If that amount of stored soil water or precipitation is not available, yield losses occur.

DROUGHT STRESS AND CROP GROWTH

Water is lost (transpired) and carbon dioxide is absorbed through small openings in the leaves called stomata. When crops are turgid, stomata are open during the day and closed at night. When stomata are open, they account for only 1 percent to 3 percent of the leaf area. Nevertheless, water loss through this small pore area is nearly the same as the amount evaporated from an open water surface with an area equal to the total leaf area, because diffusion through small pores is proportional to the perimeter of the pore rather than the pore area. Thus, a turgid crop loses a great deal of water through the stomata during the day, and this amount of water can not be reduced without also reducing the uptake of carbon dioxide and, therefore, crop growth.

A measure of the total water requirement of the crop includes evaporation from the soil surface and transpiration. These processes combined are called evapotranspiration. Factors increasing transpiration of water from the leaves and evaporation of water from the soil surface include high soil water availablility and environmental conditions such as high temperatures, low humidity, and rapid air movement. During the very early stages of crop development, evaporation from the soil surface can be a greater source of water loss than transpiration. However, when vegetative leaves develop and expecially when the leaf surface area is equal to or greater than the land surface area, transpiration is the greater source of water loss.

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Water transpired from the stomata of leaves must be absorbed by the roots or the crop will lose turgor and the stomata will close. The closing of stomata reduces transpiration and minimizes further reductions in cell turgor. Turgor frequently is lost during the day but may not be visible. Generally turgor is regained that evening when water absorption exceeds transpiration. If transpiration sufficiently exceeds absorption, the turgor deficit will be visible as the plant wilts.

The water potential of a cell is an indication of whether that cell is filled completely with water or if it can absorb more water. Pure water has a water potential of zero, and plant cells have potentials less than zero. The major factor causing a more negative water potential of mature plant cells is the loss of water through transpiration. Water moves from a higher potential to a lower potential (more negative), and in a plant this is from the roots to the stems to the leaves.

The presence of certain plant traits increases the probability for crop growth and production in drought stress environments. These traits can be categorized as traits resulting in drought escape or drought tolerance. The most common drought escape trait is early maturity, which allows the crop to mature before the drought stress becomes severe. Maximum crop productivity is limited by this trait because early maturity makes use of a shorter proportion of the growing season. Drought tolerance traits can be subdivided into dehydration postponement and dehydration tolerance.

Dehydration tolerance involves traits that allow the plant to tolerate some degree of dehydration without major reduction of metabolic activities. The physiological basis for dehydration tolerance is not known but is probably at the molecular level, such as membrane structure or enzyme activity.

Dehydration postponement involves traits that allow the plant to reduce transpiration or increase water absorption. These traits leading to the postponement of drought effects include an extensive root system, leaf rolling or folding, and osmotic adjustment. Improving osmotic adjustment has the greatest potential for developing drought tolerant varieties (3).

Osmotic adjustment is the accumulation of various solutes (sugars, ions, etc.) in the cells in response to the onset of a drought. The accumulation of solutes maintains cell turgor until later in the drought stress when low leaf water potentials are reached (Fig. 1). A small decrease in turgor potential as a result of water stress signals the plant to alter many growth processes. Thus, maintaining cell turgor will keep stomata open, maintain metabolic activity including photosynthesis, maintain root growth into deeper soil zones, and

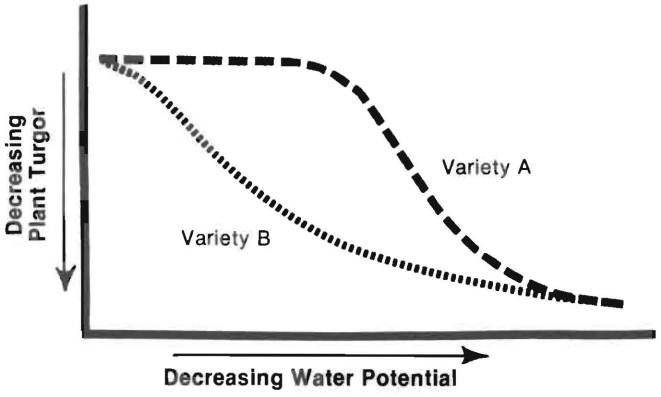

Figure 1. Effect of osmotic adjustment on plant turgor during a decreasing water potential (drought stress). Variety A exhibits osmotic adjustment and variety B does not.

maintain soil water extraction at low soil water potentials. Full or partial maintenance of turgor through osmotic adjustment is important for developing drought tolerant crops.

