

Physiological and biochemical response of common bean varieties treated with salicylic acid under water stress

Nelson Barbosa Machado Neto^{1*}, and Maria Amélia Barbosa Durães²

Received 07 July 2006

Accepted 29 August 2006

ABSTRACT - *There is genetic variability in Phaseolus vulgaris L. with regard to sensitivity to environmental stress situations, such as heat stress and water deficiency. This variability may have been originated by natural selection or plant breeding processes. The objective of this experiment was to evaluate some physiological and biochemical characteristics in common bean plants submitted to water stress and to different salicylic acid doses. Two varieties were grown in 20L pots until the development of the third leaf. The plants were treated with salicylic acid (zero, 0.025, 0.05 and 0.1mM) every three days for nine days. Then the plants were not watered for nine days until they reached the wilting point. The effective and potential quantum efficiency and electron transport rate were measured during and after stress. Proline content, SOD activity, soluble protein content and dry matter was measured 24h after rehydration. Salicylic acid induced alterations in the population Guarumbé while Aporé reacted insensitively to salicylic acid regarding photosynthetic parameters.*

Key words: salicylic acid, water deficit, common bean, photosynthesis.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is considered one of the most important grains for human alimentation and is worldwide planted on approximately 12 million hectares. Common bean is sensitive to severe environmental stress situations, such as heat and water deficiency. (Bajji et al. 2001, Parry et al. 2002).

There is great variability regarding drought tolerance among species (Pimentel and Hérbert 1999). This fact must be analyzed in depth, since more than half of the worldwide common bean production is grown in regions of occurrence of water deficit (Souza et al. 2003). The variability could be the result of natural or artificial selection processes that may have resulted in

different tolerance or resistance levels (Allard 1988, Araus et al. 2002, Gepts 2002, Salamini et al. 2002, Diamond 2002).

Genotypes, be it wild populations or commercial cultivars whose alleles were selected for different purposes, must be evaluated as allele sources for improvement programs (Allard 1988, Araus et al. 2002, Gepts 2002, Diamond 2002). Cultivated plants were selected for yield in detriment of the defense systems while wild plants evolved towards resistance or tolerance to environmental fluctuations in detriment of high seed yields. The adaptation of plants to unfavorable environments is based on the extreme need of survival. (Gimalov et al. 1996) and in many plants, gradual

¹ Universidade do Oeste Paulista (UNOESTE), Rodovia Raposo Tavares, km 572, 19.067-175, Presidente Prudente, SP, Brasil. *E-mail: nbmneto@unoeste.br

² UNOESTE

changes of the environmental conditions induce tolerance to extreme situations.

Although plants do not have the defense of free movements, it is known that adaptations and alterations can occur in the cell metabolism, as the synthesis of defense proteins, expressed by specific genes, activated by complex mechanisms (Busam et al. 1997, Pinheiro et al. 1999), influencing plant resistance and survival directly or indirectly.

Salicylic acid (SA) is a natural molecular signal that plays an important role in the regulation of a number of physiological plant processes (Shi et al 2005). The SA accumulation is one of the transduction signals of the main paths for the Systemic Acquired Resistance (SAR) which involves the accumulation of PRPs (Pathogenesis-Related Proteins) as induced defense mechanisms with salicylate-dependent induction (White 1979, Métraux et al. 1991, Palva et al. 1994, López-López et al. 1995, Andarwulan and Shetty 1999) The exact function of SA is however uncertain; it can be the activation/inactivation of catalase isoforms (Hunt et al. 1996) or a defense signal, well-defined in studies with tobacco plants (Ganesan and Thomas 2001).

The activity of this elaborated system of plant defense and adaptation is threefold: i) constitutive, unspecific or static resistance that occurs even without the action of aggressor agents; it is received by genetic inheritance and immunizes plants to certain pathogens (Goto 1990, Sticher et al. 1997); ii). localized resistance activated at the point where aggression occurs and iii) SAR, which protects the plant against subsequent attacks.

H₂O₂ has diverse functions within the cell, from the production of free radicals over lignification and production of phenolic compounds, up to the action of an inducer of SAR responses (Hammerschmidt and Kuc 1982, Siegel 1993, Hammerschmidt and Kuc 1995, Benhamou and Nicole 1997, Jung et al. 2000, McCue et al. 2000) where salicylic acid could be responsible for initiating the SAR response by inhibiting specific peroxidases.

