

## EFFECTS OF WATER STRESS ON LEAF WATER RELATIONS OF YOUNG BEAN PLANTS

## ВЛИЯНИЕ НА ВОДНИЯ СТРЕС ВЪРХУ ВОДООБМЕНА В ЛИСТАТА НА МЛАДИ РАСТЕНИЯ ФАСУЛ

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### ABSTRACT

The effects of water stress on water relations in the leaves of young common bean (*Phaseolus vulgaris* L.) plants were studied. Water stress was imposed 14 days after the emergency by withholding water until soil water potential reached  $-0.9$  MPa. Water deficit led to a noticeable decrease in both the osmotic potential at full hydration and turgor loss point in the primary and the first trifoliolate leaves of all the cultivars. The lowest calculated values for osmotic adjustment are found in droughted plants of cv. Dobrudjanski ran ( $-0.29$  MPa for the primary and  $-0.42$  MPa for the first trifoliolate leaf). In contrast, high osmotic adjustment was found in cultivars Plovdiv 10 and Prelom. The three bean genotypes displayed significant differences in their adaptive response to drought. This study indicated that osmotic adjustment is one of the major adaptive mechanisms of *Phaseolus vulgaris* to survive drought. The main difference among cultivars appears to be due to turgor maintenance, which may be more representative of the physiological status of the leaves in these cultivars.

**KEY WORDS:** bulk elastic modulus, *Phaseolus vulgaris* L., pressure-volume curves, water potential, water stress.

### РЕЗЮМЕ

Проучено е влиянието на водния стрес върху водообмена в листата на млади растения фасул (*Phaseolus vulgaris* L.). Водният стрес е приложен 14 дни след поникването на растенията, чрез прекратяване на поливането, до достигане на потенциал на водата в почвата  $-0.9$  MPa. Водният дефицит предизвиква значително понижение в осмотичния потенциал при пълно насищане с вода, както и на точката на загуба на тургор в първичния и първи сложен лист в растенията и от трите сорта. С най-ниска стойност на осмотичното приспособяване са растенията от сорт Добруджански ран ( $-0.29$  MPa за първичния и  $-0.42$  MPa за първи сложен лист). При сорт Прелом и сорт Пловдив 10 степента на осмотично приспособяване е най-висока. Растенията от трите генотипа показват значителни различия в реакцията им към приложеното засушаване. Установено е, че осмотичното приспособяване е един от основните адаптивни механизми при фасула за преодоляване на приложеното засушаване. Основното различие между растенията от трите сорта е в способността им да поддържат тургора, което е показателно за функционалното състояние на листата и на растенията като цяло.

**КЛЮЧОВИ ДУМИ:** воден потенциал, воден стрес, криви на водообмена, модул на еластичност на клетъчните стени, *Phaseolus vulgaris* L.

**DETAILED ABSTRACT**

The effects of water stress on water relations in the leaves of young common bean (*Phaseolus vulgaris* L.) plants (cvs. Plovdiv 10, Dobrudjanski ran and Prelom) were studied. Plants were cultivated in pots as soil culture in a growth chamber. Control plants were grown at soil water content of 41% (0.410 g H<sub>2</sub>O g<sup>-1</sup> dry soil) corresponding to a soil water potential ( $\Psi_{\text{soil}}$ ) of -20 kPa. It is considered that soil is well watered and there is no water stress if  $\Psi_{\text{soil}}$  is above -30 kPa. Water stress was progressively induced in 14-day old plants by withholding water supply for 10 days until soil water content reached 23% (0.230 g H<sub>2</sub>O g<sup>-1</sup> dry soil) corresponding to a soil water potential of -0.9 MPa. The measurements were made at the end of stress period on the primary and first trifoliolate leaves, which were fully matured. Water stress induced significant changes in relative water content (RWC) and leaf water potential ( $\Psi_w$ ) in the leaves of young bean plants (Table 1). In the primary leaf, the highest accumulation and increase in proline content is registered in cv. Prelom (with 276% rise) and the lowest accumulation and increase in Dobrudjanski ran (with 195 % rise). Genotype Plovdiv 10 exhibited an intermediate behavior. The same tendency is observed in the first trifoliolate leaf. The PV curves drawn with the data of  $\Psi_w$  and RWC, obtained under control and water stress conditions, exhibited different slopes for primary and first trifoliolate leaves in all the genotypes studied (Figs. 1 – 3). Water deficit led to a noticeable decrease in both the osmotic potential at full hydration ( $\Psi_o^{100}$ ) and turgor loss poin (TLP) in the primary and the first trifoliolate leaves (Tables 2 and 3). The lowest calculated values for osmotic adjustment (OA) were found in droughted plants of cv. Dobrudjanski ran (-0.29 MPa for the primary and -0.42 MPa for the first trifoliolate leaf). In contrast, high osmotic adjustment was found in cultivars Prelom and Plovdiv 10. The mean maximum leaf bulk elastic modulus ( $E_{v_{\text{max}}}$ ) in cv. Prelom and cv. Dobrudjanski ran was higher in the droughted plants compared with control ones. In Plovdiv 10  $E_{v_{\text{max}}}$  is slightly reduced. That should indicate that water stress induced changes in cell wall properties, rendering them less elastic in cvs. Prelom and Dobrudjanski ran. The three bean genotypes displayed significant differences in their adaptive response to drought. This study indicated that osmotic adjustment is one of the major adaptive mechanisms of *Phaseolus vulgaris* to survive drought. The main difference among cultivars appears to be due to turgor maintenance, which may be more representative of the physiological status of the leaves in these cultivars.

