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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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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
(サリチル酸、アスコルビン酸、ジャスモン酸、トレハロースの外部投与によるアブラナ属実生における短期乾燥ストレスの改善：抗酸化防御系とグリオキサラーゼ系の役割)

学位論文要約：
Dissertation Summary

The effect of drought stress in different species of *Brassica* seedlings was studied. The roles of exogenous application of salicylic acid, ascorbic acid, jasmonic acid and trehalose in improving drought stress tolerance of *Brassica* species were also studied considering the growth parameters, physiological attributes, antioxidant defense and glyoxalase systems.

Considering the adverse effect of drought stress which is one of the most severe abiotic stresses several experiments were conducted. The background of the study has been described here. Drought has been distinguished to cause substantial growth reduction and crop failure. Impairment of physiological and biochemical processes, such as transportation water, ion and nutrients; stomatal movement, transpiration, photosynthesis, respiration, translocation of assimilates are also common under drought stress. Like other abiotic stresses, drought stress results in generation of other reactive oxygen species, ROS (superoxide, $O_2^{\bullet-}$; hydrogen peroxide, H_2O_2; hydroxyl radicals; OH•, etc.) causing severe oxidative damages to cellular and sub-cellular components. Plants have well organized antioxidant defense system comprising of non-enzymatic and enzymatic components which can efficiently and readily detoxify ROS. The enzymatic antioxidant defense mechanisms are represented by the enzymes, superoxide dismutase (SOD); 4 enzymes of the ascorbate-glutathione cycle: ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR); catalase (CAT); glutathione peroxidase (GPX); and glutathione S-transferase (GST). Non-enzymatic antioxidants include carotenoids, ascorbte (AsA), glutathione (GSH), tocopherol, flavanones, anthocyanins etc. Without the ROS, methylglyoxal (MG) is another cytotoxic compound, production of which is increased many times under abiotic stresses including drought stress and through glyoxalase system, plants detoxify MG. In plants, MG is detoxified mainly by the maintenance of GSH homeostasis via glyoxalase system which comprises 2 enzymes, namely, glyoxalase I (Gly I) and glyoxalase II (Gly II). Gly I converts MG to S-D-lactoylglutathione (SLG), utilizing GSH, while Gly II converts SLG to D-lactic acid. During the latter reaction, GSH is regenerated. Enhanced antioxidant and glyoxalase system can prevent oxidative damage effects of ROS and MG on biomolecules, enzymes, lipid, protein for which plants' physiological processes and structural components are prevented. That is why, both the antioxidant and glyoxalase system are necessary to obtain substantial tolerance against oxidative stress induced by abiotic stresses including drought.

Uses of hormones, growth regulators or signaling molecules are now being popular to enhance abiotic stress tolerance in plants. Salicylic acid (SA) is an endogenous growth regulator having vital roles in physiological processes both in normal growing conditions and in abiotic stress conditions. Treatment of drought stressed plants

with exogenous SA was found to be effective in modulating physiological processes, both enzymatic and non-enzymatic components of antioxidant defense system. Salicylic acid can modulate other phytohormones, osmoregulators, performing a signal actions those made it potent to counteract drought stress.

Ascorbate (AsA), the vital non-enzymatic antioxidant is effective and indispensible in ROS detoxification system. Endogenous AsA has been implicated in the promotion of plant growth and development by involvement in a complex and enigmatic array of phytohormone-regulated signaling networks that ties together different environmental stresses. Enhanced synthesis of AsA enhances drought tolerance in plants. Exogenous application of AsA influences the antioxidant defense system and plants' biochemical processes and confers abiotic stress tolerances including drought.

Jasmonic acid (JA) is a plant-signaling molecule having wide range of plant responses that has effects on morphological to molecular level. Cell division, plant growth, stomatal conductance and photosynthetic processes are diversely affected by JA. Jasmonic acid is also actively involved in drought related gene expression. Foliar application of JA was modulate plant physiology towards development of drought stress tolerance or other abiotic stress tolerances.

