

Mechanisms of Desiccation Tolerance in Wheat (*Triticum aestivum* L.) Seedlings

Atif Hassan Naim¹ and Faisal El Gasim Ahmed^{2*}

Abstract-- Mechanisms of desiccation tolerance were evaluated in wheat seedlings. The possible role of exogenously added sugars on desiccation tolerance of four- and six-day old wheat seedlings was investigated under controlled conditions. Desiccation tolerance was expressed as percentage of survived seedlings following severe dehydration for 96 h. The seedlings of spring wheat germinated in water and dehydrated to about 20% relative water content (RWC) survived almost completely up to the fourth day following imbibition but in the six-day old seedlings, only less than half of seedlings subjected to dehydration survived. Glucose and fructose, at concentration as low as 5 mM, increased considerably the survival of the six-day old seedlings. Growth at similar concentration of mannose or sucrose decreased significantly the survival of either four- (by 40% or 23%, respectively) or six-day old seedlings (by 13% or 10%, respectively). In the six-day old seedlings, the total pool of soluble sugars in seed remnants was significantly higher than in four-day old seedlings whereas in shoots and roots it was the same for both seedlings age. Our analysis of soluble sugars evidenced a possible role of raffinose on desiccation tolerance in wheat seedlings.

Keywords-- Desiccation tolerance, sugars, wheat, *Triticum aestivum*

I. INTRODUCTION

DESICCATION tolerance is one of the most outstanding features in the plant kingdom. It can be defined as the ability of the cell to rehydrate successfully after the removal of 80-90 % of protoplasmic water, reaching moisture content below 0.3 g H₂O /g dry matter [1]. Seeds that possess such an attribute, the so-called orthodox seeds, can be dried and stored for many years without significant loss of viability. Most crop species such as wheat, rice, maize and soybean produce orthodox seeds. In contrast, seeds that lack this characteristic, the so-called recalcitrant seeds, shed at high water content, metabolically active and have short life spans [2].

Orthodox seeds acquire desiccation tolerance during their development and dry mature seeds are extremely tolerant to desiccation [3]. During germination; however, desiccation tolerance is rapidly lost depending upon the species. For example, the seedlings of wheat up to fourth day following imbibition are able to survive severe dehydration. This

desiccation tolerance period for other species is either shorter as in soybean [4], or longer as in pea [5].

Although the phenomenon of desiccation tolerance at the early stage of wheat seedling development is well known [6] the induction mechanism of desiccation intolerance (sensitivity) is not fully understood. Various authors point to the role of soluble sugars in the protection against stresses. Mobilization of storage reserves in the endosperm of cereal seeds is tightly regulated and has a primary pivotal role in the interaction among sugar, ABA and gibberellins pathway responsible for the response to drought [7]. A central role of sugars depends not only on direct involvement in the synthesis of other compounds, production of energy but also on stabilization of membranes, acting as regulators of gene expression and signal molecules [1],[8]. Some authors [5],[9] observed accumulation of oligosaccharides including raffinose in response to dehydration but others are of the opinion that coincidence of dehydration tolerance with raffinose content has not been proved [10]. The aim of this work is to study the effect of exogenously added sugars on the desiccation tolerance of wheat seedlings at different stages of growth. The changes in soluble sugars pool and raffinose content in response to water deficiency are also investigated in various parts of wheat seedlings.

II. MATERIALS AND METHODS

A. Plant materials

Spring wheat (*Triticum aestivum* L. cv. Eta) seeds were surface-sterilized with 1% sodium hypochloride (NaOCl) for 20 min and then rinsed several times with distilled water. After soaking in water overnight at 4°C in the dark, seeds were transferred to plastic containers with wire gauze as mechanical support. The hydroponics system was continuously aerated and growth media changed every day. Seedlings were grown in a growth chamber at day/night temperature of 22/18 °C, relative humidity of 60/70 % and irradiance of 100 μmole m⁻² s⁻¹ PPFD for 8h. In order to evoke water deficit in seedlings, water was drained off and seedlings were allowed to dehydrate on filter paper for four days in the growth chamber under the same conditions. This period of drying has been used for comparative characteristics of the four- and six-day old seedlings. After these dry treatments, seedlings were rehydrated by distilled water on wet filter paper. Three days after rehydration, the percentage of survived seedlings was calculated as the number of seedlings resuming growth i.e. roots and shoots were able to elongate. Similar experiments

¹Faculty of Agric. and Environmental Sciences, University of Gadarif, Sudan

²Faculty of Agriculture, University of Khartoum, 13314 Shambat, Sudan

*Corresponding author E-mail:Fgahmed2005@yahoo.com Cell phone number: +249912988847

were performed with seedlings grown alternatively in 5 mM solutions of glucose, fructose, mannose and sucrose throughout the whole experimental period. It has been previously found that at this concentration germination was not affected [11].