An increasing number of studies show the phenomenon of cross-tolerance in plants, i.e., the exposition of tissues to moderate stress induces resistance to another stress type, where for instance, water stress conveyed resistance to intense cold in winter crops or to frosts in rice (Song et al. 2005).

Proline is an aminoacid that seems to be the most

widely distributed solute in organisms, especially in those exposed to environmental stress situations (Trotel et al. 1996, Hoai et al. 2003, Yokota 2003, Rizhsky et al. 2004), so it can be used as osmotic regulator, enzymatic protector, carbon skeleton, and as amine reserve for *de novo* synthesis and growth restart when the stress ceases (Sudhakar et al. 1993, Al-Karaki et al. 1996, Hoai et al. 2003).

Proline accumulation has been suggested as the result of degradation or synthesis (Sudhakar et al. 1993), inhibition of the protein synthesis while in common bean it can be related to degradation mechanisms (Andrade et al. 1995). Proline synthesis can be related with photoinduction and/or photoinactivation of the enzymes associated with their catabolism. Stewart et al. (1966), Bates et al. (1973) and Stewart and Larher (1980) pointed out the role of proline as solute during stress, where an increase in the proline content would indicate resistance or tolerance to water deficit, serving as parameter for the selection of highly resistant cultivars. But Maggio et al. (2002) demonstrated that proline-accumulating genotypes were susceptible to this type of stress.

The objective of this study was to evaluate physiological and biochemical characteristics in common bean plants subjected to water stress and the application of different doses of salicylic acid.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse, at the UNOESTE - Universidade do Oeste Paulista, in Presidente Prudente, state of São Paulo. Two common bean varieties (*Phaseolus vulgaris* L.) were used: the cultivar Aporé (EMBRAPA) and a wild genotype, Guarumbé, found in the Parque Nacional do Iguaçú. The seeds were sown in pots with 20L substratum and after germination thinned to one plant per pot. The plants were grown until the development of the third trifoliolate, ferigated with 300 mL Hoagland nutrient solution per pot. Upon appearance of the third trifoliolate the plants were sprayed and irrigated with 250mL of the salicylic acid solution at four different concentrations: zero; 0.025; 0.05 and 0.1 (mM) every three days, for nine days. After this period, the plants were maintained for another nine days without irrigation to enter in water stress. One lot of each plant variety was maintained under normal conditions and without acid treatment as

control, corresponding to a factorial design of two varieties with four treatments plus one control (2x4+1).

The potential quantum efficiency of PSII, relative quantum efficiency of PSII and electron transport rate were measured by a fluorometer of modulated light (model FMS-2, Hansatech, UK) before and after the rehydration of the plants. Finally, the plants were harvested and the dry weight obtained by oven-drying at 65 °C for 48h.

After the fluorometer readings, the third trifoliolate of one plant of each treatment was removed and cut up. Part of the trifoliolate was ground in 0.1M cold (4 °C) phosphate buffer (pH 7.8), containing 0.4g polyvinylpyrrolidone, 2mM dithiothreitol and 0.1mM EDTA, and centrifuged at 12,000g for 20 minutes. This was divided in aliquots and the protein quantified according to Bradford (1976). The remaining supernatant was divided in four fractions that were immediately installed in the trials, when possible, or stored at -80 °C. The activity of superoxide dismutase (SOD, EC.1.15.11) was evaluated in 50µL extract to which 0.1M phosphate buffer (pH 7.8) was added, containing 1.3µM riboflavin, 13mM methionine and 63µM nitro blue tetrazolium (NBT). The tubes were incubated at 25 °C for 15 minutes under illumination of fluorescent lamps, measuring the absorbance at 560 nm. Tubes containing the same medium and not subjected to light were used as control. One unit SOD was defined as the enzyme activity able to inhibit the photoreduction of NBT to blue formazan in 50% and was expressed in units of SOD (mg protein)⁻¹.

The proline content in a part of the third trifoliolate was analyzed, according to the methodology of Bates et al.(1973) and modified by Machado Neto et al. (2004).

The data variance analysis was performed by the F test and afterwards the analysis of polynomial regression of the effect of the salicylic acid doses. The data comparison with the control was performed by Dunnet's test.

RESULTS

The common bean plants of the variety Guarumbé and cultivar Aporé presented, in general, a differentiated performance, based on the evaluated variables.

In an analysis of the potential quantum efficiency of photosystem II (Figure 1A), during the water stress,

the response of the variety Guarumbé increased with the applied acid doses. After the stress, both varieties presented response to the doses, with a maximum point between 0.025 and 0.05 mM (Figure 1B).