**INTRODUCTION**

Drought is one of the most important constrains for bean production but improvement of drought tolerance is very difficult because of the set of mechanisms involved. Crop plants have developed many mechanisms to survive water deficit, including escape, tolerance, and avoidance of tissue and cell dehydration [15], [30]. Avoidance of stress includes rapid phenological development, increased stomatal and cuticular resistance, changes in leaf area, orientation and anatomy, among others [12], [17]. Plants tolerate drought by maintaining sufficient cell turgor to allow metabolism to continue under increasing water deficits. Tolerance to stress involves at least two mechanisms, osmotic adjustment and changes in the elastic properties of tissues [19], [25].

Osmotic adjustment is generally thought to be the major mechanism to maintain cell turgor in many species as the water potential decreases, enabling water uptake and the maintenance of plant metabolic activity and therefore growth and productivity [5], [8], [22]. Lowering of the osmotic potential of the cells accumulating solutes is considered to be due to osmotic adjustment if the buildup of compounds is not merely the result of tissue dehydration [4].

Several reports suggest that plant metabolic processes are in fact more sensitive to turgor and cell volume than to absolute water potential [12]. Among the physiological mechanisms that act to maintain leaf turgor pressure, decreased osmotic potential resulting either from a decrease in osmotic water fraction or from an osmotic adjustment (net accumulation of solutes in the symplast) was pointed out [13]. Changes in tissue elasticity in response to drought, which modify the relationship between turgor pressure and cell volume, might contribute to drought tolerance, as observed in black spruce [3], and sunflower [16]. Leaf water relations data may provide a useful indication of the capacity of species to maintain functional activity under drought [32].

The aim of this work was to study the effects of water stress on water relations in young bean plants and to investigate whether some aspect of the plant water relations differed among the studied genotypes. The capacity for leaf osmotic adjustment and changes in cell tissue elasticity, water potential and turgor pressure were analyzed through pressure-volume curves in order to compare their behavior in response to water stress.

**MATERIALS AND METHODS****Plant material and growth conditions**

For this study three genotypes of common bean (*Phaseolus*

vulgaris L.) were used: cv. Plovdiv 10, cv. Dobrudjanski ran and cv. Prelom. Seeds were washed in distilled water, surface sterilized and germinated on moist filter paper, in Petri dishes, maintained at 28 °C, in the dark, for 3 days. After germination seedlings with well developed roots and having approximately the same morphological aspect were selected and cultivated in pots as soil culture in a growth chamber. Dissolved nutrients were added to the soil 15 days before planting: 280 mg Ca(NO<sub>3</sub>)<sub>2</sub> kg<sup>-1</sup> dry soil, 180 mg KNO<sub>3</sub> kg<sup>-1</sup> dry soil and 220 mg NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> kg<sup>-1</sup> dry soil. One seedling was maintained in each pot. The environmental conditions in the growth chamber were: photosynthetic photon flux density (PPFD) of 150 μmol m<sup>-2</sup> s<sup>-1</sup>, day/night temperature 25±2/17±2 °C, photoperiod of 14 h, and relative air humidity between 65-70 %. Pots were watered daily to maintain control soil water content of 41% (0.410 g H<sub>2</sub>O g<sup>-1</sup> dry soil) corresponding to a soil water potential ( $\Psi_{\text{soil}}$ ) of -20 kPa. It is considered that soil is well watered and there is no water stress if  $\Psi_{\text{soil}}$  is above -30 kPa [1]. Water stress was progressively induced in 14-day old plants by withholding water supply for 10 days until soil water content reached 23 % (0.230 g H<sub>2</sub>O g<sup>-1</sup> dry soil) corresponding to a soil water potential of -0.9 MPa. Relative water content (RWC) in the primary leaf was < 65% and in the first trifoliolate leaf RWC was < 75% in all the studied genotypes. The measurements were made at the end of stress period on the primary and first trifoliolate leaves, which were fully matured.

