Carbohydrates in Germination Salt Tolerant and Non-Salt Tolerant Alfalfa Seed

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Abstract

Alfalfa which is extremely salt tolerant during germination has been developed by researchers at the University of Arizona. Carbohydrates were analyzed in the original parental germplasm 'Mesa-Sirsa' and Cycle$_5$Syn$_2$ and Cycle$_8$Syn$_2$ seed to determine why this seed could germinate in extremely saline conditions.

Raffinose and sucrose were both significantly higher in the salt-tolerant germplasm compared to the parental germplasm; however, the magnitude increase of these free sugars was not sufficient to explain the increased ability of the seed to absorb water in a stress environment. The galactomannan content was not different among the alfalfa germplasms.

Introduction

The absorption of water by germinating seed must occur whether the seed is planted in a non-stress or stress environment. Brewley and Black (1985) suggested that the absorption of water during the initial stages of germination occurs in three phases. The imbibition stage (first phase) is due to the seed matrix potential. The static phase and the radicle elongation phases are closely associated with the metabolic processes of the embryo. The second or static phase is characterized as the stage of rapid anabolic activity including enzyme synthesis and is often referred to as the activation stage (Hegarty, 1978). Allen (1984) suggested that osmotic water uptake in viable seed is more important after initial imbibition occurs. Bliss, et al. (1986) also confirmed the importance of water relations in the seed beyond the matrix phase or initial imbibition.

Alfalfa seed contain several forms of carbohydrates which definitely influence the amount of water a seed absorbs during germination in drought or saline environments. McCleary and Matheson (1974) reported that raffinose was the first carbohydrate depleted during germination. Stachyose was the major reserve oligomer found in alfalfa seed. Raffinose, sucrose, glucose and fructose were also found in smaller concentrations. Gonzalez-Murua et al., (1985) reported that arabinose and xylose were among the neutral sugars released from the endosperm of alfalfa seed after acid hydrolysis.

This research project was designed to determine the concentration and relative differences in carbohydrate found in alfalfa seed which had been developed for germination salt tolerance.

Materials and Methods

Germination salt tolerant alfalfa seed developed at the University of Arizona (Dobrenz et al., 1989) and the original parental population 'Mesa-Sirsa' were used as experimental germplasm. Mesa-Sirsa, Cycle$_5$Syn$_2$ and Cycle$_8$ Syn$_2$ seed were all produced at the Campus Research Center, Tucson, Arizona, in 1988.

Three replications of 50 unscarified seed from each germplasm were ground with a mortar and pestle and placed in centrifuge tubes. Five ml of 95% ethanol was added to each tube and boiled in a water bath for 15 minutes. Each sample was centrifuged for 15 minutes. The solubilized free sugars were pipeted from each tube. This extraction was repeated three times on each tube. Acid hydrolysis of the remaining residue (primarily galactomannan) was accomplished as described by Albersheim (1967) and Grimes (1976).

Samples were purified with cation and anion exchange columns and a one μl sample was injected into a High Performance Liquid Chromatograph (HPLC) equipped with a Bio-Rad HPX-87C carbohydrate analysis column. Sugars were detected in a refractive index and quantified with an electronic integrator.

Results

The germination salt tolerance of alfalfa has been increased dramatically. $Cycle_8Syn_2$ seed germinated 87% better than Mesa-Sirsa at a salinity level of -1.7 MPa (17,000 ppm) NaCl (Figure 1).

Percentage stachyose and glucose were not significantly different among the germplasm sources (Figure 2). However, both raffinose and sucrose were significantly higher in the $Cycle_8Syn_2$ seed. These free sugars could be important in the osmotic adjustment for increased water absorption during the static and radicle elongation phases of germination. However, the differences in the concentration of these soluble sugars were not of a magnitude that would explain the ability of the seed to absorb water in a highly saline environment.

The galactomannan, glucose and arabinose were not different in seed of Mesa-Sirsa, $Cycle_5Syn_2$ and $Cycle_8Syn_2$ (Figure 3). The galactomannan carbohydrate is a very important component of the alfalfa seed endosperm. Since this endosperm layer is located adjacent to the seed coat a higher concentration of this reserve polymer would certainly improve the ability of a seed to absorb water in saline soils. Since no differences exist in the galactomannan concentration of Mesa-Sirsa and the $Cycle_8$ seed, we must conclude that the enhanced salt tolerance is not due to this carbohydrate.

References

Allen, S. G. 1984. Physiology of salt tolerance in alfalfa. Ph.D. diss., Univ. of Arizona, Tucson.

Bewley, J. D. and M. Black. 1985. Seeds: physiology of development and germination. Plenum Press, New York, NY.

Bliss, R. D., K. A. Platt-Aloia and W. W. Thomson. 1986. The inhibitory effect of NaCl on barley germination. Plant, Cell and Environ. 9:727-733.

Dobrenz, A. K., D. L. Robinson, S. E. Smith, D. C. Poteet. 1989. Registration of AZ-GERM SALT-II nondormant alfalfa germplasm. Crop Science 29:493.

Gonzalez-Murua, C., M. Sanchez-Diaz, P., Aparicio-Tejo, A., Munoz-Rueda and J. S. G. Reid. 1985. The effect of NaCl and water stress on germination and alpha-galactosidase activity in germinated seeds of *Medicago sativa*, *Trifolium repens* and *T. brachycalycinum*. J. Plant Physiol. 119:317-326.