Neither during nor after the stress there was a statistically significant response of the varieties to the salicylic acid doses applied in relation to the effective quantum efficiency of PSII (Figures 1C and 1D). However, there was an increase of this efficiency when the plants were under stress.

Neither during nor after the stress there was a statistically significant response of Aporé to the salicylic acid doses applied with regard to electron transport (Figures 2A and 2B). But Guarumbé presented increasing responses of the rate after stress.

The variety Guarumbé presented significant responses to the salicylic acid doses for proline synthesis/accumulation (Figure 3A) as much as for SOD activity (Figure 3B), with a minimum point close to the dose of 0.05 mM. For proteins, the response of Guarumbé (Figure 3C) was opposite to the previous, that is, soluble proteins increased up to the dose of 0.05 mM and decreased thereafter. Aporé did not present response in the SOD activity, but presented a significant response in proline accumulation.

In relation to dry weight, the variety Guarumbé (Figure 4A) presented an increasing response to the salicylic acid doses during the stress for root growth, but did not present the same response in the shoot (Figure 4 B). Cultivar Aporé did not present significant responses, neither for root dry matter nor for dry matter of the shoot (Figures 4A,B).

When compared with the control, cultivar Aporé did not present variations in the potential and effective quantum efficiency, electron transport rate, SOD activity and total soluble proteins. There was a significant response for shoot and root dry matter as well as for the root – shoot ratio. The proline accumulation occurred at the doses zero and 0.025 mM, while it decreased under higher doses (Table 1).

The variety Guarumbé, compared with the control, did not present significant responses by Dunnet's test at 5% for electron transport rate, dry matter of shoot and for the characteristics of effective and potential quantum efficiency, 24h after the stress ended (Table 1). The effective quantum efficiency of PSII during stress was lower at all doses of the treatment and the potential quantum efficiency of PSII and the ratio dry

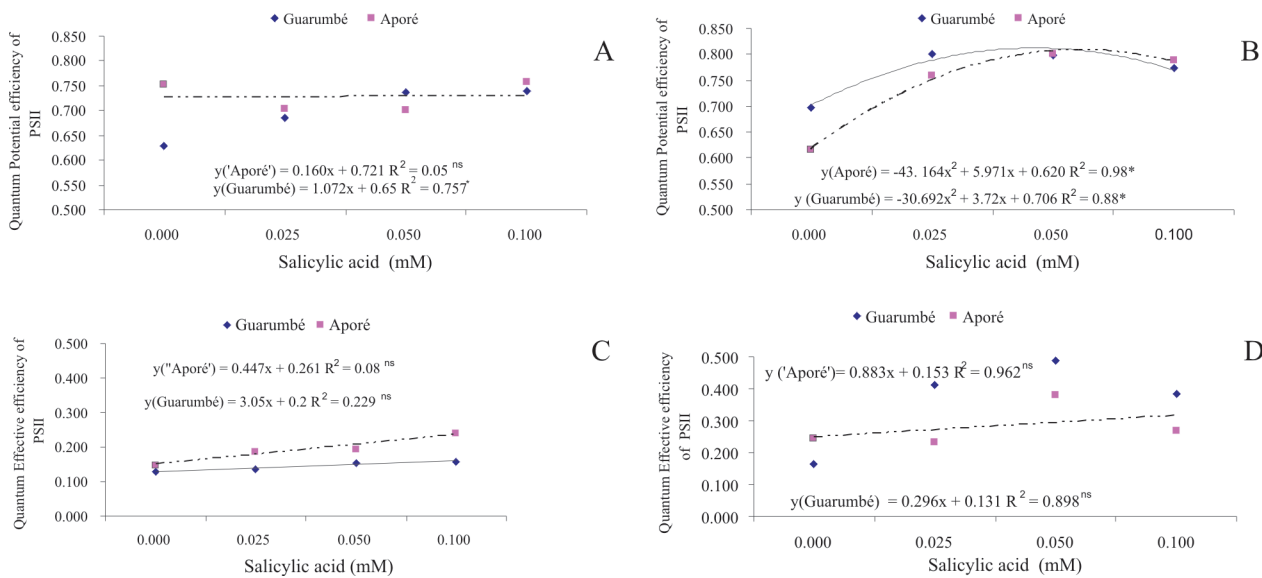


Figure 1. Quantum efficiency, potential (A, B - Fv/Fm) and effective (C, D - δ PS2), of PSII in common bean plants of the cultivar Aporé and the variety Guarumbé treated with different doses of salicylic acid under water stress (A, C) and 24h after rehydration (B, D)