### Plant water relations

Leaf water potential ( $\Psi_w$ ) was determined in five leaves from different plants with pressure chamber EL 540-305 (ELE-International, Hemel Hempstead, England) using pressure chamber technique [26]. The relative water content (RWC) was determined according to Morgan [18], on five leaf discs ( $\phi=10$  mm) from leaves of the same age and size as the ones used in the measurements of  $\Psi_w$ .

In order to study the tissue water relations and osmotic adjustment, pressure-volume (PV) curves were done on three individual fully hydrated leaves of control and stressed plants, using the pressure chamber technique [23], [26]. Leaves were cut from each of the plants and then, as soon as possible, cut again under distilled water to remove any vapour gap in the xylem. Leaves were allowed to reach full turgor by storing them at 9 °C in a closed dark container with the petioles in distilled water. After a 4 h rehydration period, each leaf was weighed for determining the initial fresh weight and wrapped in a humidified plastic bag to minimize both temperature fluctuations and tissue water loss [31], and inserted in the pressure chamber. The inside surface of pressure

chamber was lined with moist filter paper. The initial leaf water potential ( $\Psi_w$ ) ( $\Psi_w = -P$ , balance pressure) was then recorded. After establishing the balancing pressure, chamber pressure was successively raised by 0.5 MPa increments. This resulted in exudation of sap from the cut surface. Expressed sap was collected in preweighed cotton-filled plastic vials, which were reweighed to give the weight of the exudates sap and to evaluate the weight of leaf. The elevated pressure was sustained for 5 min. After each sap collection, the chamber pressure was slowly reduced to the previous balance pressure. The new balance pressure was then determined. The pressurization rate was about 0.025 MPa s<sup>-1</sup> [29]. After the PV measurements, the leaves were oven dried at 80 °C for at least 48 h and leaf dry weight was determined.

Pressure-volume data were plotted as the water potential ( $\Psi_w$ ) versus RWC<sup>-1</sup> (type I transformation) [23]. Such plots have an initial non-linear phase and a subsequent linear section. The beginning of the linear section indicates the turgor loss point (state of incipient plasmolysis). The Richter plot has advantages over the usual presentation of data on inverse water potential ( $\Psi_w^{-1}$ ) versus RWC graph (type II transformation, Tyree plot) since the graph will cover the entire range of turgid state. Using the type II transformation, however, the relation between  $\Psi_w$  and RWC cannot be expressed since for RWC=1,  $\Psi_w^{-1}=\infty$ . Furthermore, the effects of errors in the measurement will be lower, since the inverse term is much more sensitive to inaccurate measurements, and RWC can be measured with more precision than the water potential [14]. The Richter plot was used to determine the turgor potential ( $\Psi_p$ ), the turgor loss point (TLP), the bulk elastic modulus ( $E_{\text{vmax}}$ ) and the structure coefficient ( $A_{\text{pmax}}$ ) by the method of Stadelmann [27] and Kim and Lee-Stadelmann [14].

### Proline content

The free proline content was determined spectrophotometrically according to Bates et al. [2].

### Statistical analysis

Values obtained were expressed as mean ± SE from five replications of each variant. The Student's t-test was used to evaluate the differences between control and stressed variants.

## RESULTS

The data in Table 1 show that the water stress induced significant changes in RWC,  $\Psi_w$  and proline content in leaves of young bean plants. RWC in the primary leaf decreased with 29% to 37%, being most significant reduced in cv. Dobrudjanski ran (37%). The decrease in

RWC in the first trifoliolate leaf is lower – 19% for cv. Plovdiv 10 and 32% for cv. Dobrudjanski ran. Cv. Prelom showed an intermediate behavior.