Water deficit in seedlings was calculated according to the relative water content (RWC) formula:

$$RWC (\%) = \frac{(Fresh\ mass - Dry\ mass) \times 100}{(Full\ turgor\ mass - Dry\ mass)}$$

Where:

Full turgor mass represented the mass following submersion in water overnight in the dark and dry mass was determined after drying at 70 °C overnight.

B. Soluble sugar analysis

Seedlings were harvested at the end of a fourth and of a sixth day following imbibition and at the end of a fourth day of dehydration and at the end of a third day of rehydration for further analyses. For sugar determination, seedlings (shoots and roots) and remnant seed tissues were processed separately. Samples of 500 mg fresh weight were ground in liquid nitrogen to a fine powder and then extracted with 2.5 ml of 80% ethanol with 50 µl of 20 µM fructose as an internal standard. Extraction was carried out for 30 min at 40 °C. The samples were then centrifuged for 10 min at 17000 rpm. Supernatants were evaporated and pellets were dissolved in 2 ml of deionized water and then desalted on Dowex 50W and Amberlit IRA-145 columns (Alltech Associates, Inc, 4.0ML, 50/PK). Carbohydrates were separated by HPLC on Carbohydrate Analysis TM columns (Waters) using 85% acetonitril as an eluent. Qualitative and quantitative analyses of sugars were carried out refractometrically [12].

C. Statistical analysis

Data were subjected to analysis of variance using SPSS statistical packages (version 15). The results represent the average of three independent experiments in three replicates. A significance of differences was computed as the Least Significant Difference (LSD) and the mean values obtained were compared by the Tukey's Honestly Significant Difference Test ($P \leq 0.05$).

III. RESULTS

A. Effect of exogenously applied sugars on desiccation tolerance of wheat seedlings

Desiccation tolerance was expressed as the percentage of survived seedlings following severe dehydration for 96 h. This treatment led to the same water deficit (about 20% RWC); irrespective of the seedling age [11]. The seedlings of spring wheat germinated in water and dehydrated to about 20% RWC survived almost completely up to the fourth day following imbibition but in the six-day old seedlings, only less than half of seedlings subjected to dehydration survived (Table I).

TABLE I
SURVIVAL PERCENTAGES OF FOUR- OR SIX-DAY OLD WHEAT SEEDLINGS GERMINATED IN WATER AND 5MM SOLUTIONS OF SUGARS AFTER DEHYDRATION FOR 96H.

Medium	Seedling age	
	4 days	6 days
Water	96.6b*	43.3b
Fructose	100.0a	76.7a
Glucose	100.0a	70.0a
Mannose	56.7d	30.0b
Sucrose	73.3c	33.0b
LSD	3.0	19.4

*Means with the same letter in each column are not significantly different at 5% level of probability according to LSD test.

Glucose and fructose, at concentration as low as 5 mM, increased considerably the survival of the six-day old seedlings (Table I). Growth at similar concentration of mannose or sucrose, however; decreased significantly the survival of either four- (by 40% or 23%, respectively) or six-day old seedlings (by 13% or 10%, respectively).

B. Effect of dehydration on the content and composition of soluble sugars pool in wheat seedlings

The content of total soluble sugars in seed remnants of wheat seedlings germinated in water (i.e., hydrated) was about 10 times higher than in shoots and about 15 times higher than in roots (Fig.1). Differences in the soluble sugars pool between the four- and six-day old seedlings were evident only in seed remnants of both fully hydrated and dehydrated seedlings. In this regard, the pool of soluble sugars in seed remnants of the six-day old seedlings was significantly higher than that of four-day old seedlings whereas in shoots and roots there was no difference in sugars pool (Fig. 1). Dehydration reduced the pool of soluble sugars in seed remnants by about 55% and 65% in the four- and six-day old seedlings, respectively. In addition, the pool of soluble sugars decreased only by about 15% in shoots and remained at the same level in roots as a result of dehydration (Fig. 1). In contrast, no significant differences were found in the size of soluble sugars pool in seed remnants, shoots and roots of both types of seedlings recovered from water deficiency (i.e., rehydrated). The total sugars pool in seed remnants was recovered on rehydration by 50%, although statistically insignificant, in the four-day old seedlings (Fig. 1). In shoots, the pool of soluble sugars in rehydrated seedlings was about 50% higher as compared to the hydrated seedlings and remained at the same level in roots of four-day old recovered seedlings (Fig. 1).

