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*J. Plant Biol.*, Vol. 27 (1), April, 2000, pp. 39–42.


ARTICLE

# Water Stress Effects on Membrane Damage and Activities of Catalase, Peroxidase and Superoxide Dismutase Enzymes in Black Pepper (*Piper nigrum* L.)

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*MS received 22 November 1999; accepted 22 February 2000*

**An experiment was conducted in black pepper to investigate the effect of water stress on membrane damage, relative water content and the activities of catalase, peroxidase, superoxide dismutase and polyphenol oxidase enzymes. Water stress was imposed by withholding irrigation and the treatment continued till plants started showing wilting symptoms. Membrane damage increased and relative water content decreased with stress intensity. Catalase and superoxide dismutase showed decreased activity under stress while peroxidase and polyphenol oxidase showed increased activity. The results suggest the possibility of utilizing some of these parameters as a measure of water stress tolerance.**

***Keywords:*** Water stress, relative water content, cell membrane damage, catalase, peroxidase, superoxide dismutase, polyphenol oxidase, black pepper.

## Introduction

Black pepper is a spice of great economic importance. The export of the spice depends upon its production. Many factors influence production, among which soil moisture plays an important role. This is more so in black pepper because more than 90% of the area under black pepper is rainfed. The crop suffers from acute soil moisture shortage during summer months leading to a fall in productivity. Hence investigating the physiological and biochemical changes that occur during water stress may help in understanding the effect of water stress. This would further help in identifying the genotypes/accessions tolerant to water stress.

Various physiological and biochemical parameters such as membrane leakage (Blum and Ebercon, 1981), relative water content (Rahman *et al.*, 1999), enzyme activities, viz. catalase, superoxide dismutase, peroxidase (Pederson and Aust, 1973; Kellog and Fridovich, 1975; Chempakam *et al.*, 1993) and polyphenol oxidase and lipid peroxidation (Chempakam *et al.*, 1993) have been evaluated to assess the relative tolerance of crops to water stress. This paper aims to investigate whether some of these parameters could be utilized to assess the relative tolerance of black pepper accessions to water stress.

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## Materials and methods

Ten black pepper accessions were selected for the study. Ten cuttings each of the selected accessions were raised in pots containing forest soil, sand and farm yard manure in 3:1:1 proportion and grown in pots for 3–4 months with normal irrigation. Once they attained sizeable growth, water stress was imposed by withholding irrigation. The stress treatments continued till the plants started showing wilting symptoms. Observations on relative water content (RWC), membrane leakage, enzyme activities such as catalase, peroxidase, superoxide dismutase (SOD) and polyphenol oxidase (PPO) were recorded on day 4th and day 8th after stress imposition.

## Preparation of the enzyme extract

The procedure used by Dhindsa *et al.* (1981) was followed with minor modifications. Matured leaves were collected and cleaned thoroughly by rubbing with cotton to remove the particles adhering to the leaf surface. One gram of the leaf material was ground under ice cold conditions in 3 ml of 0.1 M sodium phosphate buffer (pH 7.2) containing 1% insoluble PVP and 0.1% sodium metabisulphite. The homogenate was filtered through four layers of cheese cloth and then centrifuged at 10,000 rpm for 20 min. The supernatant was directly used for enzyme assay. An aliquot of the extract was used to determine protein content.

*Enzyme activity determination*

Catalase was assayed by measuring the initial rate of disappearance of hydrogen peroxide according to Dhindsa *et al.* (1981). SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) according to Dhindsa *et al.* (1981). Peroxidase activity was measured according to Putter (1974). Polyphenol oxidase activity was measured according to the method of Kar and Mishra (1976). RWC and membrane leakage were determined as per the standard procedures.

## Results and discussion

Relative water content decreased and membrane leakage increased due to water stress in different black pepper genotypes (Table 1). The reduction in relative water content ranged from 4.7 (acc 1466) to 12% (acc 1411) after 4 days of stress and from 16% (acc 1466) to 46.3% (acc 1411) after 8 days of stress. Similarly, the increase in membrane leakage ranged from 35% (acc 1368) to 83.4% (acc 1409) after 4 days of stress and from 105% (acc 1368) to 253.5% (acc 1411) after 8 days of stress. In general, acc 1466 and 1368 maintained both lesser reduction in RWC and lesser increase in membrane leakage compared to the other accessions.

