

Apoplastic and symplastic solute concentrations contribute to osmotic adjustment in bean genotypes during drought stress

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Abstract: The present study investigates changes in the inorganic ions, proline, and endogenous abscisic acid (ABA) contents of the apoplastic and symplastic compartments of leaves from drought-tolerant (Yakutiye) and drought-sensitive (Zulbiye) cultivars of the common bean (*Phaseolus vulgaris* L.). Drought stress caused a decrease in leaf water potential and stomatal conductance in both cultivars. Concentrations of proline in the drought-tolerant and drought-sensitive cultivars increased in response to drought stress in both compartments. The symplastic K⁺ concentration decreased in both cultivars. However, the opposite trend was observed concerning K⁺ concentrations in the apoplastic areas. While the symplastic Na⁺ concentrations significantly decreased in the drought-tolerant cultivar, the apoplastic Na⁺ concentrations increased during drought stress. However, Na⁺ concentrations did not significantly change in either of the compartments in the drought-sensitive cultivar. The Ca²⁺ concentrations in the sensitive cultivar significantly decreased in both compartments during drought stress. In the tolerant cultivar, the Ca²⁺ concentration significantly increased in the symplast but decreased in the apoplast. Cl⁻ concentrations in the tolerant cultivar did not significantly change in either compartment. In the sensitive cultivar, the Cl⁻ concentration increased in the apoplastic area but decreased in the symplastic area. In addition, while the symplastic sap of the leaves exhibited a constant pH value, it diminished in the apoplast during drought stress. Symplastic and apoplastic ABA concentrations significantly increased in both cultivars. It might be said that inorganic ions (especially Na⁺, K⁺, and Ca²⁺) and ABA concentrations changed between the apoplastic and symplastic spaces to contribute to osmotic adjustment under drought stress. In addition, the drought-tolerant cultivar showed a much higher capacity to maintain osmotic adjustment between the symplast and the apoplast.

Key words: Apoplast, symplast, drought stress, tolerance, inorganic ions, abscisic acid

Kuraklık stresi sırasında fasülye çeşitlerinde apoplastik ve simplastik alanlardaki çözünen madde konsantrasyonu osmotik regülasyona katkı sağlar

Özet: Kuraklığa hassas ve dayanıklı 2 fasülye çeşidinin içsel absisik asit (ABA), prolin ve inorganik iyon içeriklerindeki değişim yaprak apoplastik ve simplastik alanlarında araştırıldı. Kuraklık stresi her iki çeşitte yaprak su potansiyeli ve stoma iletkenliğinde azalışa neden oldu. Kuraklığa dayanıklı ve hassas çeşitlerdeki prolin konsantrasyonu her iki alanda kuraklık stresine cevap olarak arttı. Simplastik K⁺ konsantrasyonu her iki çeşitte azaldı. Buna karşılık K⁺ konsantrasyonu ile ilişkili ters bir eğilim apoplastik alanda görüldü. Simplastik Na⁺ konsantrasyonu dayanıklı çeşitte önemli ölçüde azalırken apoplastik Na⁺ konsantrasyonu arttı. Diğer taraftan, Na⁺ konsantrasyonu hassas çeşitte her iki alanda önemli ölçüde değişmedi. Kuraklık stresi sırasında hassas çeşitte Ca²⁺ konsantrasyonu her iki alanda da azaldı.

Dayanıklı çeşitte Ca^{2+} konsantrasyonu simplastta arttı apoplastta ise azaldı. Dayanıklı çeşitteki Cl^- konsantrasyonu her iki alanda önemli ölçüde değişmedi. Hassas çeşitteki Cl^- konsantrasyonu ise apoplastik alanda arttı simplastik alanda azaldı. Ayrıca kuraklık stresi sırasında simplastik pH değişmezken, apoplastik pH azaldı. Simplastik ve apoplastik ABA konsantrasyonu her iki çeşitte önemli ölçüde arttı. Sonuç olarak, inorganik iyonlar (özellikle Na^+ , K^+ ve Ca^{2+}) ve ABA konsantrasyonu kuraklık stresi sırasında osmotik regülasyona katkı sağlamak için apoplastik ve simplastik alanlar arasında değişti. Ayrıca kuraklığa dayanıklı çeşidin apoplastik ve simplastik alanlar arasında osmotik regülasyonu devam ettirebilmek için çok daha büyük bir kapasiteye sahip olduğu sonucuna varıldı.