The difference in the size of sugars pool detected in this study was associated with significant changes in its composition (Fig.1). In this respect, although sucrose remained a predominant component of the soluble sugars pool in shoots and roots irrespective of seedlings age, in seed remnants sucrose content was significantly lower than the sum of glucose and fructose. Moreover, dehydration decreased sucrose content to more than 50 % and fructose to undetectable trace in seed remnants (Fig. 1). On rehydration treatment the share of fructose was regained in seed remnants. A nearly double increase of the pool size in rehydrated shoots was due to sucrose content; while in roots the content of

sucrose decreased and it was not recovered on rehydration (Fig. 1).

C. Effect of dehydration and exogenously applied sugars on raffinose content in wheat seedlings

Raffinose was undetectable in seed remnants of both types of seedlings. However, in shoots and roots of six-day old seedlings germinated and grown in water its level was about four to five times lower than in four-day old seedlings (Table II). Dehydration increased the level of raffinose about two times in shoots and roots of six-day old seedlings compared to their respective values in hydrated treatments. Addition of fructose and glucose to growth media increased significantly the content of raffinose both in shoots and roots of hydrated

seedlings whereas mannose and sucrose containing media had an opposite effect (Table II). In dehydrated shoots and roots, fructose or glucose in the growth media increased raffinose content whereas mannose and sucrose had no significant effect i.e., both in shoots and roots the level of raffinose was the same as in shoots and roots of seedlings grown under sufficient water supply. In seedlings recovered from dehydration the content of raffinose was lower both in shoots and roots and did not change significantly in seedlings germinated on mannose or sucrose media (Table II). Glucose and fructose, however, increased the content of raffinose in shoots and roots of seedlings recovered from dehydration.