VARIETAL IMPROVEMENT AND DROUGHT TOLERANCE

The goal of varietal improvement is to improve a crop variety for one or more characteristics such as production efficiency, yield potential, quality, stress resistance, disease resistance, and adaptation to the environment. The ultimate goal is to increase the profitability of producing a high quality crop. In addition to directly improving yield potential or quality, reducing the risk to drought stress by developing a drought tolerant variety also would improve profitability.

The first step in developing a drought tolerant variety is the creation of new genetic variability for drought tolerance. In traditional plant breeding, this is done by identifying and crossing suitable parents. The second step is the selection of drought tolerant progeny during several generations of selfing to develop a true-breeding (homozygous) line. In traditional plant breeding, this is a complex process. A procedure for identifying the drought tolerant progeny must be developed, and the breeder must retain the many desirable plant traits, in addition to drought tolerance. The third and last step is field testing the selected homozygous lines to determine the level of success. Because of the complex nature of drought tolerance and the tenuous identification of drought tolerant parents or progeny, the first two steps of the breeding process generally cannot be conducted with the breeding objective of improving drought tolerance. Nevertheless, lines may be identified during the third step that apparently are more adapted to the drought prone crop producing areas.

In addition to traditional plant breeding, various applications of biotechnology provide new opportunities for the first two steps of varietal improvement - creation of new genetic variability and selection of desirable progeny (1). Biotechnology techniques with beneficial applications to varietal improvement include cell culture with plant regeneration and/or transfer of specific genes to a crop through nonsexual means. These techniques are possible because each plant cell contains the complete genetic information (DNA) necessary to produce a whole plant, and the DNA of all species is written in the same language. Thus, whole plants can be produced from individual cells, and genes from one species can function in a different species.

The biotechnology technique of transferring specific genes to a crop requires detailed biochemical knowledge of the desired trait, gene identification and isolation, and the means to correctly insert the gene into the crop DNA. These requirements are not met for drought tolerance, although research to develop the techniques for inserting genes is progressing rapidly. Research leading to detailed biochemical knowledge of drought tolerance is progressing at a much slower rate. Drought tolerance is complex and the number of involved genes undoubtedly is large. The same probably can be said for the promising drought tolerant trait of osmotic adjustment, although the biochemical knowledge of osmotic adjustment in the various plant tissues presently is inadequate to fully understand the complexities involved.

The biotechnology technique of cell culture involves initiating cultures from a small piece of a plant part in a medium containing the minerals, vitamins, hormones and sugar necessary for the cells of the plant part to divide and grow. The medium prevents plant formation, and the dividing cells form a callus growth. As these cells divide, some will undergo genetic change (1). Changing the media components will cause many of the cells to produce a plant. One callus will produce many plants. These small regenerated plants then can be grown to maturity. Since a portion of the individual cells change genetically during callus growth prior to plant regeneration, the regenerated plants will exhibit these changes. These genetically altered plants may supply useful new genes to breeding programs.

Cells can be selected directly for a desired specific genetic change, if the specific trait is expressed at the cell level. This selection at the cell level greatly reduces the number of whole plants that must be evaluated. Certain drought tolerant traits such as the type of root system or leaf rolling is not expressed by individual callus cells. However, osmotic adjustment is expressed by single cells. Several million cells can be grown in a few petri dishes or flasks and odds are reasonable that at least one cell will undergo a genetic change(s) causing improved osmotic adjustment. Altering the media to stimulate drought stress would cause cells without osmotic adjustment to stop dividing and eventually die, whereas the desired cells would continue to grow and divide. The growing cells could be identified even though detailed biochemical knowledge of osmotic adjustment is not available. Thus, selection for the specifically altered cell through cell culture and regenerating a plant from that altered cell should be an effective method for improving osmotic adjustment. These plants may provide the basis for drought tolerant varieties.