Anahtar sözcükler: Apoplast, simplast, kuraklık stresi, tolerans, inorganik iyonlar, absisik asit

Introduction

Drought is one of the most important stresses in crop production because it affects almost all plant functions (1). The decrease in osmotic potential in response to water stress is a well-known mechanism by which many plants adjust to drought stress (2). Stressed plants diminish osmotic potential by accumulating low-molecular-weight, osmotically active compounds called osmolytes. Under drought conditions, plants exhibit physiological, biochemical, and molecular responses at both the cellular and whole-plant levels (3). Generally, the plants accumulate some kind of organic and inorganic solutes in the cytosol to raise the osmotic pressure, thereby maintaining both turgor and the driving gradient for water uptake (4). One such solute is proline. Proline accumulation is an important indicator of drought stress tolerance in bacteria, algae, and higher plants (5,6). In addition to its role as a compatible compound for osmotic adjustment, proline contributes to the stabilization of subcellular structures, scavenging of free radicals, and buffering of cellular redox potential under stress conditions (7). Absciscic acid (ABA) is thought to play an important role in the adaptation of plants to environmental stress. In addition to its well established role in closing stomata, there is also evidence that ABA increases the influx of ions across membranes in the root and encourages the synthesis and accumulation of osmotically active solutes (8).

The cell wall apoplast, as the extraplastic matrix of the plant cell in the leaf, has an ion and metabolite composition distinct from other cellular compartments. Furthermore, the composition of the apoplastic solution is influenced by the physicochemical properties of the cell wall, transport characteristics of the plasma membrane of neighboring cells, apoplastic water transport,

solute transport, and environmental factors (9). The apoplast is the first plant compartment to encounter environmental signals in plants (10) and it contributes to plant development (11). Apoplastic pH exerts a strong influence on turgor and wall loosening, possibly via the control of hydrolytic reactions and intermolecular interactions between structural carbohydrates and proteins (12). In addition, it has been reported that the inorganic minerals K^+ , Na^+ , and Ca^{2+} account for the osmolality of the apoplastic fluid (11).

The common bean (*Phaseolus vulgaris* L.) is an important crop from the family Fabaceae that is cultivated worldwide for human consumption. A water deficiency during any of the growth stages of the bean species often results in a loss of yield (13). Therefore, it is important to identify the drought tolerance mechanisms of these species in order to improve its agronomic performance and to obtain more resistant cultivars (14). In the current study, we investigated the extent to which ABA synthesis and the accumulation of apoplastic and symplastic ions may contribute to drought tolerance in 2 bean cultivars differing in their tolerance to drought. We determined the changes of compounds contributing to osmotic adjustment in the apoplastic and symplastic areas of the bean cultivars during drought stress.

Materials and methods

Growth of the plants and stress application

The seeds of common bean (*Phaseolus vulgaris* L.) cultivars Zulbiye (drought-sensitive) and Yakutiye (drought-tolerant), whose tolerance levels are known, were obtained from the Anatolian Agricultural Research Institute in Eskişehir, Turkey. Plants were

grown by daily irrigation in plastic pots (16 cm high, 18 cm in top diameter, and 12 cm in bottom diameter) containing peat and sand (5:1) in a greenhouse (temperature: 25 ± 2 °C, relative humidity: $60 \pm 5\%$, light intensity: $400 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 30 days. Drought stress was applied by withholding irrigation at the flowering stage for 10 days. The following parameters were measured in the apoplastic and symplastic spaces.

Leaf water potential

Leaf water potential (Ψ_{leaf}) was measured with a thermocouple psychrometer at 27 ± 1 °C (PSYPRO, Wescor, Inc., Logan, UT, USA). Disks of approximately 6 mm in diameter were cut from the youngest fully expanded leaves of the plants and sealed in the C-52 psychrometer chamber. Samples were equilibrated for 60 min before the readings were recorded by a water potential data logger in the psychrometric mode. The values of Ψ_{leaf} were measured as MPa.