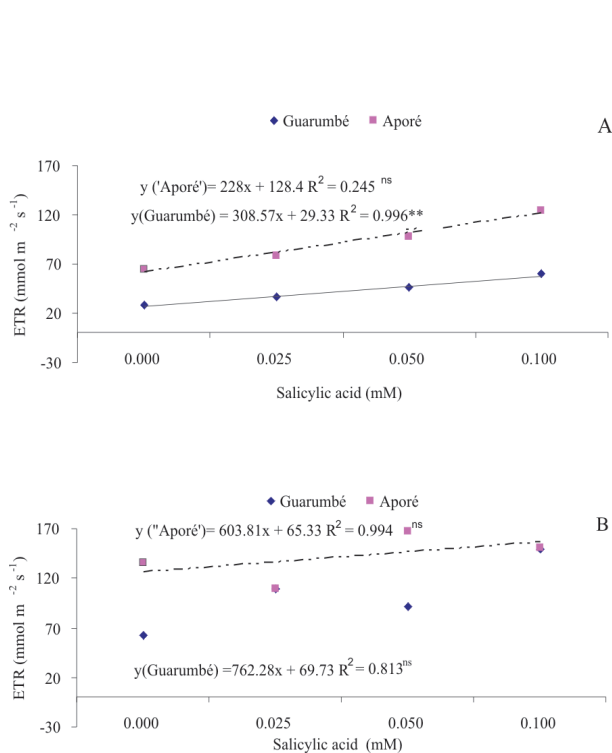


Figure 2. Electron transport rate (ETR) in common bean plants of cultivar Aporé and variety Guarumbé treated with different doses of salicylic acid under water stress (A) and 24h (B) after rehydration

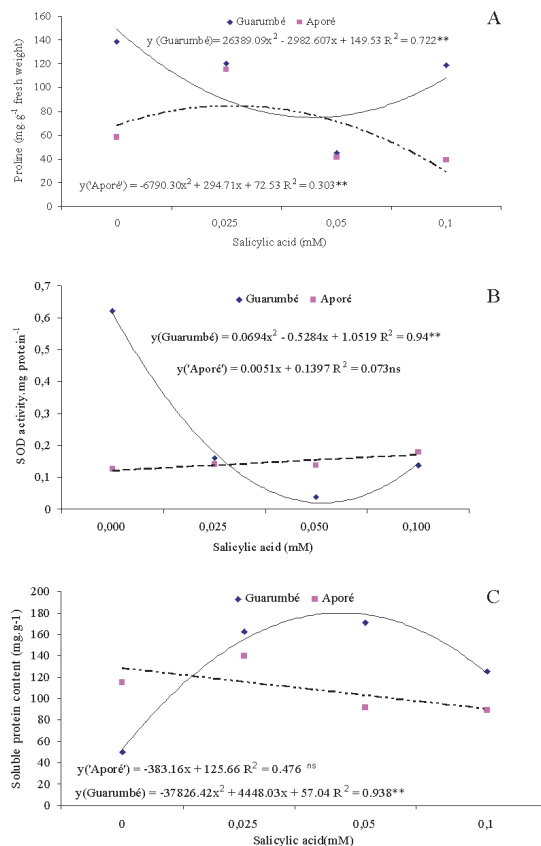


Figure 3. Proline content (A), superoxide dismutase activity (B) and soluble protein content (C) in common bean plants of the cultivar Aporé and variety Guarumbé treated with different doses of salicylic acid under water stress

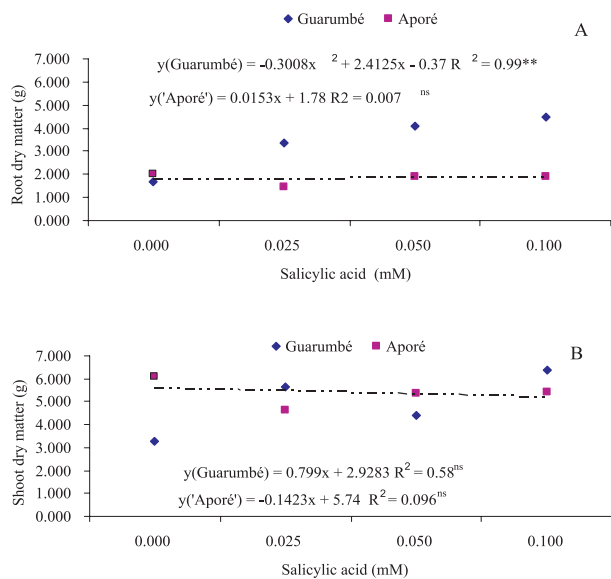


Figure 4. Dry weight data of root (A) and shoot (B), obtained from common bean plants of the cultivar Aporé and variety Guarumbé treated with different doses of salicylic acid under water stress

