

PROLINE ACCUMULATION UNDER PEG INDUCED WATER DEFICIT STRESS  
IN THE LEAF DISCS OF SELECTED BLACK PEPPER (PIPER NIGRUM L.)

T. VARGHESE THOMAS\*, T. JOHN ZACHARIAH\* and A. RAMADASAN\*

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ABSTRACT

Proline accumulation potential of ten selected cultivars of black pepper (Piper nigrum L.) was studied in isolated leaf discs under water deficit stress of -2.5 MPa using PEG (Polyethylene glycol) 6000 solution. Accumulation of proline was higher and earlier in cultivars Kottanadan and Neelamundi when compared with unstressed control. Maximum decline in the accumulated proline during water stress relief was observed in cultivars Neelamundi, Kottanadan and Aimpiriyan in decreasing order.

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Accumulation of free proline in Plant tissues has been reported in response to stress factors including drought and other pathological conditions (Aspinal and Paleg, 1981). Even though a negative correlation contradicts the proline concept on drought tolerance, the functional role of proline in the osmoregulation and energy conservation during any kind of stress is not ruled out (Stewart, 1981). An attempt has been made in the present study to characterise ten selected cultivars of black pepper (Piper nigrum L.) in relation to their proline accumulation capacity using leaf discs floated on -2.5 MPa PEG (Polyethylene glycol) 6000 as osmoticum to induce water deficit stress.

Leaf discs of youngest fully opened leaves collected from two year old vines of ten black pepper cultivars grown in well watered tubs were used for this experiment. Leaf discs of 2 cm diameter weighing 70 mg/disc were floated on 30 ml PEG 6000 solution (-2.5 MPa)

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\*National Research Centre for Spices, Marikunnu, P.O., P.B.No. 1701, Calicut-673 012, Kerala, India. Contribution No. 125



in a petridish. Ten leaf discs of each cultivars were withdrawn from the petridishes at 0,4,6,8,12 and 24 hr incubation period. The leaf discs were floated to remove PEG and the proline content was estimated by the method described by Bates *et al.* (1973). The level of accumulation of proline during each period of osmotic stress is presented in Fig.1.

The proline content in all the pepper cultivars increased significantly over the unstressed control(0h). The time course of proline accumulation shows that rate of accumulation of proline was maximum in cvs. Kottanadan and Neelamundi at the end of 6 h stress period. The increase in proline content was over 4 to 6 fold over unstressed control (Fig.1). In cv. Narayakodi and Panniyur-I the maximum proline content was registered at the 4th h and the remaining cultivars at 6th and 8th h stress period. Floating of leaf discs in desired osmoticum to induce water deficit stress has been suggested as a method to counter correlative influence of intact plants on proline accumulation (Stewart, 1981). Studies in excised leaves in other plants showed that proline level continued to increase and reached the maximum level during 24-48 h after imposition of stress and declined thereafter (Roy Chowdhury and Choudhury,1986). This was attributed to the metabolic consequences of excision (Stewart and Hanson,1980). In pepper an early and high accumulation of proline during water stress and decline thereafter could be due to the differential sensitivity of the plant to PEG induced stress.

The leaf discs after 6 h stress induction period were floated on distilled water for 6 h and proline at the zero h were estimated. This was done to analyse the capacity of the pepper cultivars in recouping after the removal of stress. The data on the percentage decline in proline level is presented in Table 1.

It was observed that in cvs. Kottanadan, Neelamundi and Aimpiriyan the percentage decline in the accumulated proline were 66%, 73% and 56% respectively. Among the remaining cultivars the decline in proline level ranged from 3 to 37%. The capacity to accumulate proline at high water deficit stress and oxidize it after the release of the stress could be considered as desirable characters for drought resistance.



FIG. 1. PROLINE ACCUMULATION IN BLACK PEPPER (*PIPER NIGRUM* L.) LEAF DISCS UNDER WATER DEFICIT STRESS (-2.5 MPa POLY-ETHYLENE GLYCOL 6000 SOLUTION).



Table 1. Percentage decline in the level of accumulated proline after the release of induced water deficit stress (-2.5 MPa PEG 6000) after 6 h\*

Cultivars	% Proline depletion
Karimunda	22 ± 3
Kottanadan	66 ± 2.5
Neelamundi	78 ± 2
Thommankodi	37 ± 1.5
Naranyakodi	08 ± 1
Kuthiravalli	11 ± 2.5
Kalluvally	15 ± 3
Arakulamunda	07 ± 2
Aimpiriyan	56 ± 4
Panniyur - I	33 ± 2.5

\* Average of six replications.

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