CELL CULTURE/PLANT REGENERATION AND DROUGHT TOLERANCE IN WHEAT

The author and co-workers have attempted this cell selection procedure for drought tolerance utilizing Angus and Chris cultures. These two wheat varieties were selected because much is known about their performance in culture and both produce regenerated plants with a high proportion of altered plants relative to other tested varieties.

The general culture procedures were similar to those previously described (1,2) and involved initiating callus growth from immature scutellar tissue placed on a solid agar medium containing the necessary minerals, vitamins, and hormones. The desirable calli were transferred to fresh

medium after four weeks of growth. After an additional four weeks, the calli, which were about one-half inch in diameter, were divided into small pieces and placed on fresh medium with or without the addtion of polyethylene glycol to simulate drought stress. A similar number of both Angus- and Chris-derived calli were grown on normal and simulated drought stress media (Table 1).

Table 1. Number of plants regenerated from spring wheat cell cultures grown on normal and simulated drought stress media.

Parent variety	Media	Number of cultures	Number of regenerated plants
Angus	Normal	32	348
	Drought stress	30	169
Chris	Normal	18	65
	Drought stress	19	31

When calli were placed on the simulated drought stress medium, most cells senesced and died, whereas cells with a genetic change providing tolerance to the simulated drought continued to grow. After three weeks, living cells were removed from both the stressed and nonstressed media and placed on shoot initiation medium followed by an additional transfer to root initiation medium. The plants then were transplanted to small pots and grown under humid, low-light conditions. After two weeks, the plants were transplanted into soil and grown to maturity in the greenhouse.

More plants were regenerated from Angus-derived calli than from Chris-derived calli and more from calli on normal medium than drought stress medium (Table 1). The progeny of these regenerated plants must be tested for osmotic adjustment or drought tolerance to determine if the genetic alteration that allowed the cell to survive is also functioning at the whole plant level.

The progeny of these regenerated plants were compared to the parent variety (Angus or Chris) for visible plant characteristics during the 1987 growing season. Regenerated plants that produced 15 or more kernels were selected for this field comparison. Partial sterility is sometimes a result of the culture process. The kernels from a single regenerated plant were seeded in a short row and control rows were dispersed randomly among the progeny rows. Based upon the visible plant characteristics, 129 progeny rows were selected for further evaluation. The kernels from a single plant from each selected row were advanced an additional generation during the 1988 growing season prior to evaluating the lines for drought tolerance in the laboratory.

DROUGHT STRESS AND THE 1988 GROWING SEASON

The 1988 growing season was characterized by drought stress from seeding to plant maturity. This experiment was seeded May 17 and harvested July 29. The total rainfall for the four-month duration from April 1 to July 31 was 3.5 inches, which was 7.0 inches below normal rainfall for the same period. During the time of plant growth, continuous moisture from the soil surface to the subsoil was never present. This severe drought stress was due to very low rainfall and higher than optimum temperatures, and was made worse by minimal crown root development due to the continual presence of dry soil at the crown depth.

Improved osmotic adjustment could not be expected to restore productivity under these severe conditions, as osmotic adjustment is most beneficial under the more common low to moderate drought stress conditions. In addition, field variability generally is high under severe stress conditions, which makes it difficult to observe or measure genetic differences for stress tolerance. Nevertheless, it was difficult not to attempt to utilize the severe 1988 drought conditions as an opportunity for identifying plants with that beneficial genetic change(s) leading to osmotic adjustment and drought tolerance.

The combination of drought and high temperature stresses resulted in a very low number of kernels present in each spike, although the weight of each kernel was near normal. The more common North Dakota drought is milder, occuring during the grain fill period, and predominantly reduces kernel weight. However, the 1988 full-season drought severely reduced the number of tillers (spikes) and the number of kernels per spike, which allowed the few kernels to fill more normally even though the stress was severe during grain filling.

Each progeny row was compared to the parent variety for number of kernels per spike and kernel weight. The mean of those rows derived from calli on normal or drought stress media were less than the mean of control (parent variety) rows for both kernels per spike and kernel weight (Table 2). This was not surprising because genetic changes detrimental to crop growth are more likely to occur than beneficial changes (1). The range of kernels per spike and kernel weight was greater for the progeny from regenerated plants (from both normal and drought stress media) than for the parent variety. Progeny rows were identified that exhibited numerically more as well as fewer kernels per spike and larger as well as smaller kernel weights (Table 2).