matter of root - shoot were lowest at the doses zero and 0.025mM during the stress period. The root dry matter was reduced by all salicylic acid doses. The SOD activity and proline quantity presented responses to dose zero, with increased enzyme activity and proline content, and at 0.05 mM with a reduction in both parameters. Dose zero reduced the quantity of soluble proteins in this genotype.

DISCUSSION

The fluorescence of chlorophyll has been considered a tool for the determination of the photoinhibition of photosynthesis and as an indicator of oxidative stress (Shim et al, 2003). The observed variations can therefore indicate a greater sensitivity of the wild variety Guarumbé as compared with cultivar Aporé when subjected to stress. The genotypes obtained by the genetic improvement and recommended for cultivation, such as Aporé, go through an analysis of phenotypic stability and therefore little variation is observed in these genotypes, even under environmental oscillations. According to Gepts (2002) and Diamond (2002) these adaptations were selected additionally to the agronomic treatments during domestication. Thus,

in the selection for yield, the genotypes evolved from the natural in different forms and the phenotypic selection used fixed traits that were not being selected directly.

Some reports show a positive correlation between proline accumulation and adaptation to salt and water stress. However, some questions remain unanswered, for example if proline accumulation contributes to the cell adaptation of the plant to salt stress or whether the function is restricted to the osmotic cell adjustment (Yokota 2003). The variety Guarumbé was already identified as proline accumulator during germination in stress situations, different from cultivar Aporé (Machado Neto et al. 2004), and as resistant to water stress (Souza et al. 2003), regardless of not differing in the cardinal germination temperatures (Machado Neto et al. 2006).

Plants under stress conditions tend to increase the peroxidase activity and it is sometimes the first enzyme with an alteration in activity, independently of the substratum or the applied stress. Peroxidase can be considered a biochemical marker of biotic as well as abiotic stress situations; moreover, it seems to be a key molecule of plant adaptation, or of some plant organs separately, to environmental changes (Rossi et al. 2001).

However, in this report, the SA application caused a diminution in the SO activity or in proline accumulation, but raised the soluble protein content in the variety Guarumbé. Compared with the control plants, the SOD activity as well as the proline content increased in plants not treated with SA and dropped with 0.05mM of salicylic acid.

According to Métraux et al. (1991) and Andarwulan and Shetty (1999), salicylic acid acts as elicitor of the SAR-related systems. The responses observed in ‘Guarumbé’ would be according to these observations, where genotypes not treated with SA activated the normal defense forms, increasing the proline concentration and SOD activity. This leads to an increase of the osmolarity of the cytoplasm and to a higher H₂O₂ production, which is related with different resistance forms such as lignification, production of free radicals and of phenolic compounds that are linked to resistance to pathogens and herbivorous animals. The proline increase could further be directly related with a sinking concentration of soluble proteins (Stewart

Table 1. Comparison significance of salicylic acid treatments, before and after water deficit, and untreated plants maintained under conditions of no stress (control) by Dunnett's bilateral test