Figure 1 shows the activity of catalase as affected by water stress. Catalase activity decreased with stress intensity in all the accessions. Significant differences among accessions were recorded for catalase activity only after 8 days of stress. Lowest reduction in catalase activity was recorded by acc 1466 (79.8%) followed by acc 1421 (80.4%) and highest decrease was noticed in acc 1390 (90.5 peroxidase activity increased with stress intensity in all the accessions (Figure 2)). Highest increase was noticed in acc 1409 (111.7%) after 8 days of stress. Enzyme activity among accessions differed during both 4 and 8 days after stress induction.

PPO activity also showed an increase with stress intensity. The enzyme activity among accessions differed only after 8 days of stress (Table 2).

The highest increase was noticed in acc 1411 (42.5%) and the lowest in acc 1409 (24.8%) followed by acc 1466 (28.6%). SOD activity showed a general

**Table 1.** Relative water content and cell membrane leakage of black pepper accessions as affected by moisture stress.

| Accession no. (Genotype) | Relative water content (% decrease over control) | | Cell membrane leakage (% increase over control) | |
|---|---|---|---|---|
| | 4 days after stress | 8 days after stress | 4 days after stress | 8 days after stress |
| 1371 | 8.7 | 34.5 | 69.4 | 201.8 |
| 1377 | 9.6 | 37.5 | 38.7 | 107.4 |
| 1411 | 12.0 | 46.3 | 82.5 | 253.5 |
| 1421 | 11.9 | 46.1 | 76.3 | 223.4 |
| 1466 | 4.7 | 16.0 | 40.1 | 117.5 |
| 1409 | 11.0 | 41.9 | 83.4 | 238.3 |
| 1368 | 5.9 | 20.2 | 35.0 | 105.0 |
| 1373 | 9.7 | 37.0 | 70.0 | 206.7 |
| 1394 | 8.3 | 30.6 | 36.7 | 108.0 |
| 1390 | 4.9 | 16.9 | 60.4 | 178.9 |
| CD ($P < 0.01$) | 1.9 | 4.7 | 12.3 | 20.1 |

**Table 2.** Superoxide dismutase and polyphenol oxidase activities as affected by moisture stress.

| Accession no. (Genotype) | SOD activity (% decrease over control) 4 days after stress | SOD activity (% decrease over control) 8 days after stress | PPO activity (% increase over control) 4 days after stress | PPO activity (% increase over control) 8 days after stress |
|---|---|---|---|---|
| 1421 | 7.8 | 11.7 | 21.4 | 41.4 |
| 1466 | 12.8 | 16.8 | 17.5 | 28.6 |
| 1409 | 4.4 | 14.7 | 18.4 | 24.8 |
| 1368 | 9.7 | 10.0 | 22.5 | 39.8 |
| 1373 | 4.5 | 10.8 | 14.5 | 36.4 |
| 1394 | 7.6 | 18.4 | 21.6 | 38.7 |
| 1390 | 3.8 | 7.60 | 24.5 | 41.5 |
| 1371 | 11.8 | 12.1 | 19.4 | 30.5 |
| 1377 | 1.4 | 13.8 | 27.8 | 32.6 |
| 1411 | 5.4 | 14.4 | 21.4 | 42.5 |
| CD ($P < 0.01\%$) | NS | NS | NS | 4.2 |