Stomatal conductance

Stomatal conductance (g_s) was monitored with a dynamic diffusion porometer (AP4, Delta-T Devices, Cambridge, UK) after it was calibrated with a standard calibration plate following the manufacturer's instructions. The values of g_s were measured as $\text{mmol m}^{-2} \text{s}^{-1}$.

Cell membrane stability (CMS)

Measurements of CMS were taken following the protocol of Blum and Ebercon (15). Samples were washed 3 times in deionized water to remove electrolytes adhering to the surface. The samples were then kept in a capped vial (20 mL) containing 10 mL of deionized water and incubated in the dark for 24 h at room temperature. The conductance was measured with a conductivity meter (YSI Model 345, Yellow Springs, OH, USA). After the first measurement, the vials were autoclaved for 15 min to kill the leaf tissue and release the electrolytes. After cooling, the second conductivity reading was taken. These 2 measurements were carried out individually for all of the samples from both the control and stress treatments. The control gave a measure of leakage solely due to the cutting and incubation of leaf disks. The conductance of the stress sample was a measure of electrolyte leakage due to drought stress and was assumed to be proportional to the degree of injury to

the membranes. CMS was calculated as the reciprocal of cell-membrane injury based on the method of Blum and Ebercon (15): $\text{CMS\%} = [(1 - (T_1/T_2))/(1 - (C_1/C_2))] \times 100$, where T and C refer to the stressed and control samples, respectively, and the subscripts 1 and 2 refer to the initial and final conductance readings, respectively.

Analysis of apoplastic and symplastic proline

Apoplastic washing fluid (AWF) was extracted using the vacuum infiltration method described by Nielsen and Schjoerring (16). Fresh leaves were cut into lengths of 1 cm, washed with deionized water, and infiltrated with 320 mM sorbitol. The leaves were then blotted dry with thin paper tissues and the apoplastic solution was collected in microcentrifuge vials by centrifuging the leaf pieces at $1450 \times g$ for 15 min at 4 °C.

For the proline extraction of residual leaf, dried ground leaves (0.25 g) were also used. Samples were homogenized in 5 mL of 3% sulfosalicylic acid and extracts were centrifuged at $8000 \times g$ for 15 min. Proline determination in the apoplast and symplast was carried out according to the method described by Bates et al. (17). The proline concentration was determined using a standard curve.

Apoplastic and symplastic ions

The extraction of the apoplastic solution for measurement of the ions was performed as described in the section on proline. Following the collection of AWF, the residual leaf (0.5 g) was homogenized with liquid nitrogen in 5 mL of deionized water. The symplastic homogenate was boiled in a water bath for 10 min. The precipitate was removed by centrifugation (18). Symplastic and apoplastic ion contents (K^+ , Ca^{2+} , Cl^- , and Na^+) were measured with a pH/mV/temperature meter (Jenco 6230N, Jenco, San Diego, CA, USA).

Apoplastic and symplastic pH

Apoplastic pH was directly measured with the pH/mV/temperature meter. Samples (0.5 g FW 20 mL) were treated for 120 min in a basal solution containing 0.5 mM CaSO_4 , 5 mM DCMU, and 20 mM MES, adjusted to the required pH (routinely pH 6, unless otherwise indicated) with H_2SO_4 depending on the presence of the weak bases. At the end of the treatments, the leaves were washed for 3 min at 0 °C

with 0.5 mM CaSO_4 to clear the free space from the external medium, blotted on filter paper, transferred to plastic syringes, and frozen at $-30\text{ }^\circ\text{C}$ for at least 3 h. The pH was directly measured with the pH/mV/temperature meter in the cytoplasmic sap obtained by squeezing the leaves after freeze-thawing (19).

Determination of apoplastic and symplastic ABA

The extraction of the apoplastic solution for the measurement of ABA was performed with a pressure pump (20). Leaves were ground in liquid nitrogen, homogenized in distilled water at a ratio of 1:7 (w/v), and placed overnight in the dark at $4\text{ }^\circ\text{C}$. The extracts were centrifuged at $10,000 \times g$ for 10 min at $4\text{ }^\circ\text{C}$, and the resulting supernatant was diluted 4 times in standard TBS buffer. The ABA in these extracts was quantified using the Phytodetek ABA ELISA kit (Agdia Biofords, Evry, France) according to the manufacturer's instructions.

