

## PEROXIDASE ACTIVITY AS AN INDICATOR OF WATER STRESS IN SWEET PEPPER PLANTS

**Antonio Evaldo Klar<sup>1</sup>; Sidnei Osmar Jadoski<sup>2</sup>; Giusepina Pace Pereira Lima<sup>3</sup>**

<sup>1</sup>*Departamento de Engenharia Rural, Faculdade de Ciências Agrônômicas, Universidade Estadual Paulista, Botucatu, SP, klar@fca.unesp.br.*

<sup>2</sup>*Universidade Estadual do Centro Oeste, Centro de Ciências Agrárias e Ambientais, Guarapuava, Pr*

<sup>3</sup>*Departamento de Bioquímica, Faculdade de Ciências Agrônômicas, Universidade Estadual Paulista, Botucatu, SP*

### 1 ABSTRACT

The purpose of the study was to evaluate the physiological and biochemical behavior of sweet pepper (*Capsicum annuum* L.) plants under different soil water availability conditions and the efficiency of the peroxidase (EC. 1.11.1.7) activity as an indicator of water stress in plants. The experiment was carried out at the *Faculdade de Ciências Agrônômicas – UNESP, Botucatu, SP*. Sweet pepper plants were grown for 230 days after transplanting of seedlings and arranged in a completely randomized experimental design with 4 treatments, two irrigation managements (50 and 1500 kPa) and two soil surface managements (presence or absence of black polyethylene covering), and six replications. Physiological activities, such as stomatal transpiration and resistance to water vapor diffusion, were evaluated as well as biochemical activities, such as peroxidase activity and total soluble protein in foliar tissues. It was observed that soil water availability may lead to physiological and biochemical alterations in plants. Successive water stress cycles may promote the development of characteristics responsible for improving plant tolerance to periods of low water availability. The peroxidase enzyme activity showed to be an efficient indicator of water stress in sweet pepper plants.

**KEYWORDS:** enzymatic activity; soil moisture; vegetable physiology; soluble protein; *Capsicum annuum* L.

**KLAR, A. E.; JADOSKI, S. O.; LIMA, G. P. P. A PEROXIDASE COMO INDICADOR DE DÉFICITS HÍDRICOS EM PLANTAS DE PIMENTÃO.**

### 2 RESUMO

O objetivo do presente estudo foi avaliar o comportamento fisiológico e bioquímico do pimentão (*Capsicum annuum* L) sob diferentes condições de disponibilidade de água no solo, além da eficiência da peroxidase (EC 1.11.1.7) como indicador de déficits hídricos nas plantas. O experimento foi desenvolvido na Faculdade de Ciências Agrônômicas – UNESP, Botucatu, SP, durante 230 dias após o transplante das plântulas em condições de ambiente protegido com quatro tratamentos: dois níveis de irrigação (-50 e – 1500 kPa como potenciais mínimos de água do solo) e dois manejos de cobertura do solo (com e sem cobertura de lonas de polietileno preto) com seis repetições. As atividades fisiológicas, como a resistência difusiva dos estômatos e a transpiração foram avaliadas, assim como a peroxidase e a proteína

solúvel, nos tecidos foliares. Os resultados mostraram que os déficits hídricos alteraram os fatores fisiológicos estudados e promoveram respostas positivas para a tolerância à déficits hídricos. A peroxidase mostrou ser um eficiente indicador de estresse hídrico em plantas de pimentão.

**UNITERMOS:** irrigação, cobertura morta, potencial de água no solo, proteína solúvel.

### 3 INTRODUCTION

Peroxidases are enzymes naturally found in plants. They are composed of 25% carbohydrates that protect them from proteolytic degradation and stabilize their protein structure (Siegel, 1995). Gaspar *et al.* (1985) described peroxidases as isoenzymes associated to changes in physiological processes of plants under stress. Chang & Kao (1998) considered that the high enzyme activity in stressed plants may indicate the ability of certain genotypes to degrade toxic substances, such as free radicals released under such conditions.