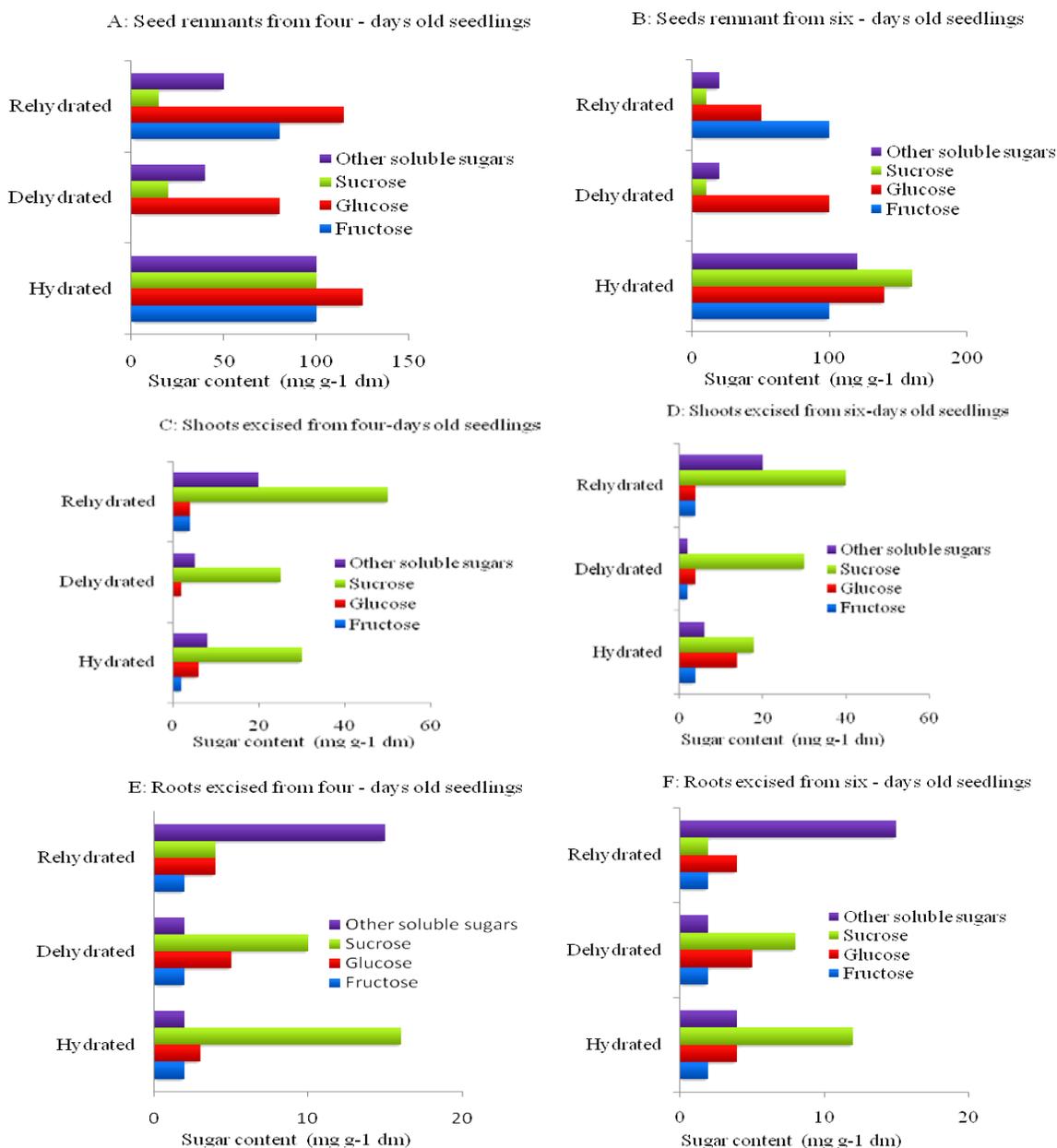


Fig.1. Sugar content in seed remnants (A, B), shoots (C, D) and roots (E, F) of four- and six-day old wheat seedlings subjected to full hydration, 96 h dehydration and 72 h rehydration

TABLE II
RAFFINOSE CONTENT (MG G-1 DM) IN FOUR- AND SIX-DAY OLD WHEAT
SEEDLINGS GERMINATED IN WATER AND 5 mM SOLUTIONS OF SUGARS

Medium	Age (days)	Hydrated		Dehydrated		Rehydrated	
		Shoot	Root	Shoot	Root	Shoot	Root
Water	4	4.1	2.7	7.9	4.6	2.6	1.0
	6	0.9	0.5	1.8	0.9	1.0	0.4
Fructose	4	14.4	7.6	17.6	9.6	22.5	15.0
	6	10.9	5.2	10.2	3.1	16.4	8.9
Glucose	4	9.5	4.4	10.8	4.9	19.3	9.9
	6	8.0	2.5	8.3	1.6	14.6	7.2
Mannose	4	0.4	0.2	0.7	0.1	0.7	0.4
	6	0.6	0.1	0.6	0.1	0.7	0.2
Sucrose	4	0.4	0.1	0.6	0.1	0.8	0.2
	6	0.2	0.1	0.3	0.1	0.5	0.1
LSD _{0.05}		1.7	0.3	2.1	0.7	1.1	1.2

IV. DISCUSSION

The observed inability of the six- day old seedlings to tolerate the same water deficit as compared to the four-day old seedlings seems to be associated with the stage of plant development. This is consistent with the opinion that desiccation tolerance is a developmentally regulated process which is modulated by sugars and controlled by hormones [13]. Therefore it seems interesting to compare seedlings metabolism at these two developmental phases of growth [14].

A complex essential role of soluble sugars in plant metabolism is well known and recently it has been claimed that even sugar flux may be a signal for metabolic regulation [15]. The question has been raised to what extent examination of different sugar distribution in the seedlings at different stage of development, and also of different response to water deficiency could provide information on regulation of desiccation tolerance. The observed divergent action of added sugars on seedling survival (increase by fructose and glucose and decrease by sucrose and mannose) illustrates integration into mechanism of desiccation tolerance. In this regard, the supply of glucose and fructose had profound positive effect on survival of six-day old seedlings indicating that growth up to this stage depends on seed storage reserves. There are reports on inhibition of seed germination by exogenous sugars among them glucose at low physiologically relevant concentration and the effect being independent of osmotic stress [15]. However, the concentration of sugars used in our experiments, namely 5mM, was lower than those used by the aforementioned authors. At this concentration, unable to cause osmotic stress, the applied sugars did lower neither the rate nor the energy of germination as reported by many researchers [16], [17].