Hegarty, T. W. 1978. The physiology of seed hydration and dehydration, and the relation between water stress and the control of germination: a review. Plant, Cell and Environ. 1:101-119.

McCleary, B. V. and N. K. Matheson. 1974. Alpha-D-galactosidase activity and galactomannan and galactosylsucrose oligosaccharide depletion in germinating legume seeds. Phytochemistry 13:1747-1757.

Figure 1: Percent germination of Mesa-Sirsa and Cycles 1 through 9 in a control and a -1.7 MPa NaCl solution.

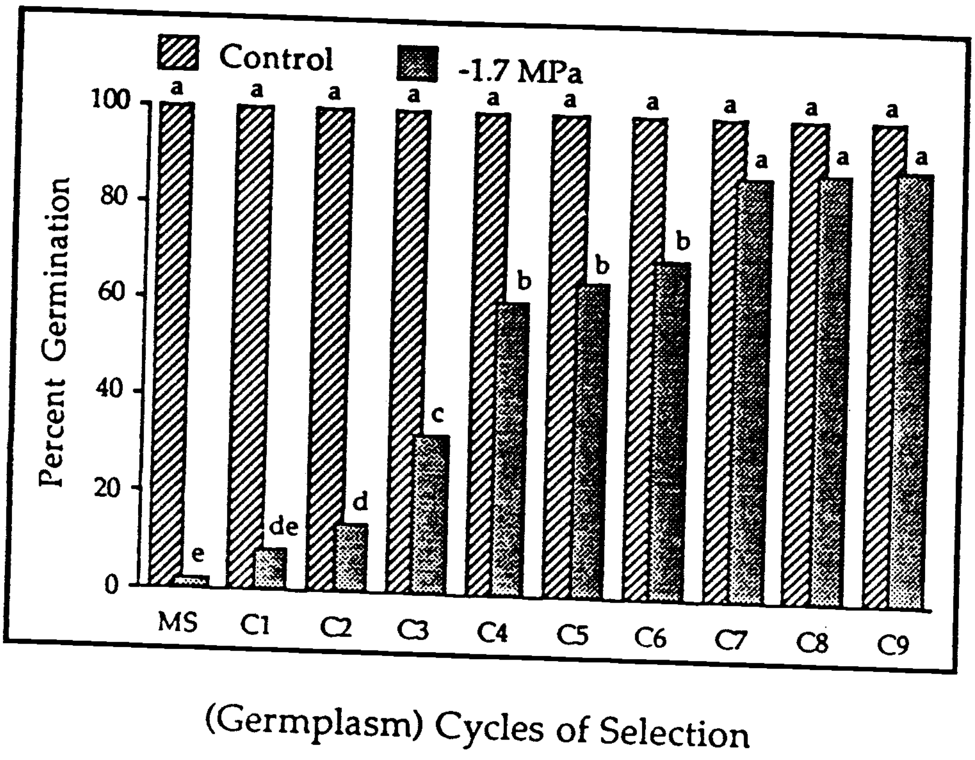
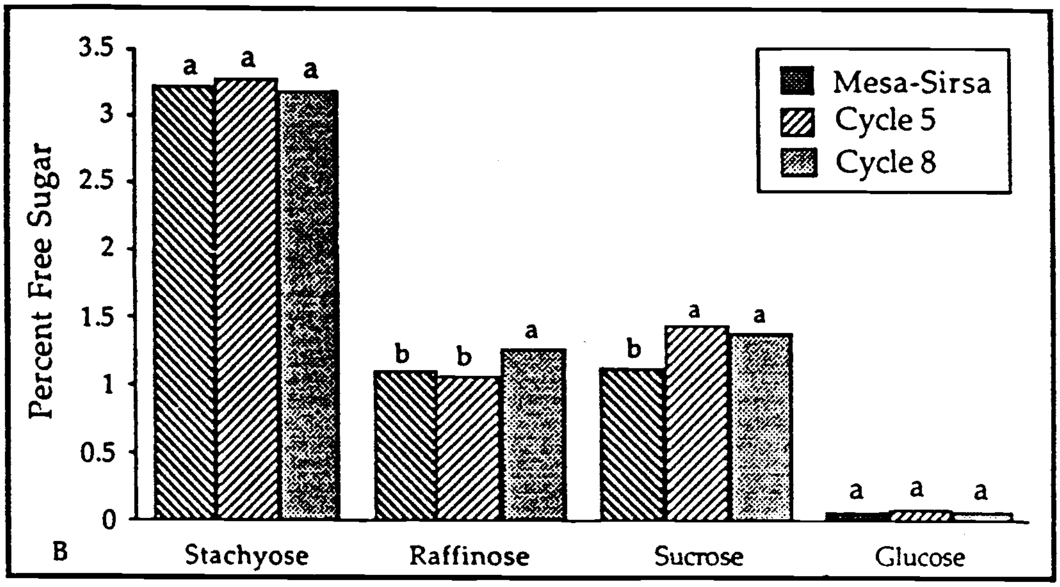


Figure 2: Percent free sugar of Mesa-Sirsa and Cycles 5 and 8.



(Germplasm) Cycles of Selection

Figure 3. Percent seed sugar of Mesa-Sirsa and Cycles 5 and 8 after acid hydrolysis.