Statistical analysis

Each analysis was repeated 3 times on a mixture of leaves from 3 individual plants with 3 replicates. Variance analysis of the mean values was performed

using Duncan's multiple comparison test (2-way ANOVA) with SPSS for Windows (Ver. 10.0, SPSS Inc., Chicago, IL, USA), and the significance level was 5% ($P < 0.05$).

Results and discussion

Leaf water status and stomatal conductance

The water potential of leaves (Ψ_{leaf}) decreased in both cultivars during exposure to drought stress. However, the reduction of the water potential in the drought-sensitive cultivar was stronger than that observed in the tolerant cultivar. For example, drought stress caused a 10-fold reduction of Ψ_{leaf} in the drought-sensitive cultivar, but the reduction in Ψ_{leaf} in the drought-tolerant cultivar was only 5.6-fold when compared to the control (Figure 1).

Stomatal conductance (g_s) was also observed to decrease in both cultivars. It was reduced by 8.4-fold over the control in the sensitive cultivar, while the decrease was approximately 4.7-fold in the tolerant cultivar (Figure 2).

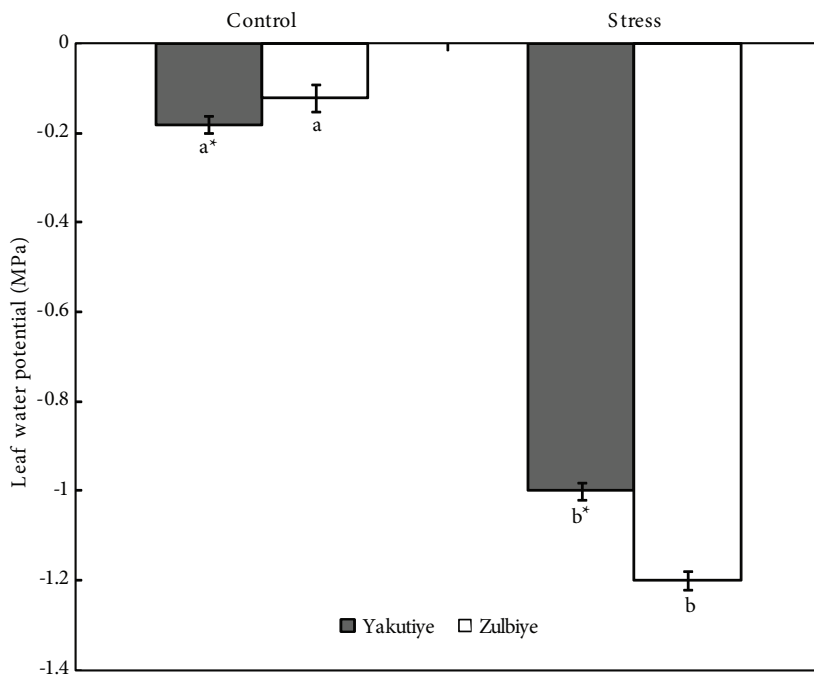


Figure 1. Effects of drought stress on leaf water potential in the leaves of bean cultivars. All values are means of triplicates \pm SD. Different letters denote significant differences at $P < 0.05$. The asterisks denote significant differences between cultivars for control and drought application.