The divergent effect of sugar media on seedling survival is closely connected with sucrose synthesis and hydrolysis. On glucose or fructose containing media synthesis of sucrose was intensified as shown by the high activity of sucrose phosphate synthase, while on sucrose medium sucrolysis was a prevailing pathway of sucrose metabolism due to the induction of invertases [18]. Higher activity of these last enzymes over that of sucrose phosphate synthase in the six-days old seedlings resulted in a higher glucose + fructose/sucrose ratio than in younger seedlings and consequently lower survival of older seedlings. Accumulation of hexoses, especially in the six-days

old seedlings grown in the mannose medium was caused by a poorly metabolized mannose 6-P formed by hexokinases [16].

The glucose + fructose/sucrose ratio was higher in younger seedlings since the effect of α -amylase activity up to fifth day of germination predominates the activity of sucrose phosphate synthase which was significantly higher in older seedlings both in hydrated and dehydrated state and the share of glucose being higher than that of fructose. The content of soluble sugars in roots did not differentiate the four-day old seedlings from the older ones; however differences in composition of this pool demonstrate changes in sucrose metabolism. The glucose + fructose/sucrose ratio in contrast to that in seed was lower in the four-day old seedlings than in the six-day old seedlings. This hold both for shoots and roots but in dehydrated shoots this ratio was lower while in roots it was elevated indicating different sensitivity of roots and shoots to dehydration [6].

The decrease in sucrose content in vegetative organs has been ascribed to the increased activity of invertases (β -fructofuranosidase) which may counteract the accumulation of sucrose in vacuoles due to a higher requirement of intensively growing seedlings on the monosaccharide [16]. It should be noted that in rehydrated shoots the significant increase of the content of soluble sugars even exceeding that in hydrated shoots was associated also with increase in the glucose + fructose/sucrose ratio. Changes of the hexose/sucrose ratio i.e. "cellular energy state" in developing seedlings, observed in monocots and legumes is supposed to be one of the main reason of the "break" in dehydration tolerance and signal transition from heterotrophy to autotrophy [8].

The importance of the glucose + fructose/sucrose ratio in dehydration tolerance was confirmed in the experiments with exogenous sugars in growth media. This ratio was significantly lower in the glucose and fructose containing media in which seedling survival was prolonged compared to seedlings grown in the sucrose media (higher ratio). In addition, the results obtained with sugar media draw attention to the role of sugar flux in seedling development and dehydration tolerance. Glucose and fructose stimulated transport of soluble sugars to shoots, since increased sucrose content in seedlings grown in these media induced sucrose transport [16], although less effectively in dehydrated seedlings. In contrast, this effect was not observed in the mannose and sucrose containing media.

The observed differences in sucrose metabolism in the two analyzed heterotrophic phases of seedling development are the result of the sugar-hormone interaction in a complex regulatory web of enzymes induction and signaling. In water deficient plants ABA level is elevated mainly by induction of genes coding for enzymes catalyzing ABA biosynthesis [19]. There are evidences that glucose participates in this induction and also retards ABA degradation [15]. This action by increasing ABA levels leads to regression of metabolism to dehydration "storage-dominates metabolic site" [20]. It seems, however, that ABA response may not be mediated by sugars [7], [21] and that sugars signaling may be ABA independent [15].

Desiccation sensitivity is closely connected with changes in subcellular organization and membrane structure [5],[6]. Our analysis of soluble sugars evidenced a possible effect of raffinose on dehydration tolerance in wheat seedlings. This non-reducing oligosaccharide is believed to protect macromolecular structures by replacing water molecules between the polar head-groups of phospholipids [12] or form a protective vitreous state [22]. Raffinose undergoes rapid degradation during germination by galactosidases [10] and therefore raffinose content in the four-days old seedlings was 4 times higher than in the six-days old seedlings. Dehydration increased raffinose content in the four- and six-days old seedlings and may be factor opposing dehydration damage. In view of the role of raffinose in dehydration tolerance it is interesting that glucose and fructose which retards sucrose metabolism stimulated raffinose synthesis in dehydrated and rehydrated seedlings independent of their age. Knowledge on the enzymes involved in raffinose metabolism could give an answer.

