Pak. J. Bot., 33(2): 197-202, 2001.

EFFECTS OF DROUGHT STRESS ON ABSCISIC ACID, MANNITOL, K^+ AND Ca^{2+} CONTENT IN TWO LINES OF WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

Experiments were conducted to determine the level of ABA, K^+ and Ca^{2+} mineral content and mannitol in the leaves of two lines of wheat (*Triticum aestivum* L.) under field condition in order to find any relationship between them and the tolerance of the plant against drought stress and to know if we can use one or more of them in determining tolerant or sensitive plant. The resistance of the plants was affected by the studied factors and all of them changed under drought conditions. Both K^+ and Ca^{2+} have an important role in determining the stomatal situation, however, the ratio of K^+/Ca^{2+} may regulate the position of stomata in different conditions. The existence of an inverse relation between this ratio and the rate of mannitol confirms the validity of this hypothesis. When the ratio is low, stomata are closed and when it is higher than the control, the stomata are open. Thus we could suggest this ratio to determine tolerant and sensitive lines, as well as the tolerant or sensitive stages of the plant development.

Introduction

Environmental stresses come in many forms, yet the most prevalent stresses have in common their effect on plant water status. The availability of water is important for its biological roles as a solvent and transport medium.

Plant species vary in their sensitivity and responses to the decrease in water potential caused by drought, low temperature or high salinity (Bohnert & Nelson, 1995). Water availability is one of the most important factors in plant growth. Higher plants exhibit a range of biochemical, physiological and morphological adaptations in their responses to water stress, including accumulation of abscisic acid (ABA) as one of them. ABA is known to influence a variety of processes characteristic of water stress (Roberts, 1998), and a number of metabolites, such as fructan, Pro., Gly., betaine, trehalose and mannitol. As a group, these and other metabolites which alone or in combinations accumulate in a number of stress-tolerant plants, have been termed compatible solutes, because they do not interfere with normal metabolic reactions even at high concentrations (Shen & Jensen 1997).

Under drought conditions an increase in the ABA concentration of the apoplastic compartment of the leaf is an important determinant of stomatal behavior and there is evidence that ABA receptors are located at the external surface of the plasmalemma of the guard cell. When bound, the receptors induce changes in membrane ion transport and reduce the osmolarity of the guard cell such that it loses turgor pressure, which leads to stomatal closure (Wilkinson & Davies, 1997; Harrison & Walton, 1975). Stomatal conductance may be influenced under drought conditions via changes in leaf-water potential and via metabolic changes in the leaf. There is evidence for non – hydraulic root-to-shoot communication on soil water status, which causes stomata to close without changes in the water potential and the turgor of the leaf (Gollan & Schurr, 1992).

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The role of ABA in plant growth and development is multifunctional; it is involved in stomatal function, seed development and germination, and the plant's responses to drought, salinity and cold stresses (Hong & Bary, 1992). Dehydration of plants has been shown to cause ABA levels to increase up to 50-fold. This increase is due to denovo biosynthesis. A key factor that triggers ABA accumulation is a loss of cell turgor or cell membrane perturbation which occurs as pressure potentials approach zero (Guerrero & Mullet, 1986). In response to these abiotic stresses, all of which disturb the intracellular water balance, many plants and bacteria synthesize and accumulate osmotically active, low molecular weight compounds such as sugar alcohols. Although their exact function in plants is unknown, numerous studies suggest these osmolytes may protect the plant from abiotic stresses. An example of such an osmolyte is a sugar alcohol, mannitol, which occurs widely in plants and animals. Mannitol accumulates in leaves and roots (Tarczynski & Jensen, 1993).

The regulation of stomatal apertures by guard–cell osmotic potential was established well before the turn of the century. Early physiologists explained guard-cell osmoregulation on the basis of the starch sugar hypothesis. However, the K^+ hypothesis dominates contemporary thinking, in stomatal physiology. Numerous studies have documented K+ uptake during stomatal opening (Talbott & Zeiger, 1996).

Ca^{2+} has been shown to play a key role in regulation of guard-cell turgor and stomatal aperture. Increase in Ca^{2+} is known to promote stomatal closure and Ca^{2+} has been shown to act as a second messenger in the response of stomata to both opening and closing stimuli. Therefore in the absence of other compensatory mechanisms, it can be predicted that factors that interfere with cellular Ca^{2+} homeostasis will have a marked effect on stomatal behavior (Mcainsh & Clayton, 1996). The present paper describes the effects of water deficit on the changes of ABA, Ca^{2+}, K^+, mannitol and $K+/Ca^{2+}$ content in leaves of two wheat lines and also their roles in tolerance of the plant against drought.