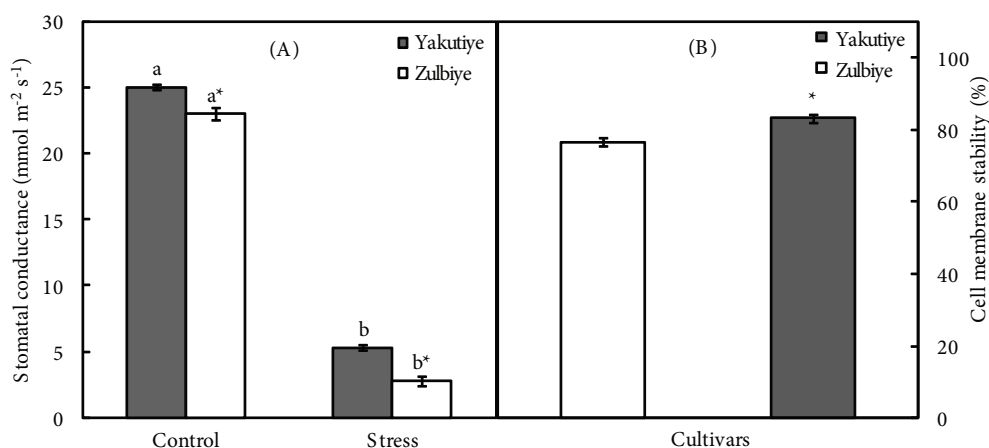


Figure 2. Effects of drought stress on stomatal conductance (A) and cell membrane stability (%) (B) in the leaves of bean cultivars. All values are means of triplicates \pm SD. Different letters denote significant differences at $P < 0.05$. The asterisks denote significant differences between cultivars for control and drought application.

Cell membrane stability

CMS was significantly influenced by drought stress. A significant difference was observed in the CMS in both bean cultivars. The drought-tolerant cultivar expressed a higher CMS than the sensitive cultivar. The mean CMS values for the tolerant and sensitive cultivars were 83.2% and 76.7%, respectively (Figure 2).

Proline changes in apoplastic and symplastic areas

Symplastic proline concentrations significantly increased in both cultivars during exposure to drought stress. In the sensitive cultivar, the ratio of the increase compared to its control was 4-fold in the symplastic area of the leaf, but the increase in the corresponding ratio of the tolerant cultivar was 1.5-fold compared to the control. As for the apoplast, proline concentrations significantly increased in both cultivars during drought stress. When compared to the controls, these increases were 1.4- and 1.5-fold in the tolerant and sensitive cultivars, respectively. The symplastic proline concentration of the leaf was further noted to be higher than that of the apoplast in both cultivars (Figure 3).

Ion and pH changes in apoplastic and symplastic areas

Symplastic K^+ concentrations decreased in the drought-tolerant and drought-sensitive cultivars

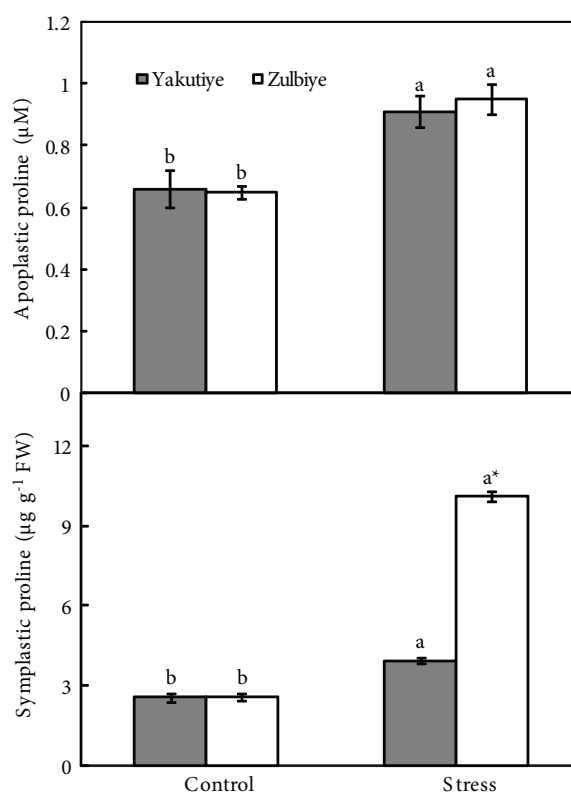


Figure 3. Changes in apoplastic and symplastic proline during drought stress. All values are means of triplicates \pm SD. Different letters denote significant differences at $P < 0.05$. The asterisk denotes significant differences between cultivars for control and drought application.

under stress conditions. As compared to the controls, the rates of decrease were 1.6- and 1.12-fold in the sensitive and tolerant cultivars, respectively.