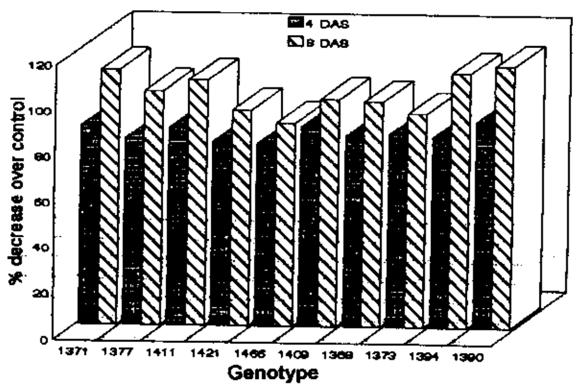


**Figure 1.** Catalase activity as affected by moisture stress.

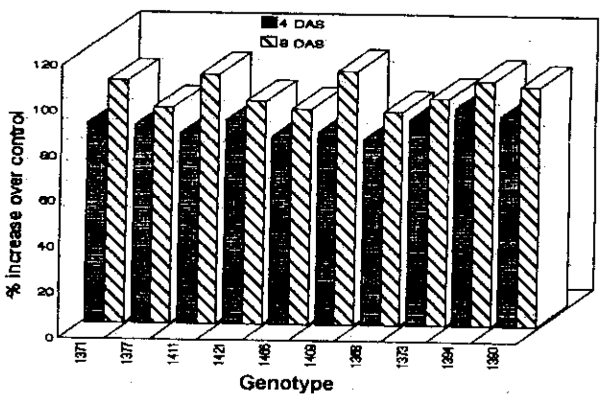


**Figure 2.** Peroxidase activity as affected by moisture stress.

decrease with stress intensity and the activity among accessions was on par both during 4 and 8 days after stress induction. The decrease was lowest in acc 1377 (1.4%) followed by acc 1390 (3.8%) after 4 days of stress. The highest decrease was noticed in acc 1394 (18.4%).

The environment under which the plant is growing has a major role on its growth and productivity. Among these, availability of water is the major factor influencing the growth, development and productivity. RWC has been in use to screen varieties/germplasm for drought tolerance. Stomatal conductance, transpiration rate, photochemical efficiency and RWC declined during drought progression in fescue and the severity of reduction varied with cultivars (Huang and Gao, 1999). Water stress reduced water potential of tomato leaves and the RWC followed the similar pattern as that of leaf water potential (Rahman *et al.*, 1999). RWC is related to the water potential of the same tissue though the relationship is dependent on species and stages of growth. Large variation existed among black pepper accessions for RWC, especially after 8 days of stress induction. This makes RWC a suitable parameter for screening black pepper germplasm. Membrane leakage is known to increase under water stress. Black pepper also shows similar response (Krishnamurthy *et al.*, 1998). A wide variation was noticed among black pepper genotypes for this character and moreover, membrane damage showed very high correlation with drought tolerance suggesting the usefulness of this parameter in screening germplasm. Blum and Ebercon (1981) suggested the usefulness of cell membrane stability as a measure of drought and heat tolerance in wheat.

Table 3. Correlation between membrane leakage and catalase, peroxidase and SOD activities.

| | r-value Membrane leakage |
|---|---|
| Catalase | −0.436* |
| Peroxidase | 0.518** |
| Superoxide dismutase | −0.315 |

*Significant at $P < 0.05$, **significant at $P < 0.01$.

It has been reported that membrane damage is directly linked with the activity of catalase and SOD. Activities of SOD and catalase can determine the abundance of $O_2^-$, $H_2O_2$, $OH^\cdot$ and $O_2$ which control lipid peroxidation (Dhindsa *et al.*, 1981). Though the lipid peroxidation level has not been measured in the present study, it has been shown in many plant species that lipid peroxidation levels increase during water stress (Chempakam *et al.*, 1993; Dhindsa *et al.*, 1982; Chowdhury and Choudhuri, 1985). Decreased activities of SOD and catalase result in increased levels of $O_2^-$, $H_2O_2$, $OH^\cdot$ and $O_2$ in the tissue. This in turn increases the level of lipid peroxidation. However, Rahman *et al.* (1999) recorded increased activity of SOD during water stress and suggested the possibility of using this as a criterion for drought tolerance. In the present study, both SOD and catalase showed a negative correlation with membrane leakage though the correlation between SOD and membrane leakage was non-significant (Table 3). On the other hand, peroxidase showed significant positive correlation with membrane leakage suggesting that the peroxidase activity may be an indicator of the extent of membrane damage in black pepper.

Polyphenol oxidase enzyme activity was found to be higher during water stress. Up to 30% increase was noticed in some accessions. The genotype 1466 which maintained higher RWC and lesser membrane damage during stress also maintained relatively lesser increase in PPO and lesser decrease in catalase and slightly higher activity of SOD than the rest. On the other hand, the genotype 1409, which showed greater decrease in RWC and greater increase in membrane leakage, showed a significant decrease in catalase and increase in PPO activities. It has been reported in coconut that the activity of SOD was higher in tolerant varieties and that of PPO was higher during stress in susceptible varieties (Chempakam *et al.*, 1993). The same appears to be true in black pepper also.

Peroxidase enzyme activity increased by around 80–110% in all the accessions and all of them showed similar activity levels (non-significant difference in activity among the accessions). The activity of the enzyme must have increased because of higher substrate levels as the breakdown of $H_2O_2$ by catalase was reduced under stress. More studies are needed on the coordination of these enzyme activities and also the regulation of these enzyme activities under various kinds of stresses and during attack by pests and pathogen.

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