Trehalose (Tre) (an organic compatible solute) is a non-reducing disaccharide of glucose that stabilizes biological structures and macromolecules like proteins and membrane lipids during dehydration and other abiotic stress conditions. Over production of Tre in transgenic crop plants enhanced abiotic stress tolerance. Exogenously applied trehalose also plays significant roles as osmoprotectant and improves stress tolerance.

Brassica juncea, *B. napus* and *B. campestris* belong to family Brassicaceae are important oil yielding crops cultivated widely throughout the world as source of edible oil production. *Brassica* stands second position after soybean. These three species are the most popular cultivated *Brassica* species in many countries of the world including Bangladesh. The different species of the *Brassica* are different in terms of some phenotypic, physiological characteristics or growth habits. *Brassica* species are often face drought stress and the species have different drought tolerance capacity. So, the present study explores the differences among these species under drought stress. Numerous research studies exist on the plant responses and tolerance to drought stress, but comparative studies on the effects of drought stress on different *Brassica* species are not remarkable. Studies on exogenously applied phytohormones (SA and JA), antioxidant (AsA) and osmoprotectant or compatible solutes (Tre) to enhance drought stress tolerance in different *Brassica* species are also scare. Considering the above mentioned aspects, studies were undertaken to investigate the physiological and biochemical responses of different *Brassica* species under drought stresses; to investigate the possible biochemical mechanisms of SA, JA, AsA and Tre- induced drought stress tolerance in *Brassica* species and to investigate the roles of antioxidant defense system and glyoxalase system in conferring drought stress tolerance in *Brassica* plants.

The first experiment investigates the protective role of salicylic acid (SA) 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. Drought stress resulted in a sharp increase in lipid peroxidation (melondealdehyde, MDA which is a product of lipid peroxidation) and H_2O_2 contents, compared to control. The SA supplemented drought-stressed seedlings showed significantly lower MDA and H_2O_2 content, compared to drought exposed seedlings without SA. Leaf relative water content (RWC) of mustard seedlings was decreased significantly upon exposure to drought stress. The drought-stressed seedlings sprayed with SA showed 40 and 50% increase in RWC at 10 and 20% of PEG, respectively compared to the drought imposed seedlings which were not sprayed with SA. In this study, chlorophyll *a* content of the leaves did not changed under 10% PEG. However, it decreased significantly under 20% PEG. The SA treated control seedlings also showed decrease in Chl *a* content. Compared to drought stress alone, the seedlings exposed to 20% PEG supplemented with SA spray showed higher Chl *a* content. The Chl *b* content also significantly decreased under any level of drought stress. However, the seedlings sprayed with SA showed significantly higher amount of Chl *b* content compared to drought stress alone. Drought stress caused a profound increase in Pro content in mustard seedlings. On the other hand, SA supplemented drought stressed (20% PEG only) seedlings maintained the Pro content significantly lower than the seedlings treated with PEG only. Significantly declined AsA content was observed in drought stressed mustard seedlings, compared to control and AsA was restored in SA supplemented drought-stressed seedlings, compared to the drought stressed seedlings without SA. The GSH contents were increased under drought stress (compared to control) and SA supplemented drought-stressed seedling showed further increase in GSH content, compared to drought stress alone. The GSSG content markedly increase with increasing levels of drought, compared to control and then reduced by SA supplementation with drought stress. The GSH/GSSG ratio showed no change under mild stress (10% PEG), while it significantly decreased upon exposure to severe drought stress (20% PEG). However, SA supplemented drought stressed seedlings showed significantly improved GSH/GSSG ratio. Compared to control the activities of CAT and MDHAR did not change due to drought stress. The activity GR increased only at 10% PEG, while APX and GST activity increased at any level of stress. The activities of GPX and Gly II decreased only at severe stress (20% PEG), while DHAR) and glyoxalase I Gly I activities decreased at any level of stress. 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.

The first experiment was conducted with *B. juncea* which is one of the most popular *Brassica* species of Bangladesh and recognized as the most drought tolerant. In the later experiments, comparative studies among three most popular *Brassica* species (*B. napus*, *B. campestris* and *B. juncea*) were executed under drought stress. The roles of different protectants, such as, ascorbic acid, jasmonic acid and trehalose were studied in different species under drought stress.

