ПРОУЧВАНЕ ВЪРХУ АКТИВНОСТТА НА НЯКОИ ЕНЗИМИ ПРЕЗ ПЕРИОДА НА ЗАСУШАВАНЕ И ВЪЗСТАНОВЯВАНЕ НА ДОМАТИТЕ
STUDY ON SOME ENZYME ACTIVITY IN TOMATO PLANTS DURING DROUGHT AND RECOVERY PERIODS

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Резюме

Водният стрес оказва негативен ефект върху зеленчуковите култури, като намалява добива и качеството на плодовете. Променят се също и физиологичните процеси в растенията в резултат на формирането на активни кислородни радикали.

Целта на проучването беше да се проследи реакцията на два сорта домати към окислителен стрес, възникнал в резултат на умерен воден дефицит.

Експериментът е проведен през периода на водния стрес и след възстановяване на растенията от стреса. Установено е, че ензимите GPOD, APOD, SPOD и MDA са особено подходящи индикатори за оценка на толерантността на доматите към воден стрес.

Abstract

Water stress strongly affects horticultural cultivars, reducing the yield and fruit quality. The physiological functions of the plant are also altered by this stress, due to the formation of reactive oxygen species and water interactions.

The study examines the response of two tomato cultivars to oxidative stress generated by moderate water deficit.

Experiments were carried out during the stress period and after recovery. It was observed that the water stress had an inhibitory effect on the physiological state of tomato plants. The *GPOD*, *APOD, SPOD* and *MDA* enzymes were identified as particularly suitable indicators for assessing the tolerance of tomato genotypes to water stress.

Ключови думи: воден стрес, възстановяване, ензими, малондиалдехид.
Key words: water stress, recovery, enzymes, *malondialdehyde*.

Съкращения: ROS - реактивни кислородни видове; GPOD - гваякол пероксидаза; APX - аскорбат пероксидаза; SPOD - сирингалдазин пероксидаза; MDA - малондиалдехид.

Abbreviations: ROS, reactive oxygen species; GPOD, guacol peroxidase; APX, ascorbate peroxidase; SPOD, siringaldazin peroxidase; MDA, malondialdehyde.

INTRODUCTION

Water stress influences plant growth in several ways. For example, shoot biomass significantly decreased in wheat under drought conditions (Loggini et al.,1999). In potato plants, stem length and dry weight diminish under water stress (Ravindra et al., 1991) and (Ierna, 2006), and in the tomato plant, shoot weight and total leaf area were lower than well-watered (Tahi et al., 2008). Also, water deficit diminishes leaf size, longevity, and the number of leaves per plant (Shao et al., 2008; Gerbling et al., 1984).

The damage caused by water stress has two primary causes: first, the formation of reactive oxygen species (ROS) and, second, the alteration of water linkages within the plant. The extent to which plants can avoid or buffer these physiological processes determines the degree of resistance to water stress. Therefore the study of the metabolic and biochemical responses to water deficit is vital to the present-day agriculture in order to select plants with high yield and stability under this type of stress (Yordanov, et al.,2000).

Free ROS attack biological structures, damaging DNA, prompting the oxidation of amino acids and proteins, and provoking lipid peroxidation (Asada, 1999; Johnson, et al., 2003). To avoid such damage, plants have developed ROS-detoxification mechanisms that can be divided into enzymatic and non-enzymatic systems.

The purpose of this study was to examines the response of two tomato cultivars to oxidative stress generated by water deficit and recovery.

MATERIAL AND METHODS

The experiment was carried out on the experimental field of the Institute of Vegetable Crops "Maritsa" – Plovdiv in 2011. The determinant cultivars for medium early field production Marty and Yana were used.

The seeds were planted in the second part of March, with planting norm 1.0-1.5 g/m^{-2}. The seedlings were produced in a non-heated steel and glass greenhouse, on a high flat seed bed, without pricking in. The pricking phase lasted for 45-50 days.

The field experiment was carried out on an area of 100 m^2. The plants were planted on a permanent place on 14 May on a profiled surface – a high flat seed bed, at a planting scheme 120+40x30cm. Each cultivar was planted in two variants – under irrigation and without irrigation, in four repetitions, every 9.6m^2, 30 plants in a repetition. The plants were dried up from the flowering phase until the beginning of ripening and the irrigation norm was reduced to 50%. After 10 days the dried up plants were recovered.