Sweet pepper is considered to be a sensitive crop to water stress, as reductions in soil water availability or soil water excess jeopardize its normal development. According to Carvalho *et al.* (2001) the amount of water made available to sweet pepper is a limiting factor for the growth and development of plants, causing stress conditions that limit their growth.

The purpose of this work was to evaluate the physiological and biochemical behavior of sweet pepper plants under different soil water availability conditions and evaluate the efficiency of peroxidase activity (EC. 1.11.1.7) as an indicator of water stress.

### 4 MATERIAL AND METHODS

This work was carried out at the *Faculdade de Ciências Agronômicas* – UNESP, Botucatu, SP (22° 51'03"S and 48° 25'37"W), from November 2002 to August 2003.

Sweet pepper plants (*Capsicum annuum* L. 'Elisa'), presenting red skin and regular shape, were grown under greenhouse (27.5m long by 8.0m wide) conditions.

The experiment was arranged in a completely randomized experimental design with six replications. Four treatments were tested: T1 – irrigation at 50kPa with black polyethylene soil covering; T2 – irrigation at 50kPa without soil covering; T3 – irrigation at 1500kPa with black polyethylene soil covering; and T4 – irrigation at 1500kPa without soil covering.

Sowing was on November 3, 2002, and transplantation on November 11, 2002. Eight plants were transplanted to each experimental unit, which consisted of two rows (0.60m apart) of plants spaced 0.50m apart, yielding a final stand of 26660 plants ha<sup>-1</sup>. The crop was allowed to grow for 28 days after transplanting (DAT), when soil water availability was kept near the maximum availability limit to plants. The soil covering used consisted of a black 30µm-thick polyethylene film, which was used only for treatments T1 and T3, after the plant establishment period. Plants were conducted in a "V" shape, without pruning.

Plants were cultivated for 230 days after transplanting and this period was divided in two stages according to the crop management and procedures used.

*Stage 1:* (29 to 169 DAT) – use of irrigation management and soil covering practices. Soil water amounts were monitored daily up to 0.40-m depth using a neutron probe. Dripping irrigation was used. The maximum soil water amount at 10kPa was 0.38cm<sup>3</sup> cm<sup>-3</sup> and the

minimum water amounts at 50 and 1500 kPa were  $0.316\text{cm}^3\text{ cm}^{-3}$  and  $0.225\text{cm}^3\text{ cm}^{-3}$ , respectively, according to each treatment used.

Initial fertilization was 60g thermophosphate (17.5%  $\text{P}_2\text{O}_5$ , 0.15% B, 0.4% Zn)  $\text{plant}^{-1}$ . The maintenance fertilization was carried out via fertirrigation. Each plant received 0.136g potassium nitrate, 0.2g calcium nitrate and 0.136g monoammonium phosphate (MAP) daily from the onset of blooming. Fertilizer amounts were increased to 0.2g potassium nitrate, 0.3g calcium nitrate and 0.25g MAP from the appearance of the first fruits.

*Stage 2:* (170 to 230 DAT) – no irrigation was carried out during this period and soil covering was withdrawn from all experimental units. Peroxidase activity - E.C.1.11.1.7 (POD), total soluble protein (TSP), stomatal resistance to water vapor diffusion (Rd) and foliar transpiration were monitored during this period.

Foliar transpiration and stomatal resistance to water vapor diffusion were determined using a digital porometer at 8-day intervals between 12:00pm and 2:00pm, scheduled by means of previous experiments. Readings were carried out in a predefined plant per plot, in fully-grown leaves at two positions in the plant, upper canopy near the apex and middle canopy. Results were expressed as the average of the two readings.

POD was assessed by means of the methodology proposed by Lima (1994). Leaves were collected, weighed and processed, yielding a raw extract, which was homogenized in potassium phosphate buffer solution 0.2N, pH 6.7, and centrifuged at 10,000 rpm at  $4^\circ\text{C}$  for 10 minutes. Readings were carried out in a spectrophotometer at 505nm and the enzymatic activity was expressed as micromoles  $\text{H}_2\text{O}_2$  decomposed per minute per gram of green leaves mass ( $\mu\text{mol decomposed g glm}^{-1}\text{ min}^{-1}$ ).