Water potential, which is a principal quantity of plant water relations, was reduced in greater extent – with 81% in the primary leaf in cv. Prelom and with 78% in the first trifoliolate leaf in cv. Dobrudjanski ran. Changes in absolute values of  $\Psi_w$  varied between 1.2 and 1.7 MPa for the primary leaf and between 1.4 and 1.8 MPa for the first trifoliolate leaf (Table 1). According to Hale and Orcutt [6] these changes in  $\Psi_w$  indicated that water stress was severe.

Significant differences in proline content were observed among genotypes under water stress (Table 1). In the primary leaf, the highest accumulation and increase was registered in cv. Prelom (with 276% rise) and the lowest accumulation and increase in Dobrudjanski ran (with 195% rise). Genotype Plovdiv 10 exhibited an intermediate behavior. There were no significant differences between cvs. Plovdiv 10 and Dobrudjanski ran under control conditions. The same tendency was observed in the first trifoliolate leaf.

Analysis of PV curves allow more precision determination of plant water relations, as well as the rate of osmotic adjustment under water stress [14], [28]. The PV curves drawn with the data of  $\Psi_w$  and RWC, obtained under control and water stress conditions, exhibited different slopes for the primary and first trifoliolate leaves in all the genotypes studied (Figs. 1 – 3).

In the primary leaf the  $\Psi_o^{100}$  derived from the PV curves was significantly decreased by the drought (Table 2).  $\Psi_o^{100}$  had the highest values in the leaves of cv. Dobrudjanski ran both for control and stressed plants, followed by Plovdiv 10 and Prelom in decreasing order. Turgor loss point (TLP) was decreased under drought. TLP in cv. Dobrudjanski ran was at highest  $\Psi_w$  and RWC in control and droughted plants. In cv. Prelom TLP was at lowest  $\Psi_w$  and RWC.

The mean maximum leaf bulk elastic modulus ( $E_{vmax}$ ) of the well-watered plants was 7.39 MPa (cv. Plovdiv 10). In this cultivar drought slightly reduced the  $E_{vmax}$ .

In cv. Prelom and cv. Dobrudjanski ran  $E_{vmax}$  was higher in the droughted plants compared with control ones. That should indicate that water stress induced changes in cell wall properties, rendering them less elastic in cvs. Prelom and Dobrudjanski ran. The variation in relation of  $E_{vmax}$  to the  $\Psi_p$  (the structure coefficient  $A_{pmax}$ ) was also affected, but in different way. In cv. Plovdiv 10  $A_{pmax}$  significantly decreased, and in cv. Prelom slight decrease was obtained. In cv. Dobrudjanski ran an increase of

ca. 15% was observed. A large variation was observed among cultivars for osmotic adjustment (OA). The lowest calculated values are found in droughted plants of cv. Dobrudjanski ran (-0.29 MPa for the primary and -0.42 MPa for the first trifoliolate leaf). In contrast, high osmotic adjustment was found in cultivars Prelom and Plovdiv 10.

Generally, the trifoliolate leaves of both control and water stressed plants showed lower  $\Psi_o^{100}$  than the primary leaves of plants with the same treatment (Table 3). In the first trifoliolate leaf the  $\Psi_o^{100}$  derived from the PV curves was significantly decreased by the drought (Table 3).  $\Psi_o^{100}$  had the highest values in the leaves of cv. Dobrudjanski ran for both control and stressed plants, followed by Plovdiv 10 and Prelom in decreasing order. Turgor loss point (TLP) was decreased under drought. TLP in cv. Dobrudjanski ran was at highest  $\Psi_w$  and RWC in control and droughted plants. In cv. Prelom TLP was at lowest  $\Psi_w$  and RWC.

The mean maximum leaf bulk elastic modulus ( $E_{vmax}$ ) of the well-watered plants was 11.80 MPa (cv. Prelom). In this cultivar drought slightly increased the  $E_{vmax}$  as well as in cv. Dobrudjanski ran. In Plovdiv 10  $E_{vmax}$  was slightly reduced. The variation in relation of  $E_{vmax}$  to the  $\Psi_p$  (the structure coefficient  $A_{pmax}$ ) was also affected. In all the cultivar studied  $A_{pmax}$  decreased significantly.

