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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation　Amelioration of short-term drought stress in *Brassica* seedlings by exogenous application of salicylic acid, ascorbic acid, jasmonic acid and trehalose: A key role of antioxidant defense and glyoxalase systems

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Drought stress responses and enhancement of drought tolerance by the application of different exogenous protectants were studied in *Brassica* seedlings considering the antioxidant defense system, glyoxalase system and physiological adaptations of plants by conducting four experiments.

In our first experiment, the protective role of salicylic acid (SA) in relation to the water status, chlorophyll (chl) content, antioxidant defense and glyoxalase system was investigated in drought stressed mustard (*Brassica juncea* L. cv. BARI Sharisha 11) seedlings. Two sets of 10-d-old seedlings were subjected to two different levels of drought (10% and 20% PEG, 48h), where one set of seedlings was supplemented with 50 µM SA. The relative water content (RWC), chl *b* and chl (*a*+*b*) decreased at any level of drought, while chl *a* content decreased only at severe drought (20% PEG). Drought stress caused a sharp increase in proline (Pro) content. A sharp increase in malondialdehyde (MDA) and H_2O_2 was observed in both levels of stresses. Decline in AsA content and increase in glutathione (GSH) and glutathione disulfide (GSSG) content was observed at both levels of drought. Compared to control, the activities of catalase (CAT) and monodehydroascorbate reductase (MDHAR) did not change due to drought stress. The activity of glutathione reductase (GR) slightly increased only at 10% PEG, while ascorbate peroxidase (APX) and glutathione *S*-transferase (GST) activity increased at any level of stress. The activities of glutathione peroxidase (GPX) and glyoxalase II (Gly II) decreased only at severe stress (20% PEG), while dehydroascorbate reductase (DHAR) and glyoxalase I (Gly I) activities decreased at any level of stress. Spraying with SA alone had little influence on the non-enzymatic antioxidant and the activities of antioxidant enzymes. However, supplementation of SA in drought stressed seedlings increased the RWC and chl content, increased the AsA and GSH, decreased the GSSG content and maintained a higher ration of GSH/GSSG. Salicylic acid supplemented drought stressed seedlings also enhanced the activities of MDHAR, DHAR, GR, GPX, CAT, Gly I, and Gly II as compared to the drought-stressed plants without SA supplementation, with a concomitant decrease in H_2O_2, and lipid peroxidation level. These results suggest that the exogenous application of SA assisted the plants to become more tolerant to drought stress-induced oxidative damage by enhancing their antioxidant

defense and glyoxalase systems.

In our second experiment, the roles of ascorbic acid (AsA, 1 mM) under osmotic stress (induced by 15% polyethylene glycol, PEG-6000) were investigated by examining morphological and physiological attributes, antioxidant defense, and glyoxalase system in *Brassica* species. Osmotic stress reduced fresh and dry weight, leaf RWC, chl content; increased Pro, MDA, H_2O_2 contents and lipoxygenase (LOX) activity. Ascorbate content in *B. napus, B. campestris* and *B. juncea* decreased, increased and remained unaltered, respectively. Dehydroascorbate (DHA) content increased only in *B. napus*. The AsA/DHA ratio was reduced by osmotic stress in all species except *B. juncea*. Osmotic stress increased GSH content only in *B. juncea*, increased GSSG content and decreased GSH/GSSG ratio in all species. Osmotic stress increased activities of APX (except in *B. napus*), GR (except in *B. napus*), GST (except in *B. juncea*), and GPX, and decreased activity of CAT (in all species) and decreased MDHAR activity (only in *B. campestris*). Osmotic stress decreased Gly I and Gly II activities. Addition of AsA addition in combination with PEG improved fresh weight, RWC and chl, decreased Pro, MDA, H_2O_2. It improved AsA-GSH cycle components, improved activities of all antioxidant and glyoxalase enzymes in most of the cases. So, exogenous AsA improved physiological adaptation and alleviated oxidative damage under osmotic stress by improving the antioxidant and glyoxalase systems in all species with *B. napus* showing greater antioxidant capacity. The *B. juncea* can be recognized as naturally drought tolerant cultivar, because in response to osmotic stress and exhibited the least damage effects including the oxidative damage. The performance of *B. juncea* was also better in response to exogenous AsA addition under osmotic stress.

In our third experiment, the ability of jasmonic acid (JA) to enhance drought tolerance in different *Brassica* species in terms of some physiological parameters, antioxidants defense and the glyoxalase system. Ten-day-old seedlings were drought stressed by supplying 15% PEG either alone or in combination with 0.5 mM JA. Drought stress significantly increased the oxidative stress as indicated by increased levels of MDA and H_2O_2 and higher LOX activity. Drought stressed plants had reduced seedling biomass, chl content, leaf RWC. Other signs of drought stress were higher levels of Pro, DHA, and GSSG. Drought stress affected different species differently: in *B. napus,* CAT and Gly II activities decreased, while GST and GPX activities increased in drought-stressed compared to unstressed plants; in *B. campestris,* activities of GR, Gly I, GST, and GPX increased, while activities of MDHAR, DHAR, CAT and other enzymes decreased; in *B. juncea,* activities of APX, GR, GPX, and Gly I increased, Gly II activity decreased and other activities did not change. Spraying drought-stressed seedlings with JA increased GR and Gly I activities in *B. napus*; increased MDHAR activity in *B. campestris*; and increased DHAR, GR, GPX, Gly I and Gly II activities in *B. juncea* seedlings. Jasmonic acid improved fresh weight, chl, and leaf RWC in all species, while dry weight increased

only in *B. juncea*. *Brassica juncea* showed the lowest oxidative stress under drought stress, indicating its natural drought tolerance capacity. Addition of JA improved the drought tolerance of *B. juncea* to the highest level among the studied species.

In our fourth experiment, Comparative responses of three *Brassica* species including *B. napus, B. campestris* and *B. juncea* under polyethylene glycol induced drought stress and the protective effects of exogenous Trehalose were investigated. Although drought reduced fresh, dry weight, chl contents; increased Pro content and oxidative stresses (LOX activity, MDA, H_2O_2 contents) along with altered antioxidant and glyoxalase systems in all *Brassica* species, *B. juncea* seems to be the most drought tolerant species showing the least oxidative damage due to enhancement of some non-enzymatic and enzymatic antioxidants. Combination of Tre and drought improved performance of all species, but responses were different. In *B. juncea*, combination of Tre with drought improved seedlings' fresh weight, dry weight, leaf RWC, chl *a*, chl *b*, AsA, GSH contents, AsA/DHA (ratio of AsA and dehydroascorbate) and GSH/GSSG (reduced to oxidized GSH) ratios; enhanced APX, DHAR, GR, GPX and Gly II activities; reduced MDA, H_2O_2, Pro content, LOX activity. *Brassica napus* seedlings with Tre addition under drought showed improved seedlings' fresh weight, dry weight, GSH/GSSG ratio; upregulated CAT, GST, Gly I activities; reduced MDA, H_2O_2 contents and LOX activity. In *B. campestris* Tre supplementation with drought improved fresh weight, RWC, chl *a*, chl *b*, chl *(a+b)* contents; AsA/DHA ratio, MDHAR activity. The results suggest that *B. juncea* is naturally drought tolerant species and moreover, its drought tolerance capability is further enhanced by exogenous Tre application.

The results suggest that salysilic acid, ascorbic acid, jasmonic acid and trehalose are effective phytoprotectants those conferred drought stress tolerance in *Brassica* species as they efficiently reduced oxidative stress by improving antioxidant and glyoxalase system which helped to improve the physiological adaptations to a great extent and to improve the drought tolerance.