TSP was determined according to the methodology proposed by Bradford (1976), using the raw extract obtained described herein. Readings were carried out with a spectrophotometer at 525nm (casein standard) and protein amounts were expressed as milligrams of total soluble protein per gram of green leaves mass ( $\text{mg TSP g glm}^{-1}$ ).

The data statistical analysis was conducted using the software SAS – Statistical Analysis System.

## 5 RESULTS AND DISCUSSION

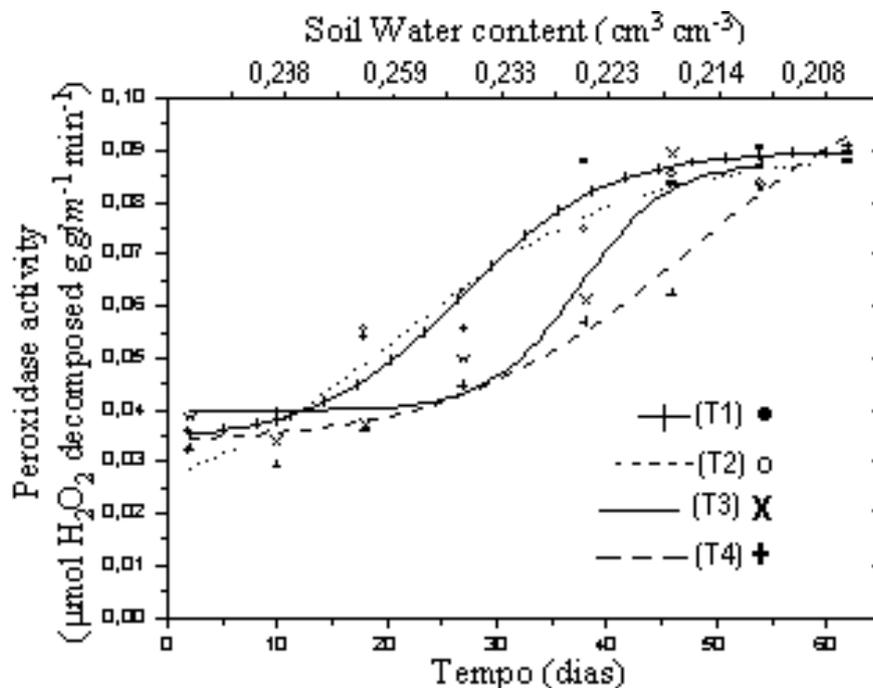
The amplitude of POD average concentrations was observed near 0.04 and 0.09  $\mu\text{moles decomposed gmv}^{-1}\text{ min}^{-1}$  (Figure 1).

Even when the soil water content is high, POD can be observed in plants, as it happened at the beginning of Stage 2, in which plants belonging to both treatments showed levels around  $0.035\text{ }\mu\text{moles decomposed g glm}^{-1}\text{ min}^{-1}$ , showing that in adequate water availability conditions plants submitted to previous water stress present biochemical levels similar to those of crop plants grown under high soil water availability levels.

With the increase of soil water potential, a quick increase in POD levels was noticed for plants belonging to treatments T1 and T2, which can be observed when such potential is approximately 100 kPa ( $0.28\text{ cm}^3\text{ cm}^{-3}$ ). For treatments T3 and T4 those changes only became evident when the soil water potential dropped to around 400 kPa ( $0.24\text{ cm}^3\text{ cm}^{-3}$ ).

It can be pointed out that the delay in the outset of changes in POD levels for plants in treatment T3, and specially in treatment T4, even under conditions of higher soil water potential (Figure 1), may probably be related to the better conditioning of plants to such conditions. However, it is important to consider the influence of a possible improvement in

the root system of these plants during periods of soil water restriction in the experimental stage 1.