The enzymatic activity was determined spectrophotometrically. Guaiacol peroxidase (GPOD, EC 1.11.1.7) was determined at 436 nm under an established methodology [Bergmeyer, 1974], Syringaldazine peroxidase (SPOD.EC 1.11.1.7) was determined at 550 nm and the enzyme included in the ascorbate-glutate cycle – the ascorbate peroxidase (APOD, EC 1.11.1.11) – at 298 nm (Gerbling et al., 1984). The lipid peroxidase was also determined spectrophotometrically in accordance with the MDA quantity (Heath and Packer, 1968).

RESULTS AND DISCUSSION

The vegetable peroxidases are present in the cell walls, the vacuoles and the cytoplasm. They catalyze the oxidation of various substances through hydrogen peroxide (H_2O_2) or organic hydroperoxides thus decomposing the latter. Guaiacol ($C_7H_8O_2$) is the most widely used donor when determining the general peroxidasic activity of the cells, due to which the so determined activity is called guaiacol-peroxidasic and the enzyme – guaiacol peroxidase (GPOD) (Fig. 1-a).

In the tested tomato genotypes at the beginning of the stress response increase in the activity of the antioxidizing enzymes is observed. A higher activity of GPOD is reported in the leaves of the plants from Marty cultivar – 40% above the control and a lower activity of the enzyme is observed in the leaves of the plants from Yana cultivar – 30%. The obtained results indicate that the applied drought causes serious oxidizing stress. An increased peroxide activity in the plants' leaves in case of water stress is also observed by other authors (Zhang and Kirkham, 1994).

In the period of recovery of the plants from the water stress the data about the enzyme are close to those of the control plants, which is more considerably expressed in Marty cultivar – the results are similar to the control.

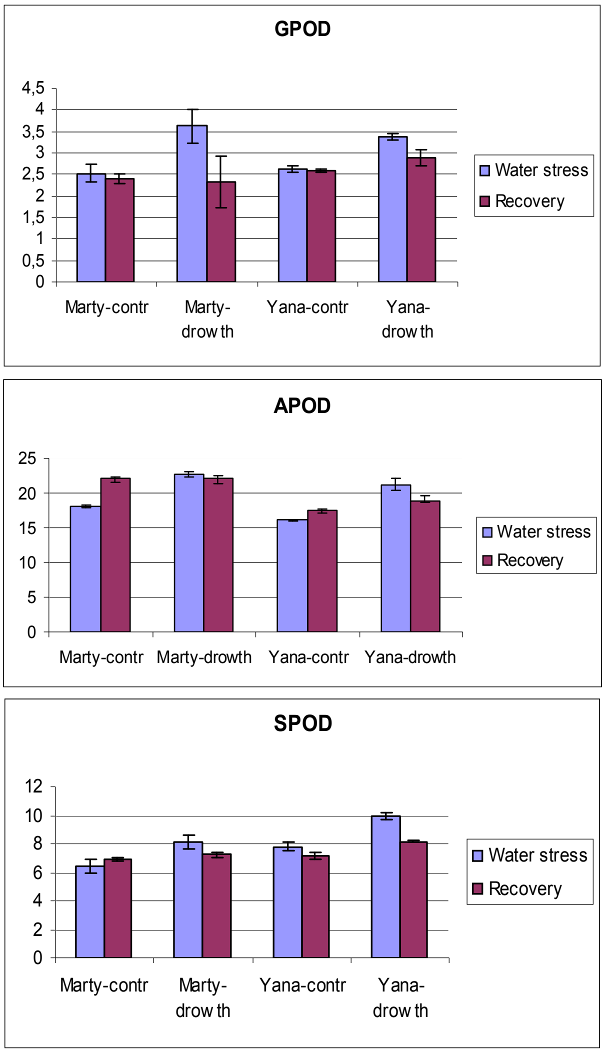
Ascorbate peroxidase (APOD) is important for the elimination of H_2O_2 in the photosynthesizing tissues. It belongs to different classes of the "extinguishing"H_2O_2 enzymes, which show different affinity to peroxide.