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Table 1. Influence of water stress on relative water content, RWC (%), water potential,  $\Psi_w$  (MPa) and proline accumulation ( $g\ g^{-1}DM$ ) in the primary and first trifoliolate leaves in young bean plants. \*, \*\* and \*\*\* - significantly different from the control plants at  $p=0.05$ ,  $p=0.01$  and  $p=0.001$ , respectively ( $n=5$ )

Таблица 1. Влияние на водния стрес върху относителното водно съдържание, RWC (%), водния потенциал,  $\Psi_w$  (MPa) и съдържанието на пролин ( $g\ g^{-1}DM$ ) в първичния и първи сложен лист на млади растения фасул. \*, \*\* и \*\*\* - статистически различни от контролните растения при  $p=0.05$ ,  $p=0.01$  and  $p=0.001$ , съответно, ( $n=5$ )

Genotype	Variants	RWC	$\Psi_w$	proline
Primary leaf				
Plovdiv 10	control	90.1±2.7	-0.3±0.01	21.2±1.1
	water stress	64.3±2.1*** (71)	-1.8±0.06*** (17)	54.7±2.1*** (258)
Dobrudjanski ran	control	88.2±2.3	-0.3±0.01	21.4±1.1
	water stress	55.3±2.0*** (63)	-1.5±0.07*** (20)	41.7±2.1*** (195)
Prelom	control	92.4±2.8	-0.4±0.01	32.8±2.1
	water stress	60.7±2.1*** (66)	-2.1±0.06*** (19)	90.5±2.1*** (276)
First trifoliolate leaf				
Plovdiv 10	control	93.2±2.8	-0.5±0.02	18.3±0.9
	water stress	75.6±2.5** (81)	-2.1±0.09*** (24)	50.1±1.6*** (274)
Dobrudjanski ran	control	91.7±2.7	-0.4±0.02	17.7±0.9
	water stress	62.4±2.1*** (68)	-1.8±0.08*** (22)	35.9±1.4*** (203)
Prelom	control	93.8±2.9	-0.6±0.02	38.9±0.9
	water stress	68.6±2.3*** (73)	-2.4±0.09*** (25)	109.7±1.6*** (282)

Table 2. Water relation characteristics for the primary leaf of control and water-stressed bean plants, derived from pressure-volume analysis.  $\Psi_{t_{lp}}$  – water potential at turgor loss point [MPa];  $RWC_{t_{lp}}$  – relative water content at turgor loss point [%];  $\Psi_o^{100}$  – osmotic potential at full hydration [MPa]; OA – osmotic adjustment [MPa];  $E_{vmax}$  - bulk elastic modulus at full hydration [MPa];  $A_{pmax}$  - structure coefficient at full hydration [MPa].

Таблица 2. Показатели на водообмена в първичния лист на контролни и подложени на воден стрес растения фасул, получени при анализ на кривите на водообмена.  $\Psi_{t_{lp}}$  – воден потенциал в точката на загуба на тургор [MPa];  $RWC_{t_{lp}}$  – относително водно съдържание в точката на загуба на тургор [%];  $\Psi_o^{100}$  – осмотичен потенциал при пълно насищане с вода [MPa]; OA – осмотично приспособяване [MPa];  $E_{vmax}$  – модул на еластичност при пълно насищане с вода [MPa];  $A_{pmax}$  - структурен коефициент при пълно насищане с вода [MPa].

Variants	$\Psi_{t_{lp}}$	$RWC_{t_{lp}}$	$\Psi_o^{100}$	OA	$E_{vmax}$	$A_{pmax}$
Plovdiv 10 – control	-0.79	74.6	-0.65		7.39	11.38
Plovdiv 10 – water-stressed	-1.82	65.8	-1.28	-0.63	6.64	5.89
Dobrudjanski ran – control	-0.60	79.4	-0.54		5.49	10.14
Dobrudjanski ran – water-stressed	-0.98	76.9	-0.83	-0.29	12.29	11.63
Prelom – control	-1.10	71.4	-0.92		6.85	7.42
Prelom – water-stressed	-2.05	60.7	-1.71	-0.79	9.69	5.67

Table 3. Water relation characteristics for first trifoliolate leaf of control and water-stressed bean plants, derived from pressure-volume analysis.  $\Psi_{\text{tpr}}$  – water potential at turgor loss point [MPa];  $\text{RWC}_{\text{tpr}}$  – relative water content at turgor loss point [%];  $\Psi_o^{100}$  – osmotic potential at full hydration [MPa]; OA – osmotic adjustment [MPa];  $E_{\text{vmax}}$  – bulk elastic modulus at full hydration [MPa];  $A_{\text{rmax}}$  – structure coefficient at full hydration [MPa].