The second experiment was conducted to investigate the roles of ascorbic acid (AsA, 1 mM) in osmotic stress (induced by 15% polyethylene glycol, PEG-6000) affected *Brassica* species (*B. napus*, *B. campestris* and *B. juncea*) by examining morphological and physiological attributes, antioxidant defense, and glyoxalase system in *Brassica* species. Osmotic stress resulted in significant decreases in seedling fresh weights and dry weight of all *Brassica* species compared to the unstressed control seedlings. Exogenous application of AsA in combination with osmotic stress resulted in increase of seedling fresh weight. The leaf RWC was reduced in osmotic-stressed *B. napus*, *B. campestris*, and *B. juncea*, respectively, compared to the unstressed controls. Exogenous application of AsA in combination with osmotic stress resulted in noticeable improvements in RWC content in all species, when compared to the seedlings grown under osmotic stress alone. The Pro content was noticeably increased in all three *Brassica* species upon exposure to osmotic stress. Addition of exogenous AsA in combination with osmotic stress resulted in decrease of Pro levels, compared to drought stress alone. The chl contents in *Brassica* seedling leaves were significantly reduced by osmotic stress, irrespective of seedling species. Addition of AsA in combination with osmotic stress prevented the loss of chl *a* content in all three species, while loss of chl *b* was prevented only in *B. campestris*. The chl (*a*+*b*) content was improved in all three species when AsA was added. Osmotic stress significantly increased oxidative stress as indicated by higher MDA, H_2O_2 levels and LOX (lipoxygenase) activity in all three, compared to control. In case of exogenous AsA application in osmotic-stressed seedlings, MDA, H_2O_2 levels and LOX activity contents decreased significantly than the seedlings treated with PEG alone. The endogenous AsA content of *B. napus* seedlings remained unchanged under osmotic stress, but the addition of exogenous AsA in combination with osmotic stress resulted in significantly enhanced AsA content. The DHA content was markedly increased in *B. napus* exposed to osmotic stress, but was unchanged in *B. campestris* and *B. juncea*. The combination of exogenous AsA and osmotic stress resulted in a reduction in DHA content in *B. napus* and in *B. juncea*, compared to the osmotic-stressed condition alone. The AsA/DHA ratio was decreased significantly by osmotic stress in both *B. napus* and *B. campestris*, compared to their respective unstressed controls, but was unchanged in *B. juncea*. When compared to osmotic stress alone, the addition of AsA in combination with osmotic stress improved the AsA/DHA ratios all *Brassica* species. The GSH content was enhanced in osmotic-stressed *B. napus*, *B. campestris*, and *B. juncea*, respectively, compared to unstressed controls. Addition of exogenous AsA in combination with osmotic stress further increased GSH levels in *B. napus and B. campestris*, but no additional effect was observed in *B. juncea*. The GSSG content was increased in osmotic-stressed seedlings of all *Brassica* species, compared to unstressed controls. Addition of AsA in combinations with osmotic stress resulted in decreased GSSG content in all three species, with the greatest decrease seen in *B. juncea*. The ratio of reduced to oxidized glutathione GSH/GSSG showed a decreasing trend under osmotic stress, which was abolished by the combination of AsA addition and osmotic stress. 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 CAT (in all species) and decreased MDHAR activity (only in *B. campestris*). Osmotic stress decreased Gly I and increased Gly II activity. Addition of AsA in combination with PEG improved AsA-GSH cycle components, improved activities of all antioxidant and glyoxalase enzymes in most of the cases. *B. napus* seedlings improved their antioxidant system better in response to exogenous AsA, compared to other species. Drought stress induced damage effects were lesser in *B. juncea*, compared to other species.