Stress	Salicylic acid (mM)	Effective Quantum efficiency of PSII ($\Delta F/F_m'$)		Potential Quantum efficiency of PSII		Electron transport rate (ETR)		Dry matter		Root - Shoot ratio		Superoxide Dismutase Activity		Proline		Soluble proteins	
		before	after	before	after	before	after	before	after	Root	Canopy	Shoot	ratio	Superoxide Dismutase	Activity	Proline	Soluble proteins
Cultivar		($\Delta F/F_m'$)	Fv/Fm	($\mu\text{mol m}^{-2} \text{s}^{-1}$)	(g)	(g g ⁻¹)											
Guarumbé	Control	0.276	0.276	0.735	0.605	143	143	9.10	7.25	1.273	0.192	107.41	153.32				
	0.000	0.130**	0.163	0.628**	0.698	29	63	1.71**	3.28	0.539**	0.622*	138.65*	50.23**				
	0.025	0.136**	0.411	0.686*	0.801	37	109	3.34**	5.65	0.606*	0.160	120.48	162.78				
	0.050	0.153**	0.486	0.738	0.799	46	92	4.06**	4.42	1.012	0.038*	44.61**	171.25				
	0.100	0.159**	0.386	0.740	0.774	59	149	4.49**	6.35	0.850	0.139	118.79	125.86				
Aporé	Control	0.415	0.351	0.784	0.712	162	106	7.90	12.64	0.624	0.194	47.33	134.08				
	0.000	0.147	0.245	0.752	0.615	65	136	2.00**	6.11**	0.330**	0.126	57.89**	115.49				
	0.025	0.186	0.232	0.704	0.758	79	110	1.47**	4.657**	0.367*	0.140	114.71**	139.59				
	0.050	0.194	0.380	0.700	0.800	98	168	1.90**	5.373**	0.378*	0.137	41.01*	91.56				
	0.100	0.240	0.269	0.758	0.788	125	151	1.91**	5.393**	0.349**	0.180	38.99**	88.93				
	D _{5%tab}																
	D _{1%tab}																

*, ** Significant at 5 and 1% probability

et al. 1966, Bates et al. 1973, Stewart and Larher 1980, Sudhakar et al. 1993, Andrade et al. 1995, and Machado Neto et al. 2004).

Aporé presented an increase in proline at SA doses of zero and 0.025mM, reducing the content in the subsequent doses. This can mean that in spite of the low responsiveness of cultivar Aporé to SA, it responded with a positive stimulus in an attempt to minimize the stress effects, an adjustment for which proline is responsible; and that higher doses of the acid could activate other defense systems or inhibit proline accumulation.

For 'Guarumbé' as much as for Aporé there was a decrease of the root dry matter accumulation, and only the latter presented reduction in the shoot as well. Wild

genotypes tend to support greater environmental variations, so that genotypes derived from improvement programs tend to be more adapted to the specific cultivation conditions, in agreement with statements of Allard (1988), Schaal and Olsen (2000), Diamond (2002) and Gepts (2002).

CONCLUSIONS

The use of SA was not effective to minimize the effects of water stress, while wild genotypes presented a biochemical response to this treatment and in cultivated genotypes the response was translated to a reduction in the dry matter accumulation.

Resposta fisiológica e bioquímica do feijoeiro-comum tratadas com ácido salicílico e submetidas a estresse hídrico

RESUMO - Existe variabilidade em feijoeiro (*Phaseolus vulgaris* L.) quanto à sensibilidade a estresses térmicos e a deficiência hídrica. Esta variabilidade pode ser oriunda de processos de seleção natural ou de melhoramento. O objetivo desse experimento foi avaliar características fisiológicas e bioquímicas em plantas de feijão submetidas a estresse hídrico, sob aplicação de diferentes doses de ácido salicílico. Duas variedades foram cultivadas em vasos de 20L até o desenvolvimento da terceira folha e tratadas com ácido salicílico nas dosagens de zero, 0,025, 0,05 e 0,1(mM). O tratamento foi feito três vezes, espaçadas de três dias, ficando sem irrigação por nove dias até atingirem ponto de murcha. As eficiências quânticas efetivas e potencial, e a taxa de transporte de elétrons foram medidas durante e após o estresse. O teor de prolina, atividade de SOD, conteúdo de proteínas totais e massa seca foram medidos 24h após a rehidratação. O ácido salicílico induziu alterações, na variedade Guarumbé. O cultivar Aporé demonstrou-se insensível ao ácido salicílico para os caracteres fotossintéticos.

Palavras chave: Ácido salicílico; estresse hídrico; feijão, fotossíntese, bioestimulantes.

REFERENCES

- Al-Karaki GN, Clark RB and Sullivan CY (1996) Phosphorus nutrition and water stress effects on proline accumulation in sorghum and bean. **Journal of Plant Physiology** **148**: 745-751.
- Allard RW (1988) Genetic changes associated with the evolution of adaptedness in cultivated plants and their wild progenitors. **Journal of Heredity** **79**: 225-238.
- Andarwulan N and Shetty K (1999) Improvement of pea (*Pisum sativum*) seed vigour response by fish protein hydrolysates in combination with acetyl salicylic acid. **Process Biochemistry** **35**: 159-165.
- Andrade JL, Larqueé-Saavedra A and Trejo CL (1995) Proline accumulation in leaves of four cultivars of *Phaseolus vulgaris* L. with different drought resistance. **Öyton** **57**: 149-157.
- Araus JL, Slafer GA, Reynolds MP and Royo C (2002) Plant breeding and drought in C3 cereals: What should we breed for? **Annals of Botany** **89**: 925-940.
- Bajji M, Lutts S and Kinet JM (2001) Water deficit effects on solute contribution to osmotic adjustment as a function of leaf ageing in three durum wheat (*Triticum durum* Desf.) cultivars performing differently in arid conditions. **Plant Science** **160**: 669-681.
- Bates LS, Waldren RP and Teare ID (1973) Rapid determination of free proline for water-stress studies. **Plant and Soil** **39**: 205-207.
- Benhamou N and Nicole M (1997) Cell biology of plant immunization against microbial infection: The potential of induced resistance in controlling plant diseases. **Plant Physiology Biochemical** **37**: 703-719.