The opposite trend was observed concerning K⁺ concentration in the apoplastic area. When compared with the controls, the rates of increase in this area were 1.7 in the sensitive cultivar and 2.0 in the tolerant cultivar. While symplastic Na⁺ concentrations significantly decreased in the tolerant cultivar, apoplastic Na⁺ concentrations increased during exposure to drought stress. However, Na⁺ concentrations did not significantly change in either of the compartments in the sensitive cultivar. On the other hand, Ca²⁺ concentrations in the sensitive cultivar significantly decreased in both compartments during drought stress. In the drought-tolerant cultivar, Ca²⁺ concentrations significantly increased

in the symplast but decreased in the apoplast. The Cl⁻ concentration in the tolerant cultivar did not significantly change in either of the compartments. In the sensitive cultivar, Cl⁻ concentrations increased in the apoplastic area but decreased in the symplastic area (Table).

Drought stress resulted in a decrease in the pH of the bean cultivars' apoplastic fluid. The symplastic pH did not significantly change during drought stress (Table).

ABA changes in apoplastic and symplastic areas

The accumulation of ABA induced by drought was significantly higher in the drought-tolerant cultivar

Table. Changes in inorganic ions and pH during drought stress. The same lower case letters are not significantly different from each other (P < 0.05) in each line. The same upper case letters are not significantly different from each other (P < 0.05) in each column. Data are means ± SD.

Potassium concentration (µM)				
	Apoplast		Symplast	
	Control	Stress	Control	Stress
Zulbiye	4.5 ± 0.9 bA	7.5 ± 0.8 aA	21.2 ± 1.8 aA	13.1 ± 0.7 bA
Yakutiye	1.7 ± 0.2 bB	3.4 ± 0.2 aB	16.0 ± 0.8 aB	14.2 ± 0.7 bA
Sodium concentration (µM)				
	Apoplast		Symplast	
	Control	Stress	Control	Stress
Zulbiye	0.44 ± 0.03 aB	0.5 ± 0.02 aB	0.27 ± 0.02 aA	0.26 ± 0.05 aA
Yakutiye	1.20 ± 0.03 bA	2.0 ± 0.10 aA	0.34 ± 0.03 aA	0.28 ± 0.02 bA
Calcium concentration (µM)				
	Apoplast		Symplast	
	Control	Stress	Control	Stress
Zulbiye	0.015 ± 0.0003aA	0.009 ± 0.0001bA	0.90 ± 0.01 aA	0.300 ± 0.01 bB
Yakutiye	0.015 ± 0.0004 aA	0.009 ± 0.0001bA	2.52 ± 0.04 bA	5.100 ± 0.008 aA
Chloride concentration (µM)				
	Apoplast		Symplast	
	Control	Stress	Control	Stress
Zulbiye	59.1 ± 0.02 bB	59.7 ± 0.02 aB	55.2 ± 0.02 aB	54.9 ± 0.02 bB
Yakutiye	63.3 ± 0.03 aA	63.6 ± 0.03 aA	56.4 ± 0.02 aA	56.4 ± 0.02 aA
pH				
	Apoplast		Symplast	
	Control	Stress	Control	Stress
Zulbiye	5.3 ± 0.04 aB	5.0 ± 0.03 bB	6.0 ± 0.2 aA	6.1 ± 0.1 aA
Yakutiye	5.6 ± 0.01 aA	5.1 ± 0.03 bA	6.0 ± 0.2 aA	6.0 ± 0.2 aA

than in the drought-sensitive cultivar. Moreover, ABA concentrations in the apoplastic and symplastic areas significantly increased in both cultivars. When compared to the control, the rates of increase in the symplast were 1.3 and 1.8 for the sensitive and the tolerant cultivar, respectively. With regard to the apoplast, ABA concentrations increased at rates of 1.3 and 1.4 for the sensitive and the tolerant cultivar, respectively (Figure 4).

In this study, changes in the ABA content and inorganic solutes of apoplastic and symplastic spaces of leaves were determined in bean cultivars differing in their tolerance to drought. As expected, the water potential of leaves (Ψ_{leaf}) decreased in both cultivars after exposure to drought stress. The capacity of the tolerant cultivar to maintain higher leaf water potential compared to the sensitive cultivar may be attributed to its ability to postpone dehydration (21). It is known that CMS is an indicator of drought tolerance (22). In our work, the CMS was significantly influenced by drought stress. The drought-tolerant cultivar expressed a higher level of CMS than the sensitive one. Stomatal conductance also decreased in both cultivars. The negative effect of drought stress on stomatal conductance was also

observed in soybean by Bunce (23). It is clear that the higher stomatal conductance observed in the tolerant cultivar compared to the sensitive cultivar could give rise to the differences in sensitivity to drought. The ability of a cultivar to keep its stomata open despite internal water stress has been considered a form of drought resistance (24).