In the third experiment, the roles 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 elevated the MDA, H_2O_2 contents and LOX activities in all *Brassica* species compared to control. Compared to drought treatment alone, MDA, H_2O_2 contents and LOX activities reduced significantly in all species in JA combined drought treatment. The fresh weights of the seedlings of three studied species were decreased significantly by drought stress. Exogenous application of JA with drought stress improved the fresh weight and dry weight of seedlings, compared to seedlings compared to drought stress alone. Leaf RWC significantly decreased by drought stress (compared to unstressed control seedlings). Application of JA in combination with drought stress improved leaf RWC in all *Brassica* species. Drought stress resulted dramatic increase in Pro contents of all the studied species compared to control seedlings and the Pro levels were significantly reduced in all species (except for *B. campestris*) by JA addition with drought stress. The chl contents were decreased significantly in all the studied *Brassica* species under drought stress. However, in JA supplemented drought stressed seedlings chl *a* content improved in *B. campestris* and in *B. juncea* seedlings, chl *b* content improved in *B. napus* and *B. campestris* seedlings and chl (a+b) content increased in all the seedlings, compared to drought affected seedlings alone. The AsA content of *B. campestris* seedlings was decreased and AsA content of *B. juncea* was increased under drought stress, compared to control seedlings. Drought did not show any effects on AsA pool of *B. napus* seedlings. The DHA level of *B. napus* increased by drought stress and in other species drought induced increase of DHA content was not significant statistically. The ratio of AsA/DHA reduced markedly by drought stress in *B. napus* and *B. campestris* compared to control seedlings. Exogenous JA application with drought stress retrieved the AsA level only in *B. juncea* and this treatment reduced the DHA level significantly in all species (compared to control seedlings). The JA application with drought stress improved

AsA/DHA ratio in all studied species, compared to the drought treated seedlings alone. The GSH levels were increased in *B. campestris* and *B. juncea* seedlings under drought stress, compared to control seedlings. Sharp increases of oxidized glutathione, GSSG levels were recorded in all species compared to control seedlings. Drought stress also reduced the GSH/GSSG in all seedlings. Addition of JA with drought stress reduced GSSG contents and enhanced the GSH/GSSG, compared to drought treatment alone. Drought stress affected antioxidant components of 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. *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.