**Figure 1.** Peroxidase activity (POD) in sweet pepper plants during experimental stage 2 – without irrigation (169 to 230 DAT).

$$T1 - Y = [(0.03-0.08)/(1+e^{(x-26.7)/6.64})]+0.09 \quad R^2=0.95^{**}$$

$$T2 - Y = [(0.18-0.08)/(1+e^{(x-20.901)/10.71})]+0.09 \quad R^2=0.96^{**}$$

$$T3 - Y = [(0.39-0.08)/(1+e^{(x-37.476)/4.01})]+0.08 \quad R^2=0.95^{**}$$

$$T4 - Y = [(0.05-0.024)/(1+e^{(x-7.63)/5.26})]+0.024 \quad R^2=0.98^{**}$$

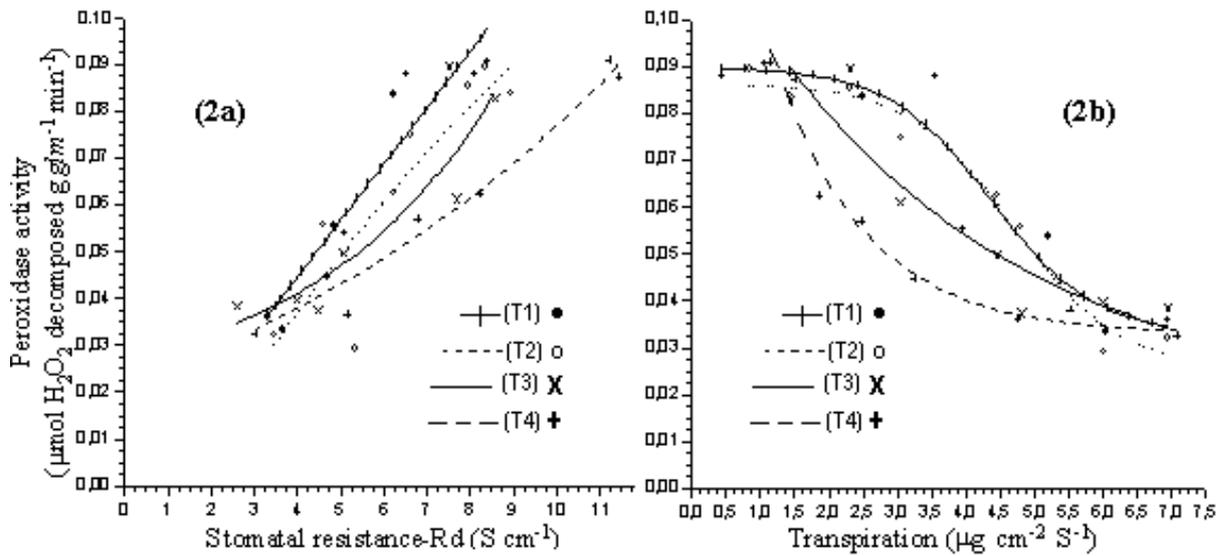
The maximum POD concentration in plants tended to stabilize at around 0.09  $\mu\text{moles decomposed g } g\text{m}^{-1} \text{ min}^{-1}$ . For plants in treatment T1, this situation could be verified when the soil water potential was around 1500 kPa (0.225  $\text{cm}^3 \text{ cm}^{-3}$ ), and for the other treatments when the potential was even higher, specially in treatment T4. Considering the days in which no irrigation was carried out (DWI – days without irrigation), this event was observed at around 38, 46, 46 and 54 DWI for treatments T1, T2, T3 and T4, respectively (Figure 1).

As POD is regarded as a defense metabolic activity against stress, the results evidenced differences among plants, mainly for plants belonging to treatment T4, which showed an increase in POD levels even in extremely severe water stress conditions, which almost did not happen with the other treatments. The results denote that the peroxidase enzyme is related to water stress in sweet pepper plants. Zhang and Kirkhan (1994) reported an increase of peroxidase in tissues of wheat plants experiencing water stress.