- Bradford MM (1976) A rapid and sensitive method for the quantitation of micrograms of protein utilizing the principle of protein dye binding. **Analytical Biochemistry** **72**: 248-254.
- Busam G, Kassemeyer HH and Mattern U (1997) Differential expression of chitinases in *Vitis vinifera* L. responding to systemic acquired resistance activators of fungal challenge. **Plant Physiology** **115**: 1029-1038.
- Cornic G, Ghashghaie B and Briantais JM (1992) Leaf photosynthesis is resistant to a mild drought stress. **Photosynthetica** **27**: 295-309.
- Diamond J (2002) Evolution, consequences and future of plant and animal domestication. **Nature** **418**: 700-706.
- Ganesan V and Thomas G (2001) Salicylic acid response in rice: influence of salicylic acid on H₂O₂ accumulation and oxidative stress. **Plant Science** **160**: 1095-1106.
- Gepts P (2002) A comparison between crop domestication, classical plant breeding and genetic engineering. **Crop Science** **42**: 1780-1790.
- Gimalov FP, Chemeris AV and Vakhitov VA (1996) Synthesis of cold shock proteins in wheat tribe seedlings of the family Poaceae. **Russian Journal of Plant Physiology** **43**: 228-231.
- Goto M (1990) **Fundamentals of Bacterial Plant Pathogens**. Academic Press, San Diego, 342p.
- Hoai NTT, Shim IS, Kobayashi K and Kenji U (2003) Accumulation of some nitrogen compounds in response to salt stress and their relationships with salt tolerance in rice (*Oryza sativa* L.) seedlings. **Plant Growth Regulation** **41**: 159-164.
- Hunt M, Neuenschwander UH, Delaney TP, Weymann KB, Friedrich LB, Lawton KA, Steiner HY and Ryals JA (1996) Recent advances in systemic acquired resistance research. **Gene** **179**: 89-95.
- Jung S, Kim JS, Cho KY, Tae GS and Kang BG (2000) Antioxidant responses of cucumber to photoinhibition and oxidative stress induced by norflurazon under high and low PFDs. **Plant Science** **153**: 145-154.
- Kaiser WSAT (1987) Effects of water deficit on photosynthetic capacity. **Physiology Plantarum** **71**: 142-149.
- López-López MJ, Liébana E, Marcilla P and Beltrá R (1995) Resistance induced in potato tubers by treatment with acetylsalicylic acid to soft rot produced by *Erwinia carotovora* subsp. *carotovora*. **Journal of Phytopathology** **143**: 719-724.
- Machado Neto NB, Custódio CC, Gatti AB, Priolli MR and Cardoso VJM (2004) Proline: use as an indicator of temperature stress in bean seeds. **Crop Breeding and Applied Biotechnology** **4**: 127-134.
- Machado Neto NB, Priolli MR, Gatti AB and Cardoso VJM (2006) Temperature effects in races of common beans (*Phaseolus vulgaris* L.). **Acta Scientiarum Agronomia** **28**: 155-164.
- Maggio A, Miyazaki S and Veronese P (2002) Does proline accumulation play an active role in stress-induced growth reduction? **Plant Journal** **31**: 699-712.
- McCue P, Zheng Z, Pinkham J and Shetty K (2000) A model for enhanced pea seedling vigour following low pH and salicylic acid treatments. **Process Biochemistry** **35**: 603-613.
- Métraux JP, Ahl Goy P, Staub T, Spelch J, Steinemann A, Ryals J and Ward E (1991) Induced resistance in cucumber in response to 2,6-dichloroisonicotinic acid and pathogens. In: Hennecke H and Verma DPS. (eds.) **Advances in Molecular Genetics of Plant-Microbe Interactions**. Dordrecht, Kluwer Academic p. 432-439.
- Oidaira H, Sano S, Koshihara T and Ushimaru T (2000) Enhancement of antioxidative enzyme activities in chilled rice seedlings. **Journal of Plant Physiology** **156**: 811-813.
- Palva TK, Hurtig M, Dainrean P and Palva ET (1994) Salicylic acid induced resistance to *Erwinia carotovora* subsp. *carotovora* in tobacco. **Molecular Plant-Microbe Interaction** **7**: 356-363.
- Parry MAJ, Andralojc PJ, Khan S, Lea P and Keys A (2002) Rubisco Activity: Effects of drought stress. **Annals of Botany** **89**: 833-839.
- Pimentel C and Hébert G (1999) Potencial fotossintético e condutância estomática em espécies de feijão caupi sob deficiência hídrica. **Revista Brasileira de Fisiologia Vegetal** **11**: 7-11.
- Pinheiro MM, Sandroni M, Lummerzheim M and Oliveira DE (1999) A defesa das plantas contra as doenças. **Revista Ciência Hoje** **147**: 25-31.
- Rizhsky L, Liang H and Shuman J (2004) **When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress.** **Plant Physiology** **134**: 1683-1696
- Rossi C and Lima GP (2001) Cádmio e atividade de peroxidase durante a germinação de sementes de feijoeiro. **Scientia Agrícola** **58**: 197-199.
- Salamin F, Özkan H, Brandolin A, Schäfer-Pregl R and Martin W (2002) Genetics and geography of wild cereal domestication in the near east. **Nature reviews: Genetics** **3**: 429-441.
- Schaal BA and Olsen, KM (2000) Gene genealogies and population Variation in plants. **PNAS** **97**: 7024-7029.
- Shi Q, Bao Z, Zhu Z, Ying Q and Qian Q (2005) Effects of different treatments of salicylic acid on heat tolerance,