Three progeny rows, each from a different Angus-derived culture grown on simulated drought stress medium, were significantly greater than the Angus parent for one of the two yield components. Two of the rows exhibited a significantly greater number of kernels per spike (16.8 and 19.6) than the mean of the parent rows (6.1), and the third row exhibited a significantly greater kernel weight (39 mg) than the parent rows (22 mg). The two progeny rows with increased number of kernels exhibited kernel weights similar to the parent and the progeny row with an increased kernel weight exhibited a kernel number similar to the parent. Thus, in all three cases productivity was improved. In addition, the three identified progeny rows were spatially separated in the field and each was surrounded by poor performing progeny rows, providing additional confidence that the identified rows represent a beneficial genetic change.

These three lines and several of the other promising lines will be further evaluated in the field and laboratory to determine the type of change(s) that occurred. If the apparent tolerance obtained and selected through cell culture is real and stable over generations, these genetic changes should be beneficial for improving North Dakota wheat varieties. However, much research would be necessary before the usefulness of the drought tolerance trait could be realized. For instance, the genetic change(s) providing for drought tolerance could be associated with an unwanted trait. In addition, the incorporation of that tolerance trait into tomorrow's varieties would take plant breeding time and effort.

Table 2. Kernels per spike and kernel weight for progeny of selected plants regenerated from Angus and Chris derived cell cultures.

Treatment	Number of parent or progeny rows	Kernels per spike		Kernel weight		No. or rows greater* than parent variety	
		Mean	Range	Mean	Range	Kernels per spike	Kernel weight
				Angus			
Parent variety	12	6.1	2.6-7.9	22	20-24		
Normal media	54	5.2	1.0-11.1	20	12-25	0	0
Drought media	52	6.0	0.5-19.6	21	9-39	2	1
				Chris			
Parent variety	3	5.3	3.4-7.1	20	19-20		
Normal media	12	4.6	1.0-7.9	18	12-25	0	0
Drought media	11	4.5	0.8-8.6	18	13-21	0	0

* 95% confident that progeny row mean is greater than the mean of parent variety rows for that yield component.
Kernel weight reported in milligrams.

SUMMARY

Plant growth is influenced by many environmental variables. However, if water is limiting, the resulting drought stress usually has a greater effect on plant growth than the other environmental variables do. Water moves from cells with a high water potential to cells of a low water potential. Thus, water movement in plants is from roots to the stem to the leaves, as water is lost predominantly through stomata in the leaf surface by the process of transpiration. Drought stress occurs in the plant when water cannot be absorbed rapidly enough by roots to replace that lost by transpiration.

Certain plant traits enable a crop to escape a drought or tolerate the effects of a drought. For crops, escaping a drought means early maturity, but this limits productivity as early maturing crops do not utilize the entire growing season. Thus, incorporating drought tolerance traits provides the greatest potential for varietal improvement and increased profitability from crop production under water limited conditions. Presently, osmotic adjustment is the most promising plant trait that would provide drought tolerance.

Due to the complex nature of drought tolerance and the tenuous identification of drought tolerant parents and progeny, traditional plant breeding has had limited success in improving drought tolerance of varieties. Various applications of biotechnology provide new opportunities for obtaining improved crop tolerance to drought. Utilizing the biotechnology technique of transferring specific genes, which would provide for drought tolerance, is not possible due to the lack of detailed knowledge of drought tolerance and the genes involved. However, the biotechnology techniques of culturing plant cells, selecting cells capable of carrying out osmotic adjustment, and regenerating whole plants from the drought tolerant cells should be an effective method for supplying new drought tolerant genes to breeding programs.

Utilizing these biotechnology techniques by growing wheat cell cultures under simulated drought stress conditions, plants were regenerated from "drought tolerant" cells. Progeny of these regenerated plants contained several promising experimental lines, especially three that were more productive than the parent variety during the severe drought of 1988. These plants may contain beneficial genes for drought tolerance and provide the basis for tomorrow's drought tolerant varieties.

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