Таблица 3. Показатели на водообмена в първи сложен лист на контролни и подложени на воден стрес растения фасул, получени при анализ на кривите на водообмена.  $\Psi_{\text{tpr}}$  – воден потенциал в точката на загуба на тургор [MPa];  $\text{RWC}_{\text{tpr}}$  – относително водно съдържание в точката на загуба на тургор [%];  $\Psi_o^{100}$  – осмотичен потенциал при пълно насищане с вода [MPa]; OA – осмотично приспособяване [MPa];  $E_{\text{vmax}}$  – модул на еластичност при пълно насищане с вода [MPa];  $A_{\text{rmax}}$  – структурен коефициент при пълно насищане с вода [MPa].

Variants	$\Psi_{\text{tpr}}$	$\text{RWC}_{\text{tpr}}$	$\Psi_o^{100}$	OA	$E_{\text{vmax}}$	$A_{\text{rmax}}$
Plovdiv 10 – control	-1.02	69.8	-0.94		8.54	9.04
Plovdiv 10 – water-stressed	-2.10	60.4	-1.58	-0.64	7.48	5.13
Dobrudjanski ran – control	-0.80	76.4	-0.64		8.13	12.60
Dobrudjanski ran–water-stressed	-1.24	71.5	-1.06	-0.42	10.54	9.97
Prelom – control	-1.31	67.6	-1.26		11.80	9.34
Prelom – water-stressed	-2.29	58.8	-2.01	-0.75	12.99	6.47

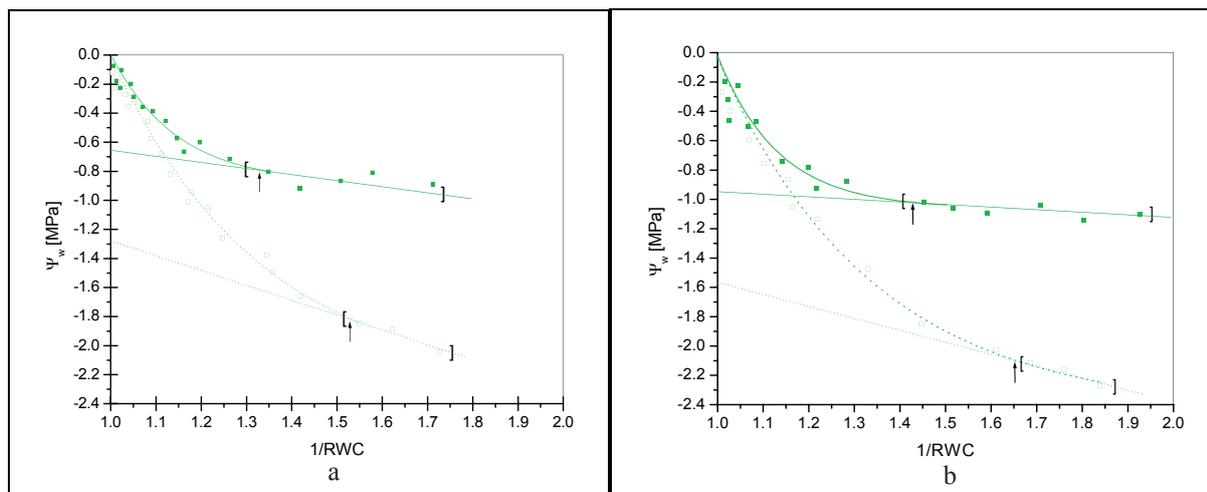


Figure 1. Pressure volume curves (Richter diagram) in young bean plants (cv. Plovdiv 10) submitted to water stress. (a) primary leaf, (b) first trifoliolate leaf. Regression line interpolated from the linear zone in brackets. Curve for the turgor potential (above turgor loss point) calculated from measured values of  $\Psi_w$  and RWC after Stadelmann (1984). Intersection of the curve and the line determines the turgor loss point, as indicated by arrow. – control,  $\cdot$  – water stress.

Фигура 1. Криви на водообмена (диаграма на Richter) в млади растения фасул (сорт Пловдив 10) подложени на воден стрес. (a) първичен лист, (b) първи сложен лист. Пресечната точка на регресионната линия и кривата определя точката на загуба на тургор, посочена със стрелка. – контроли,  $\cdot$  – засушени.