The obtained results about the activity of the enzyme ascorbate peroxidase, represented on Fig. 1-b follow the tendency of GPOD, though expressed to a less extent. It is noticed that in conditions of drought the enzymatic activity in the tomato leaves increases, probably under the influence of the increasing damage. At the end of the recovery period the enzymatic activity considerably changes and the values reach those of the control plants. The differences between the two cultivars with regards to APOD are only in the degree of the increased enzymatic activity.

The high level of endogenous APX is essential and effectively maintains the antioxidant system that protects plants from oxidative damage due to biotic and abiotic stresses (Shigeoka et al., 2002). APX activity would increase the demand for AA regeneration. In this context, it is believed that a simultaneous increase in several components of the antioxidative defense system would be necessary in order to obtain increase in the plants' protective mechanisms (Foyer et al., 1994).

Syringaldazine peroxidase (SPOD) is a typical peroxidase of the apoplast, using phenol compounds as an electronic donor. The data about this enzyme are represented on Fig. 1-c. In both tested cultivars the drought causes increase in the SPOD activity. This is more considerably noticed in the leaves of plants from Yana cultivar – 128%. In the recovered plants the SPOD level in the leaves of the same sort remains higher than that of Marty cultivar.

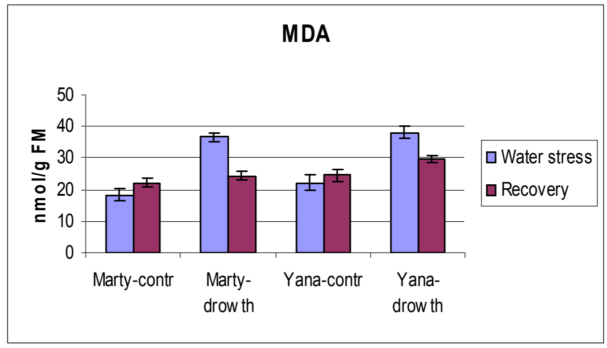
According to some authors (Smeets et al., 2005) SPOD plays an important role in the lignification of the cell wall which can be an important mechanism for adaptation to stress impacts and may lead to reduction of the leaf growth.



Фиг. 1. *Активност на ензимите а) Гваяколпероксидаза (GPOD), b) Аскорбатпероксидаза (APOD) и с) Сирингалдазинпероксидаза (SPOD) в листа от домати, в U/g FW*

Fig. 1. *Activity of a) Guacol peroxidase (GPOD), b) Asorbate peroxidase (APOD) and c) Siringaldazin peroxidase (SPOD) in tomato leaves, in U/g FW*

Upon development of the water stress the quantity of MDA in the plants increases which appears to be a product of the lipid oxidation and is an indicator of the functional activity of the membranes. The results from Fig. 2 show that the MDA level is especially high after the drought period. In Marty cultivar it is almost 100%. In the plants recovering after drought the results remain higher: for Marty cultivar – 100%, and for Yana cultivar – 120%. The MDA results correlate to those for GPOD, as represented on Fig. 1-a. The higher GPOD activity reduces the MDA level. This, according to some authors [Reddy et al., 2004] evidences the clearly expressed properties of



Фиг. 2. *Липидна пероксидация в листа от домати, изразена като съдържание на МДА в nmol/g FM*

Fig. 2. *Lipid peroxidation in tomato leaves, in content of MDA in nmol/g FM*

the enzyme as an antioxidant protection. Other authors associate the lower concentration of MDA with the plants' tolerance (Moran et al., 1994; Sairam et al., 2000). Mitler (2000) suggests that the higher levels of H_2O_2 could accelerate the Haber – Vice reaction process and therefore could stimulate the level of the lipid peroxidase.

Results show that the joint activity of the studied enzymes has an important role for the avoidance of ROS formation and the appearance of damages from oxidizing stress, which on its turn leads to better tolerance to water deficit.

The results from the experiment also show that the plants from various cultivars of one and the same type react in different ways to stress impacts and oxidizing stress. This can be an important criterion for assessment of the cultivar tolerance and for the disclosure of the physiological essence of the tolerance mechanisms in the sustainable cultivars (Reddy et al., 2004).

CONCLUSIONS

Peroxidise enzymes are especially suitable indices for assessment of the tolerance of tomato cultivars to drought.

On the basis of the presented data we can make the conclusion that the tomato plants from Yana cultivar are more tolerant to drought compared to those from Marty cultivar, which are more sensitive but are able to recover faster after drought.

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