Materials and Methods

Plant growth: Field experiments were conducted during the 1999 growing season at the Institute of Agriculture research experiment station of Karaj. Spring wheat (*Triticum aestivum* L.) lines, Boolany and Ghods, were used in this study. Boolany is a local line and Ghods is an advanced breeding line. The experimental site was divided into four plots, each measuring 3m x 5m. Individual plots were separated by 2 m buffer zones on each side to avoid seepage (Hafid & Smith, 1998). In one set, control plants were regularly watered normally and the other set of plants were subjected to water deficit by withholding water for special periods of their development. These groups were respectively watered normally until 19, 31 and 43 Zodok's growth stages and after that they resisted dry conditions.

Leaf samples were harvested 90 days after planting at medium milk stage. Only the terminal leaves were used as a source of experimental material. Frozen leaf samples were lyophilized prior to extraction.

Analysis of ABA: Lyophilized leaves were cut by scissors and extracted with 4ml of extracting ethanol (0.5% W/V), for 48h at 4°C (Daie & Wyse, 1982). ABA was quantified using a competitive – inhibitive ABA ELISA. The ELISAs were carried out at Pasteur

Institute of Iran (Bartels & Scnneider, 1990). Coupling of (±) ABA-carrier and providing of ABA polyclonal antibody was performed as described by Weiler (1986).

Analysis of mannitol: Leaf mannitol content was measured by GLC. A Shimadzu GC-16A equipped with a flame ionization detector and a 1.6m x 3.2mm i.d. glass column packed with 5% SE-30 was used. Operation temperatures were 260°C for the oven and 280°C for both the injector and detector block. Nitrogen was used as carrier gas at a flow rate of 50ml min^{-1}. The flow rates of hydrogen and air were 55 and 400 ml min^{-1}, respectively.

Determination of K^+, Ca^{2+}: For determination of minerals content, the tissues were digested using PCA (perchloric acid). Ca^{2+}, K^+ concentrations were determined using an atomic absorbtion spectrophotometer (Szabó - Nagy & Galiba, 1992).

Results

K^+, Ca^{2+} content: Changes in the concentration of K^+ in the leaves subjected to drought and control in the lines Boolany and Ghods have different patterns. Accumulation of K^+ in Boolany groups, is more than the similar groups in Ghods line. Changes of Ca^{2+} in these lines, except the first group have the same pattern. Usually the rate of Ca^{2+} in Ghods groups is more than the similar groups in Boolany (Table 1).

Table 1. Effects of water deficit on mineral elements, ABA and mannitol content in leaves of Ghods and Boolany line of wheat.

Treatment	K^+ (ppm/g)	Ca^{2+} (ppm/g)	K^+/Ca^{2+} ratio	ABA (ng/mg)	Mannitol (mg/g)
			Wheat lines Ghods		
G_1	2460c	1506b	1.6c	64.1b	0.108b
G_2	2920a	1254d	2.32a	12.4d	0.05d
G_3	2740b	2412a	1.13d	26.4c	0.132a
Control	2920a	1404c	2.07b	432.5a	0.078c
Mean square	147.268×10^3	225.133×10^3	841×10^{-3}	120.381×10^3	5×10^{-3}
	***	***	***	***	**
C.V.	0.99	1.05	7.38	2.190	8.35
			Wheat lines Boolany		
G_1	2280d	700d	3.25a	10.9b	0b
G_2	3520c	1102c	3.19a	1.4c	0.06a
G_3	3820b	1304a	2.92d	125.6a	0d
Control	4120a	1254b	3.28a	4.1c	0.04a
Mean square	1958.7×10^3	225.133×10^3	81×10^{-3}	10.871×10^3	0.3×10^{-3}
	***	***	***	***	**
C.V.	1.05	1.05	2.81	5.82	7.50

Data is average of 3 independent plots.

Significant at the 0.01 level of probability.

K^+/Ca^{2+} ratio: The ratio of K^+/Ca^{2+} indicates that in Ghods line, it's pattern of conversions is similar to K^+ and opposite to Ca^{2+} and mannitol. However, in Boolany line, it does not have a definite relation with any of the parameters studied, but it could be said that in all Boolany groups, the ratio is more than the same groups in the other line (Table 1).