- chlorophyll fluorescence, and antioxidant enzyme activity in seedlings of *Cucumis sativa* L. **Russian Journal of Plant Physiology** **52**: 793–800.
- Shim IS, Momose Y, Yamamoto A, Kim DW and Usui K (2003) Inhibition of catalase activity by oxidative stress and its relationship to salicylic acid accumulation in plants. **Plant Growth Regulation** **39**: 285–292.
- Siegel BZ (1993) Plant peroxidases - an organismic perspective. **Plant Growth Regulation** **12**: 303-312.
- Singh SP, Terá H, Munõz G, Osorno JM and Takegami JCAT (2003) Low soil fertility tolerance in landraces and improved common bean genotypes. **Crop Science** **43**: 110–119.
- Song SQ, Lei YB and Tian XR (2005) Proline metabolism and cross-tolerance to salinity and heat stress in germinating wheat seeds. **Russian Journal of Plant Physiology** **52**: 793–800.
- Souza GM, Aidar ST, Giaveno CD and Oliveira RF (2003) Drought stability in different common bean (*Phaseolus vulgaris* L.) genotypes. **Crop Breeding and Applied Biotechnology** **3**: 203-208.
- Stewart CR and Larher F (1980) Accumulation of amino acids and related compounds in relation to environmental stress. In: Stumpf, P. K. and Conn, E. E (eds.) **The Plant Biochemistry**, v. 5. Academic Press, New York. p. 609-635.
- Stewart CR, Morris CJ and Thompson JF (1966) Changes in amino acid content of excised leaves during incubation II. Role of sugar in the accumulation in wilted barley leaves. **Plant Physiology** **41**: 1585-1590.
- Sticher L, Mauch Mani B and Mettraux JP (1997) Systemic acquired resistance. **Annual Review of Phytopathology** **35**: 235-270.
- Sudhakar C, Reddy PS and Veeranjanelu K (1993) Effect of salt stress on the enzymes of proline synthesis and oxidation in green gram (*Phaseolus aureus* Roxb.) seedlings. **Journal of Plant Physiology** **141**: 621-623.
- Trotel P, Bouchereau A, Niogret MF and Larher F (1996) The fate of osmo-accumulated proline in leaf discs of rape (*Brassica napus* L.) incubated in a medium of low osmolarity. **Plant Science Limerick** **118**: 31-45.
- White RF (1979) Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. **Virology** **99**: 420-412.
- Yokota S (2003) Relationship between salt tolerance and proline accumulation in Australian acacia species. **The Japanese Forestry Society** **8**: 89–93.