In our experiment, the concentration of Ca^{2+} significantly decreased in both compartments in the sensitive cultivar. This decrease in Ca^{2+} concentration may result from its binding to peptic acids in the cell wall in order to firm the wall, making the plant more resistant to drought. On the other hand, the symplastic Ca^{2+} concentration increased in the drought-tolerant cultivar, whereas the apoplastic Ca^{2+} concentration declined. In plants, transient increases in cytosolic Ca^{2+} have been reported in response to a diverse range of abiotic and biotic stimuli (25,26), but the specificity of the physiological responses is not understood. Recent studies suggest that Ca^{2+} binding proteins known as calmodulin or calcium-dependent protein kinases serve as the primary regulators of internal Ca^{2+} levels in plant cells and function to buffer intracellular Ca^{2+} levels or translate the intracellular oscillations of free Ca^{2+} levels into

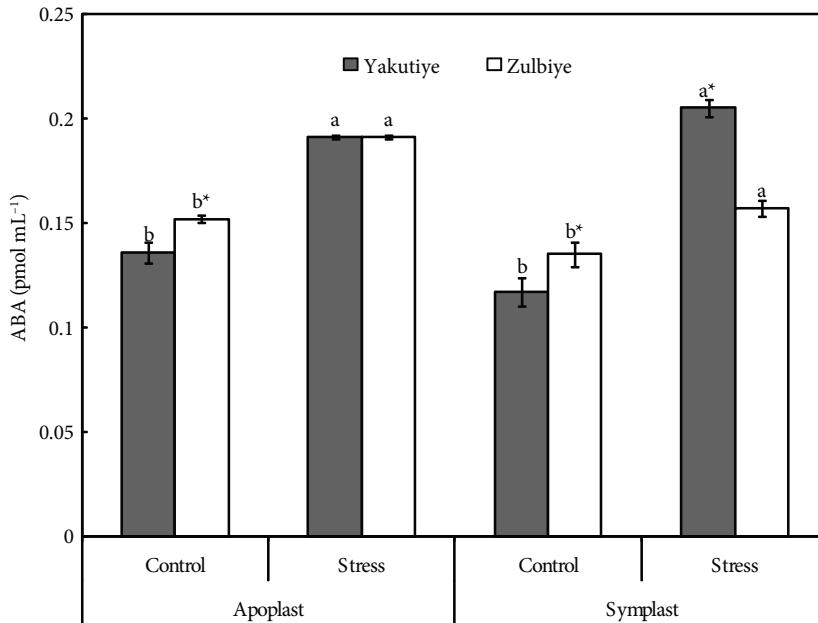


Figure 4. Changes in apoplastic and symplastic ABA in the leaves of bean cultivars during drought stress. All values are means of triplicates \pm SD. Different letters denote significant differences at $P < 0.05$. The asterisks denote significant differences between cultivars for control and drought application.

signal-specific cellular responses (27,28). On the other hand, enhanced ABA levels trigger an increase in cytosolic Ca^{2+} in guard cells, and it has been suggested that this might include Ca^{2+} influx across the plasma membrane (29). Indeed, we found that ABA increased in both compartments under drought stress. In addition, Ca^{2+} ions control the efficiency of water use by initiating stomatal closure (30). Thus, the increased symplasmic Ca^{2+} concentrations in the tolerant cultivar may be responsible for the reduction in stomatal conductance. We also found that stomatal conductance significantly decreased in the drought-tolerant cultivar.

In the current study, symplasmic K^+ concentrations decreased in both cultivars, by about 62% in the sensitive cultivar and 13% in the tolerant cultivar. Conversely, the apoplasmic K^+ concentration increased in both cultivars. A decrease in the K^+ concentration of the symplast may be associated with the influx of K^+ toward the apoplast. On the other hand, an increase in apoplasmic K^+ probably indicates a displacement of potassium from fixed anionic exchange sites in the cell wall.