ABA content: Measurment of ABA in different groups of the two lines indicated that the level of this hormone in advanced breeding line Ghods in normal condition is much more than the local line Boolany in the same condition and in other conditions in the same line. In the other situations, the changes the levels in different groups of these lines exhibit the same pattern. The accumulation of the hormone in the second line is generally low (Table 1).

Mannitol content: The results show that it has an opposite pattern in the 2 lines. Mannitol content in Ghods line, except in the second group, is much more than the other.

Discussion

On the basis of the obtained results, there is a relation between ABA and the stomatal closure. It has been suggested that when ABA increases, the water potential of guard cell decreases and the stomata become closed. The closure of stomata in plants under water stress precedes the formation of new ABA in the tissues, and it is possible that the release of ABA already present in the chloroplasts is responsible. This release would depend on a change in the penetrability of the chloroplast envelopes to ABA. The regulation by ABA could be achieved by ABA interfering with the ionic exchange of guard cell or with the metabolic events necessary for interconversions between starch and malate, because of ABA derive from pyruvat. The outcome of the action of ABA is the inhibition of both the uptake of K^+ ions and disappearance of guard cell starch, under conditions normally conductive to stomatal opening (Paleg & Aspinall, 1981).

K^+ ion plays a key role in the regulation of stomatal opening and closing. However, it was observed that its absorption had interfered with the level of ABA. There is also very convincing evidence for the involvement of Ca^{2+} in ABA responses, at least in controlling stomatal closure. Based on the current information, models of ABA signalling should incorporate a plasma membrane-localized Ca^{2+} receptor (Bowler & Chua, 1994).

Drought stress also can directly or indirectly induce oxidative stress. The stress affects stomatal behavior in a concentration-dependent manner. It inhibits stomatal opening and promotes stomatal closure. The effects appear to be the result of increases in guard-cell Ca^{2+} and changes in guard-cell Ca^{2+} homeostasis. This has the potential to affect the regulation of guard-cell turgor and stomatal aperture and the control of gas exchanges through alterations in the processes of Ca^{2+}-based signal transduction (Price & Taylor, 1994; Mcainsh & Clayton, 1996). Thus, when Ψw fell rapidly under these conditions, true photosynthesis, net photosynthesis and photorespiration all decline, although photorespiration increases as a proportion of net photosynthesis, so Mehler products that included H_2O_2 and superoxyl radical (O_2^-) increased. They react with each other to produce hydroxyl radicals (OH°), a highly reactive oxygen species capable of causing oxidative damage to lipids, proteins, pigments and nucleic acids to prevent this from occurring. The $\bar{O}_2$ H_2O_2 and radicals derived from their reaction with other chloroplast components must be effectively scavengered near their site of production (Dey & Harborne, 1997), because these compounds inactivate Calvin cycle. One of these scavenging systems is mannitol producing. Mannitol (as a polyol) increases the capacity to scavenge hydroxyl radicals in chloroplast and protects cells against oxidative damage (Shen & Jensen 1997). Based on the current information, the release of ABA already present is responsible for closing of the stomata, thus we can introduce Ghods as a tolerant line against drought.

The result of the study would suggest the possibility of using the ratio of K^+/Ca^{2+} for predicting the tolerant plants against drought. Probably, it is the result of interference between the factors and involvement of them in permeability of cell membrane, such as stomatal membrane and also the membrane of chloroplasts, which are important in the regulation of guard-cell turgor and stomatal aperture. We could introduce it as a regulator or an indicator for stomatal situation, that is when the ratio is high the stomata are open and when it is low the stomata are closed. Considering the changes of mannitol as a polyol that affects water potential of guard-cell and scavenge hydroxyl radicals under water deficit could confirm this hypothesis.

In the Boolany, the ratio of K^+/Ca^{2+} under stress and in the normal condition was the same (Table 1). Therefore we could predict that it is not a tolerant line. Analysis of mannitol level also confirms it. Boolany may be a tolerant line against other stresses like salinity. In the Ghods, the results indicate that probably at some stages, it is a tolerant line against drought. The level of K^+/Ca^{2+} ratio and mannitol studies showed that the first and third groups are tolerant and the second group that has been treated since 31 Zadok's growth stage is sensitive. However, the exact role of the ratio of K^+/Ca^{2+} on the position of stomata are not yet known which needs investigation.

Acknowledgement

We thank Dr. V. Niknam of University of Tehran for his help in going through the manuscript.

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(Received for publication 25 October 2000)