REFERENCES

- [1] F. A. Hoekstra, E. A., Golovina, and J. Buintink, Mechanisms of plant desiccation tolerance. *Trends in Plant Science*, vol. 6, pp. 431-438, 2001.
- [2] M. R .Faria, J. Buitink, A. A .M. van Lammeren, and H .W .M. Hilhorst. Changes in DNA and microtubules during loss and re-establishment of desiccation tolerance in germinating *Medicago truncatula* seeds. *Journal of Experimental botany*, vol.56, pp. 2119-2130. 2005.
- [3] N. W. Pammenter, and P. Berjak, Evolutionary and ecological aspects of recalcitrant seed biology. *Seed Science Research*, vol. 10, pp. 301-306, 2000.
- [4] T. Senaratna, and B. D. McKersie, Dehydration injury in germinating soybean (*Glycine max* L. Merr.) seeds. *Plant Physiol.*, vol. 72, pp. :911-914, 1983.
- [5] F. Corbineau, M.A. Picard, J. .A. Fougereux, F. Ladonne, and D. Come, Effects of dehydration conditions on desiccation tolerance of developing pea seeds as related to oligosaccharide content and cell membrane. *Seed Science Research*, vol.10, pp. 329-339, 2000..
- [6] J. M. Farrant, C. Bailly, J. Leymarie, B. Hamman, D. Côme, and F. Corbineau, Wheat seedlings as a model to understand desiccation tolerance and sensitivity. *Physiol. Plant*. Vol. 120, pp.:563-574, 2004.
- [7] R. Finkelstein, and T. Lynch, Abscisic acid inhibition of radicle emergence but not seedling growth is suppressed by sugars. *Plant Physiology*, vol. 122, pp.1179-1186, 2000.
- [8] S. Smeekens, Sugar-induced signal transduction in plants. *Annu. Rev. Plant Biol.*, vol. 51, pp. 49-81, 2000.
- [9] A. Whittaker, T. Martinelli, A. Bochicchio, C. Vazzana, and J. M. Farrant, Comparison of sucrose metabolism during the rehydration of desiccation -tolerant and desiccation - sensitive leaf material of *Sporobolus stapfianus*. *Physiology of Plant*, vol.122, pp. 11-20, 2004.
- [10] T. Peterbauer, and A. Richter, Biochemistry and physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds. *Seed Science Research*, vol. 11, pp. 185-197, 2001.
- [11] A. Miazek, J. Bogdan, and B. Zagdańska, Effects of water deficit during germination of wheat seeds. *Biology of Plant*, vol.4, pp.:397-403, 2001.
- [12] K. L. Koster, and A. C. Leopold, Sugars and desiccation tolerance in seeds. *Plant Physiology*, vol. 88, pp.:829-832, 1988.
- [13] P. Leon, and J. Sheen, Sugar and hormone connections. *Trends Plant Sci.*, vol. 8, pp.110-116, 2003.
- [14] E. M. Wiedenroth, G. Wernicke, and P. Hoffmann, Morphological and anatomical characterization of the coleoptile of *Triticum aestivum* with regard to the evolution of forms with different ploidy levels. *Annals of Botany*, vol. 66, pp. 531-540, 1990.
- [15] S. I. Gibson, Control of plant development and gene expression by sugar signaling. *Current Opinions in Plant Biology*, vol. 8, pp. 93-102, 2005.
- [16] E. Loreti, D. Bellis, A. Alpi, and P. Perata, Why and how do plant cells sense sugars. *Annual Botany*, vol 88, pp. 803-812, 2001.
- [17] K. Koch, Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Current Opinions in Plant Biology*, vol. 7, pp. 235-246, 2004.
- [18] L. Borisjuk, H. Rolletschek, R. Radchuk, W. Weschke, U. Wobus, and H. Weber, Seed development and differentiation: a role for metabolic regulation. *Plant Biology*, vol. 6, pp.375-386, 2004.
- [19] L. Xiong, K. S. Schumaker, and J. K. Zhu, Cell signaling during cold, drought and salt stress. *Plant Cell*, vol. 14, pp. 165-183, 2002.
- [20] F. Rook, and M. W. Bevan, Genetic approaches to understanding sugar response pathways. *Journal of Experimental Botany*, vol. 54, pp. 495-501, 2003.
- [21] J. Price, T.C. Li, S. G. Kang, J.K. Na, and J.C. Jang, Mechanisms of glucose signaling during germination of Arabidopsis. *Plant Physiology*, vol. 132, pp.1424-1438, 2003.
- [22] R. J. Williams, and A. C. Leopold, The glassy state in corn embryos. *Plant Physiology*, vol. 89, pp. 911-981, 1989.