Our data indicate an increase in the apoplasmic Na^+ concentration of the drought-tolerant cultivar after drought exposure. This result shows that the tolerant cultivar has the ability to protect its cytosol from excess sodium. It is interesting to note that the apoplast was the primary site of sodium accumulation (31). In the apoplast, the accumulation of Na^+ resulted in decreases in Ca^{2+} (Table). Additionally, high apoplasmic Na^+ and K^+ concentrations in both cultivars may be highly regulated by ion transport between the symplast and apoplast. Our findings also indicate that Cl^- was almost equally distributed between both spaces in the tolerant cultivar. The contribution of this factor to the change in osmotic potential was insignificant. In the sensitive cultivar, Cl^- concentrations increased in the apoplasmic area but decreased in the symplasmic area. The increase in apoplasmic Cl^- may show that the Cl^- was transported from the symplast to the apoplast to contribute to osmotic adjustment.

In the current study, the symplasmic sap of leaves exhibited a constant pH value of 6.0 in both cultivars during drought stress. In contrast, the pH of the

apoplast decreased in both cultivars. The apoplasmic pH has been reported many times from different species, and the majority of values vary between 5.3 (32) and 6.7 (33). It has been reported that increased symplasmic Ca^{2+} deactivates the plasmalemma H^+ /ATPase and also activates a K^+/H^+ symport. The inflow of K^+ and H^+ depolarizes the membrane, and thus the apoplast becomes less acidic (34). However, symplasmic Ca^{2+} decreased in the sensitive cultivar during drought stress. It can therefore be said that Ca^{2+} does not affect plasmalemma H^+ /ATPase activity or activate the K^+/H^+ symport sufficiently, and the apoplast becomes more acidic as a consequence. However, acidification of apoplasmic pH in the tolerant cultivar may be caused by different mechanisms, such as changes in phosphate nutrition and delivery.

During the drought period, not only the symplasmic but also the apoplasmic ABA increased. We inferred that leaf apoplasmic ABA concentrations increased and pH did not affect the distribution of ABA in the apoplasmic and symplasmic areas. In addition to the well-established role of ABA in closing stomata, there is evidence that ABA has a role in regulating solute accumulation and, thus, osmotic adjustment (35). Increases in apoplasmic ABA and the leaf osmolyte content were higher in the drought-tolerant cultivar as compared to the drought-sensitive cultivar. Therefore, it is reasonable to suppose that osmotic adjustment may respond to the ABA of the apoplasmic fraction of the leaves. In the present study, a general correlation between ABA and stomatal conductance was found ($P < 0.01$, $r = -0.97$). The decrease in g_s values during drought was accompanied by a significant rise in ABA.

It is known that proline provides an important contribution to osmotic adjustment and adaptation to stress (36). In our study, proline concentrations significantly increased in both compartments in bean cultivars, with the highest rate of increase being observed in the symplasmic area of the drought-sensitive cultivar (293%). These findings are consistent with reports of higher leaf proline in sensitive genotypes of other species (37-39). The dramatic increase in leaf apoplast osmolality in plants subjected to drought stress may be the result of a high accumulation of Na^+ and K^+ in the leaf cell.

In the symplast, however, the relative contribution of Ca^{2+} to the osmolality was the highest among all of the solutes studied. In the tolerant cultivar, it contributed to symplastic osmolality. In the sensitive cultivar, this figure was substantially lower, making it necessary for drought-sensitive plants to synthesize at least twice as much symplastic proline as drought-tolerant ones. Sensitive cultivars may need to synthesize high levels of proline to compensate for this difference to balance the intracellular osmotic potential. Moreover, extracellular proline levels can be increased by increasing intracellular proline, which provides considerable support for the role of proline in stress tolerance.

We conclude that the drought-tolerant cultivar has a much higher capacity to maintain osmotic adjustment between the symplast and the apoplast. Both cultivars showed characteristic differences regarding inorganic ions in leaves. Additionally, the Na^+ , K^+ , and Ca^{2+} concentrations in the tolerant

cultivar were high, and their relative contributions to the osmotic potential of the tolerant cultivar under drought stress were higher than that of proline. Finally, it might be said that inorganic ions and ABA concentrations changed between the apoplastic and symplastic spaces, contributing to osmotic adjustment under drought stress.

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