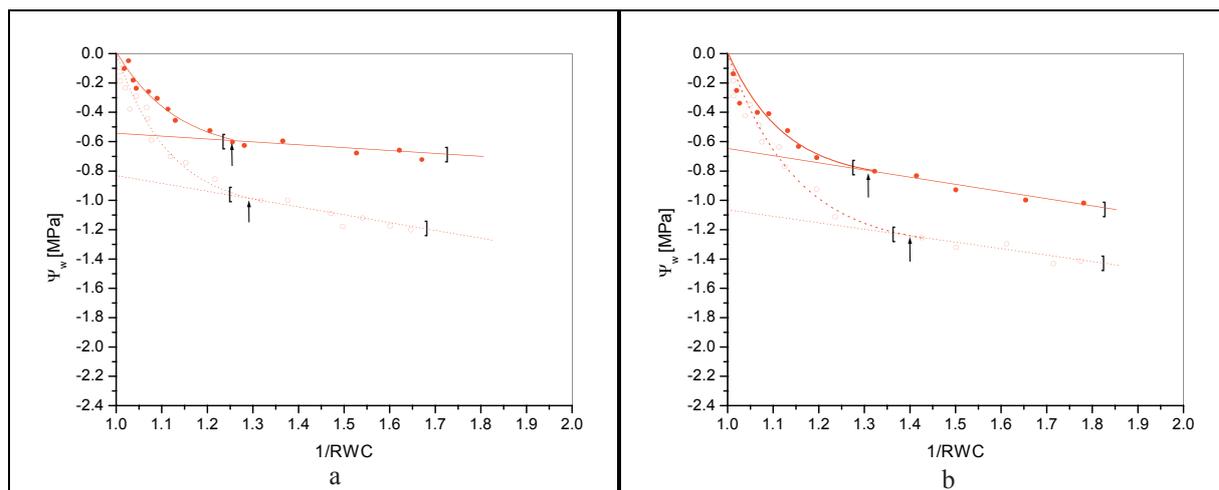


Figure 2. Pressure volume curves (Richter diagram) in young bean plants (cv. Dobrudjanski ran) submitted to water stress. (a) primary leaf, (b) first trifoliolate leaf. Regression line interpolated from the linear zone in brackets. Curve for the turgor potential (above turgor loss point) calculated from measured values of  $\Psi_w$  and RWC after Stadelmann (1984). Intersection of the curve and the line determines the turgor loss point, as indicated by arrow.  $\circ$  – control,  $\bullet$  – water stress.

Фигура 2. Криви на водообмена (диаграма на Richter) в млади растения фасул (сорт Добруджански ран) подложени на воден стрес. (a) първичен лист, (b) първи сложен лист. Пресечната точка на регресионната линия и кривата определя точката на загуба на тургор, посочена със стрелка.  $\circ$  – контроли,  $\bullet$  – засушени.