The fourth experiment investigates comparative responses of three *Brassica* species including *B. napus, B. campestris* and *B. juncea* under polyethylene glycol (PEG 15%) induced drought stress and the protective effects of exogenous trehalose (5 mM). Drought stress caused decreases in fresh and dry weights of seedlings of all *Brassica* compared to control seedlings. Exogenous application of Tre in combination with drought stress increased fresh weights and dry weights for *B. napus*, and in *B. juncea* but not for *B. campestris* compared to drought stress alone. The leaf RWC of *Brassica* seedlings were decreased significantly under drought stress, compared to unstressed control seedlings. Combination of Tre and drought stress increased leaf RWC of all species except *B. campestris*. Drought stress substantially increased Pro contents in leaves of of all *Brassica* spp comparison with the control seedlings. The Pro levels were significantly reduced in *B. campestris* and *B. juncea* seedlings by combination of Tre and drought stress. Chlorophyll *a* and chl *b* contents decreased in all *Brassica* species under drought stress which contributed to reduction in chl (*a*+*b*). As compared to drought treatment alone, Tre combined with drought stress resulted in increased chl contents in *B. campestris* and *B. juncea* seedlings. Oxidative stress induced by drought was evident in all *Brassica* species as indicated by the higher MDA, H_2O_2 levels and LOX activities, compared to non-stress control. In contrary, Tre supplementation reduced the oxidative stress or MDA, H_2O_2 levels and LOX activities significantly, compared to drought stress alone. Drought stress caused a significant decrease in AsA content of *B. campestris* seedlings but resulted in increased contents of AsA in *B. juncea* seedlings compared to control seedlings. Under water stress DHA contents *B. juncea* remained unchanged whereas those of *B. napus* and *B. campestris* increased. The ratio of AsA/DHA declined remarkably in *B. napus* and *B. campestris* but marginally increased in *B. juncea* under drought stress compared to control seedlings. Compared to drought stress alone, exogenous Tre application in combination with drought stress increased AsA level in *B. campestris* and slightly in *B. juncea.* Under the drought condition DHA level was significantly decreased in all species except for *B. napus* (compared to control seedlings). Again, as compared to drought stress alone, exogenous Tre addition with drought treatment improved AsA/DHA ratio in *B. campestris* and in *B. juncea* and in *B. napus* it was decreased. Drought stress significantly increased glutathione (GSH) levels of all *Brassica* spp seedlings, compared to control seedlings. There were significant increases in oxidized glutathione, GSSG levels in all species. Drought stress decreased the ratios of GSH/GSSG in seedlings of *Brassica napus* but remained unchanged in *B. campestris* and *B. juncea* seedlings. Addition of Tre with drought stress reduced GSSG contents and enhanced the GSH/GSSG in seedlings of all species except that of in *B. campestris*, compared to drought treatment alone. The APX activity increased in *B. campestris* and *B. juncea* under drought stress. Drought decreased MDHAR activity only in *B. campestris* as compared to control seedlings. Drought resulted in significant increase of DHAR activity in *B. napus* seedlings and significant decrease of DHAR activity in *B. campestris* seedlings, compared to control. Exogenous Tre in combination with drought stress *B. juncea* seedlings caused a significant increase in DHAR activity, compared to drought stressed seedlings alone. As compared to control seedlings, increased GR activities were observed in all three species under water stress. The GR activity was further increased only in *B. juncea* in Tre combined with water stress as compared to control or water stress treatments. There were significant increases in GST activities in all *Brassica* spp seedlings under drought stress as compared to control seedlings Tre in combination with drought resulted in a significant decrease in GST activity in *B. compestris* and *B. juncea* as compared to the control or drought seedlings. Drought resulted in increase of GPX activity in all *Brassica* spp seedlings, as compared to control seedlings. Addition of Tre with drought stress resulted in further increase of GPX activity only in *B. juncea* seedlings, compared to drought seedlings. Drought stress increased SOD activity in *B. juncea* seedlings, while in the other two *Brassica* species SOD activities did not change, compared to control seedlings. Exogenous Tre maintained the same SOD activities all species except in *B. napus* where SOD activity decreased. Drought stress decreased the CAT activity in *B. napus* and *B. campestris* whereas in *B. juncea* its activity increased, compared to the control seedlings. Trehalose treatment increased CAT activity only in *B. napus*. Drought stress significantly increased Gly I activities of all *Brassica* species compared to control seedlings. On the other hand, Gly II activities decreased under drought stress. Tre in combination with drought stress enhanced Gly I activity in *B. napus* only and in other species its activity was as high as drought treatment compared to drought stressed seedlings alone. In contrary, Tre improved the Gly II activity only in *B. juncea* under drought stress. An increase in MG contents under drought stress occurred in all species with the highest increase in *B. campestris*. As compared to water stress only, Tre addition with drought stress decreased MG contents in all species. Briefly, in *B. juncea*, combination of Tre with drought improved seedlings' APX, DHAR, GR, GPX and Gly II activities. *Brassica napus* seedlings with Tre addition under drought showed upregulated CAT, GST, Gly I activities. In *B. campestris* Tre supplementation with drought improved MDHAR activity. The *B. juncea* showed the least damage effects under drought stress and its performance, antioxidant capacity, glyoxalase system and physiological performance were also improved by exogenous Tre application.

The results of the experiments suggest that the *B. juncea* can be recognized as naturally drought tolerant cultivar, because in response to drought stress it exhibited the least damage effects, such as: the least oxidative damage and methylglyoxal production, the least reduction of growth parameters and chlorophyll content, and higher enhancement of antioxidant components, compared to other species. The results also suggest that salicylic acid, ascorbic acid, jasmonic acid and trehalose are effective phytoprotectants those conferred drought stress tolerance in *Brassica* species as they efficiently reduced oxidative stress and detoxified methylglyoxal by improving antioxidant and glyoxalase system those helped to improve the physiological adaptations to a great extent and to improve the drought tolerance. As a protectant, ascorbic acid is more efficient as almost all the antioxidant and glyoxalase system components were enhanced in all species by exogenous application of ascorbic acid with drought stress. The present study considered sort duration drought stress under laboratory environment. The performance of different *Brassica* species under different drought stress levels in field condition will explore more authentic results. The signaling roles of different protectants, biotechnological approaches to utilize these protectants under drought stress might be further recommended studies.