In figures 2a and 2b it can be seen that plants in treatments T3 and, specially, T4 showed an association of higher resistance to water vapor diffusion in leaves ( $R_d$ ) and lower transpiration with lower enzyme activity. It evidences that, within a certain limit, plants developed some characteristics related to a better control of water loss, associated with reduced stress levels.

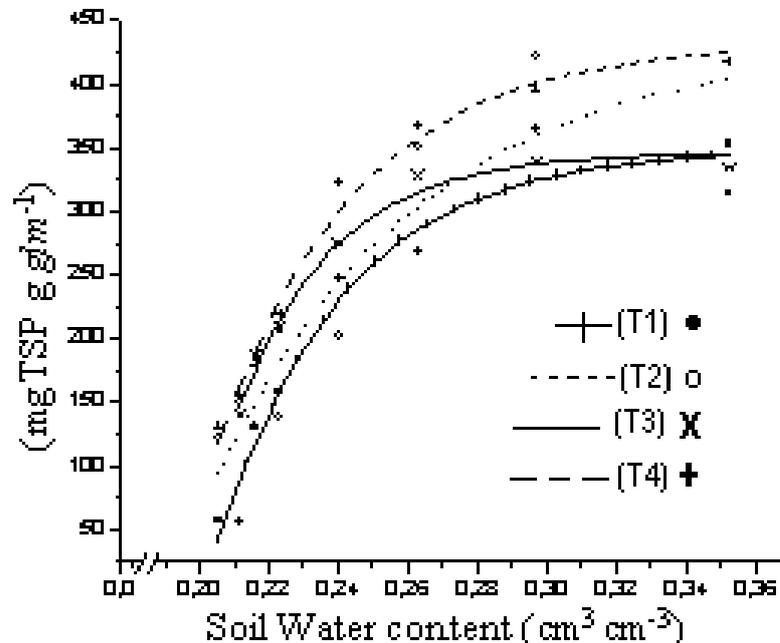
Results obtained from the analysis of total soluble protein (TSP) found in leaves are shown in Figure 3. It can be observed a decrease in TSP levels along with the increase in the water stress experienced by plants. The intensity of such process expresses differences among treatments. However, similarities can be found between variations in TSP and POD levels,

evidencing the presence of higher TSP degradation rates in leaves of plants belonging to treatments T1 and T2.



**Figure 2.** Correlation between peroxidase activity (POD), Rd (2a) and transpiration (2b) in sweet pepper plants during experimental stage 2 (169 to 230 DAT).

T1 - a) $Y = 75.40 + 75.39e^{x/6278}$	$R^2 = 0.88^{**}$	b) $Y = [(0.08 - 0.03) / (1 + e^{(x-4.8/0.5)})] + 0.03$	$R^2 = 0.89^{**}$
T2 - a) $Y = 216 - 0.237e^{-X/14.14}$	$R^2 = 0.82^{**}$	b) $Y = [(0.08 - 0.02) / (1 + e^{(x-4.8/0.7)})] + 0.03$	$R^2 = 0.97^{**}$
T3 - a) $Y = 0.018 + 0.009e^{X/4.30}$	$R^2 = 0.80^{**}$	b) $Y = 62.82 + 62.92e^{-X/6003.4}$	$R^2 = 0.93^{**}$
T4 - a) $Y = 0.0016 + 0.023e^{-X/15.15}$	$R^2 = 0.97^{**}$	b) $Y = 70.02 + 70.12e^{-X/7439.16}$	$R^2 = 0.82^{**}$



**Figure 3.** Variations in total soluble protein (TSP) amounts as a function of the reduction of soil water content during experimental stage 2 (169 to 230 DAT).