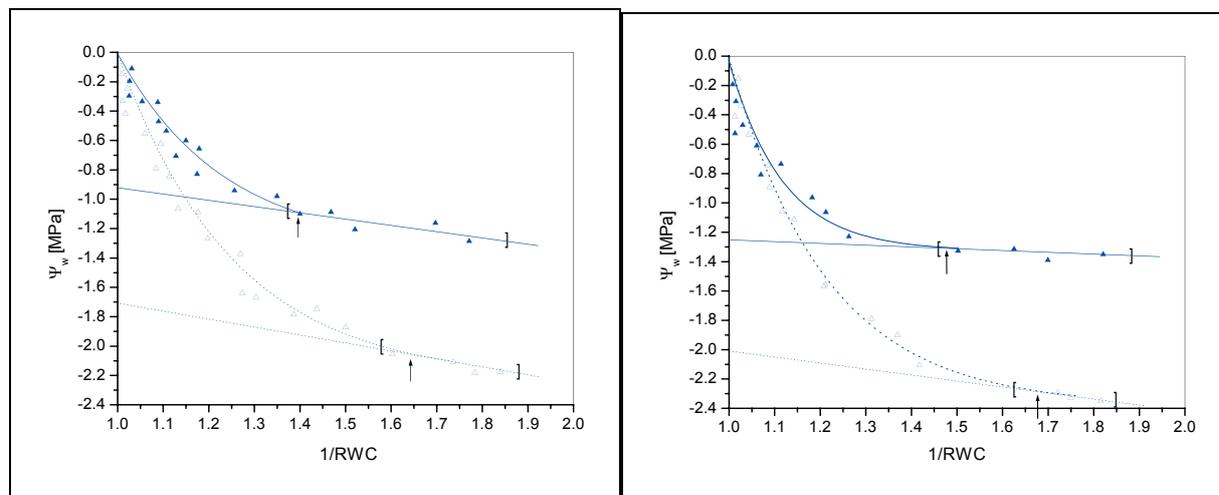


Figure 3. Pressure volume curves (Richter diagram) in young bean plants (cv. Prelom) submitted to water stress. (a) primary leaf, (b) first trifoliolate leaf. Regression line interpolated from the linear zone in brackets. Curve for the turgor potential (above turgor loss point) calculated from measured values of  $\Psi_w$  and RWC after Stadelmann (1984). Intersection of the curve and the line determines the turgor loss point, as indicated by arrow.  $\triangle$  – control,  $\blacktriangle$  – water stress.

Фигура 3. Криви на водообмена (диаграма на Richter) в млади растения фасул (сорт Прелом) подложени на воден стрес. (a) първичен лист, (b) първи сложен лист. Регресионната линия е интерполирана от линейната зона в скобите. Пресечната точка на регресионната линия и кривата определя точката на загуба на тургор, посочена със стрелка.  $\triangle$  – контроли,  $\blacktriangle$  – засушени.

## DISCUSSION

In this study, the three bean genotypes displayed significant differences in their response to drought. These differences were observed at both the primary and first trifoliate leaves.

The analysis of PV curve data showed an active osmotic adjustment in bean leaves, in response to water stress imposed slowly, at a rate of about 0.09 MPa day<sup>-1</sup>. The decrease in osmotic potential at full hydration in droughted leaves corresponded to a degree of osmotic adjustment. The capacity to maintain high RWC values under drought was observed in the cvs. Plovdiv 10 and Prelom and could be explained by their capacity to accumulate great quantities of proline and other osmotic active compounds, which participate in the reduction of  $\Psi_o$  and in osmotic adjustment. The relation between  $\Psi_o$  decrease and osmotic adjustment has already been observed by Teulat et al. [27] in barley and wheat and by Rodrigues et al. [24] in grapevine.

Stressed plants exhibited a higher value of the maximum leaf bulk elastic modulus as compared with well-watered plants both for primary and first trifoliate leaves, except cv. Plovdiv 10, where a slight decrease in  $E_{vmax}$  was observed. However, this change does not appear to be only a function of an alteration in wall structure, translated in the increase of the cell wall rigidity. Another reason is the lower solute potentials at full hydration which lead to greater maximum turgor potential.

Turgor loss point in the stressed leaves was reached at lower  $\Psi_w$  than in well-watered leaves. This indicates that they have an increased capacity to maintain turgor at lower water potentials. That parameter was higher in well-watered plants than in the stressed plants, in spite of the higher  $E_{vmax}$  of the latter. These results are in accordance with the data obtained in grasses by Wilson et al. [33] and in grapevine by the Rodrigues et al. [24].

In this experiment, we found also differences in proline accumulation during drought stress among bean cultivars. Variability for proline metabolism has been reported in various crop species, but it is not well known whether accumulation of this imino acid contributes to the susceptible or tolerant nature of the genotypes [7], [10]. Our results are in agreement with the findings of Naidu et al. [20] and Iannucci et al. [10] who reported that proline levels were more closely related to the decrease in RWC than in  $\Psi_w$ . In agreement with the reports of Navari-Izzo et al. [21], our results showed that the metabolic differences among cultivars may reflect differences in water status achieved, rather than metabolic differences at a given water status. Because the significant proline levels were observed when  $\Psi_w$  was lower than -1.5 MPa

and leaf turgor was very close to zero (Figs. 1-3), proline accumulation seems rather to be a symptom of the development of severe plant water stress. As indicated by Irigoyen et al. [11] and Iannucci et al. [10], such a relationship between turgor and proline accumulation could be useful as a possible drought-injury sensor.

## CONCLUSIONS

The results highlight the fact that water stress influenced leaf water relations in young bean plants. RWC in cv. Dobrudjanski ran was reduced in greater extent, but  $\Psi_w$  was highest. In cv. Prelom changes in RWC were intermediate, but  $\Psi_w$  was lowest, which may be considered as revealing of tolerance to drought. This study indicated that osmotic adjustment is one of the major adaptive mechanisms of *Phaseolus vulgaris* to survive drought. The main difference among cultivars appears to be due to turgor maintenance, which may be more representative of the physiological status of the leaves in these cultivars.

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