T1 - $Y = 349.9 - 84308e^{-X/0.037}$	$R^2 = 0.95^{**}$
T2 - $Y = 431.3 - 10946e^{-X/0.059}$	$R^2 = 0.87^{**}$
T3 - $Y = 346.8 - 371783e^{-X/0.028}$	$R^2 = 0.99^{**}$
T4 - $Y = 432.5 - 53226e^{-X/0.04}$	$R^2 = 0.98^{**}$

The reduction in TSP may initially be imputed to the physiological state of plants, as proteins are one of the factors related to cell growth and development (Lima, 1994). Considering that such reductions are more evidenced in plants badly affected by water stress, the hypothesis of the occurrence of TSP degradation for releasing amino acids is plausible. According to Bray (1993), this is a resource that can be used by plants in an attempt to reduce the cell osmotic potential to increase the absorption ability and water translocation. For Mudgett and Clarke (1994), protein degradation can be seen as an indicative of the stress experienced by plants.

The results presented herein are strong indicatives of the direct correlation between TSP and water stress in plants, although it is necessary to consider that other characteristics related to protein degradation may also have been affected. Mizuno (1994) emphasized the correlations between changes in vegetable regulators and the protein constitution of plants undergoing water stress.

The results evidence that the peroxidase enzyme is related to the stress resulting from water deficiency in plants. Differences observed between plants in treatments T3 and T4 may be attributed to the use of soil covering in treatment T3, as long as we consider that it is an indirect agent, whose presence was responsible for changes in water relations that affected the metabolism in plants. For treatments T1 and T2, the similarities between results are indicatives that, under high soil water availability conditions, the effect of soil covering on the water relations is little expressive for plants.

## 6 CONCLUSIONS

Variations in soil water availability are an important factor that may lead to physiological and biochemical changes in sweet pepper plants.

Successive water stress cycles may promote the development of characteristics that increase resistance and tolerance of sweet pepper plants to low soil water availability.

The peroxidase activity (E C.1.11.1.7) is a variable that can be used to indicate the development of water stress in sweet pepper plants.

## 7 REFERENCES

BRADFORD, M.M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-duepending. **Anal. Biochem.** 72:248-254,1976.

BRAY, E.A. 1993. Molecular responses to water deficit. **Plant Physiol.** V.103 p.1035-1040,1993.

CARVALHO, J.; SANTANA, M.; QUEIROZ, T.M.. Efeitos de diferentes níveis de déficit hídrico e de doses de nitrogênio sobre a produção de pimentão. **Eng. Agríc.**, v.21, p.262-269, 2001.

CHANG, C.J.; KHAO, C.H.. H<sub>2</sub>O<sub>2</sub> metabolism during senescence of rice leaves: changes in enzyme activities in light and darkness. **Plant Growth Reg.**, v.25, p.11-15, 1998.

GASPAR, T.; PENEL, C.; CASTILLO, F.J.. A two step control of basic and acid peroxidase and its significance for growth and development. **Physiol. Plant.** v.64, p.418-423, 1985.

LIMA, J.P.P. *Efeito do cálcio sobre o teor de Poliaminas, Peroxidase e Nitrato redutase em calos de arroz (Oryza sativa L. cv IAC 4440)*. Tese (Doutorado em Agronomia)-Faculdade de Ciências Agrônômicas,UNESP, Botucatu-SP, 1994.

MIZUNO, K. Inhibition of gibberellin-induced elongation, reorientation of cortical microtubules, and change of isoform of tubulin in epicotyl segments of azuki bean protein kinase inhibitors. **Plant Cell Physiol.** v.35, p.1149-1157, 1994.

MUDGETT, M. B.; CLARKE, S. Hormonal and environmental responsiveness of a developmentally regulated protein repair L-isoaspartyl methyltransferase in wheat. **J. Biol. Chem.** v.269, p.25605-25612, 1994.

SIEGEL, B.Z. Plant peroxidases – an organic perspective. **Plant Growth Reg.** v.12, p.303-312, 1993.

ZHANG, J. AND KIRKHAN, M.B. Drought stress induced changes in activities of superoxide dismutase, catalase and peroxidase in wheat species. **Plant Cell Physiol.** v.35,p.785-791, 1994.