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

氏名：
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Physiological and Biochemical Mechanisms of Polyamines-Induced Abiotic Stress Tolerance in Mung Bean (*Vigna radiata* L.): Study on Osmoregulation, Ion Homeostasis, Metal Detoxification, Antioxidant Defense and Glyoxalase Systems

学 位 論 文 題
目：
Title　　　of
Dissertation

（ヤエナリにおいてポリアミンにより誘導される非生物的ストレス耐性の生理・生化学的メカニズム：浸透圧調節、イオン恒常、金属解毒、抗酸化防御およびグリオキサラーゼ系に関する研究）

学位論文要約：
Dissertation Summary

Background of the study

Plants are subjected to various environmental stresses due to constant climate change that prevent them from reaching their full genetic potential and limit their productivity (Hasanuzzaman et al. 2012a). Crop plants, as sessile organisms, encounter unavoidable abiotic stresses during their life cycles, including salinity, drought, extreme temperatures, heavy metal toxicity, flooding, UV-B radiation, ozone, etc., all of which pose a serious challenge to plant growth, metabolism, and productivity (Fig. 1) (Ahmad and Prasad 2012a,b; Hasanuzzaman et al. 2012a). Worldwide, abiotic stresses were mentioned to reduce the overall yield of crops and plants by more than 50% (Acquaah 2007) and for only staple food crops yield reduction was recorded up to 70% (Thakur et al. 2010). Thus, abiotic stresses exert great threat for food security and for others basic needs of human.
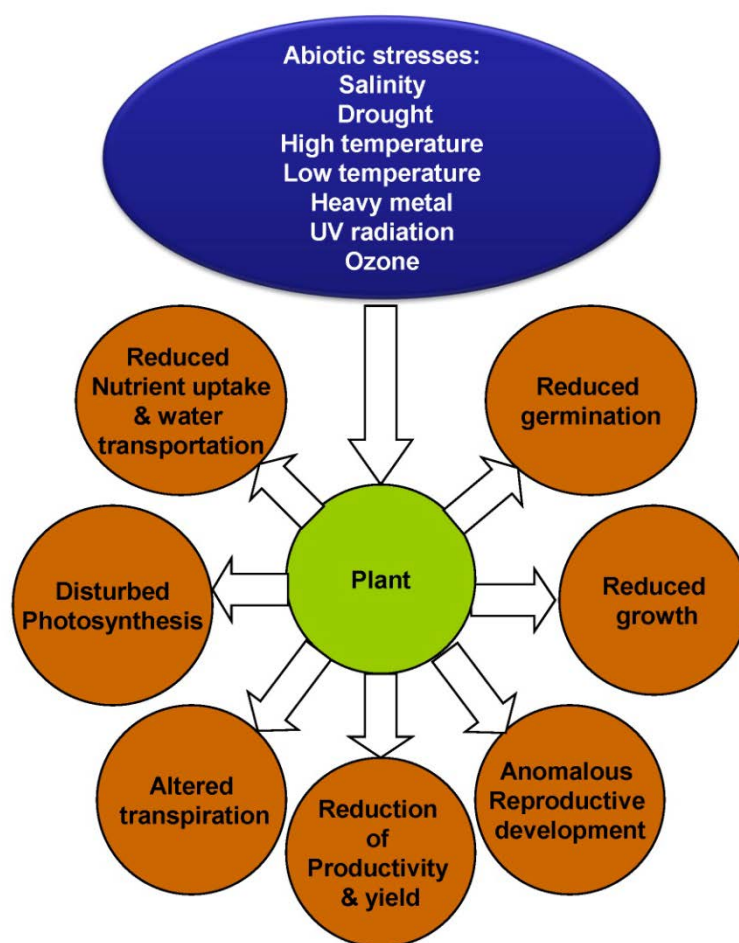
Fig. 1: Common effects of major abiotic stresses on plant

Depending upon stress type and duration; plant type, cultivar, growth stage with various other factors together act to alter the physiological processes, adversely affect growth, development. Stresses affect water and nutrient uptake and transportation, photosynthesis, respiration, transpiration, dry matter partitioning, alter phenology; altogether severely affect the yield and production of plants. (Yadav et al. 2005b; Hasanuzzaman et al. 2012c). Although the pattern of these events varied widely, there are some common effects at cellular and molecular level. These stresses cause imbalance in enzyme activity, even inactivation, ionic imbalance, accumulation of toxic ions and compounds. Production of reactive oxygen species (ROS) is one of the most common obvious effects of stresses those generally derived by high-energy exposure or electron-transfer reactions from reduction of molecular oxygen (O_2) (Gill and Tuteja 2010; Hasanuzzaman et al. 2012a). Major ROS may include oxygen centered radicals: Superoxide anion ($O_2^{\bullet-}$), hydroxyl radical (OH•), alkoxyl radical (RO•), peroxyl radical (ROO•); oxygen centered non- radicals: hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$). Nitrogen species: nitric oxide (NO•), nitric dioxide ($NO_2^{\bullet-}$), peroxynitrite ($OONO-$) are also considered as ROS (Fig. 2) doing harm to plants (Gill and Tuteja 2010; Hasanuzzaman et al. 2012a,c).
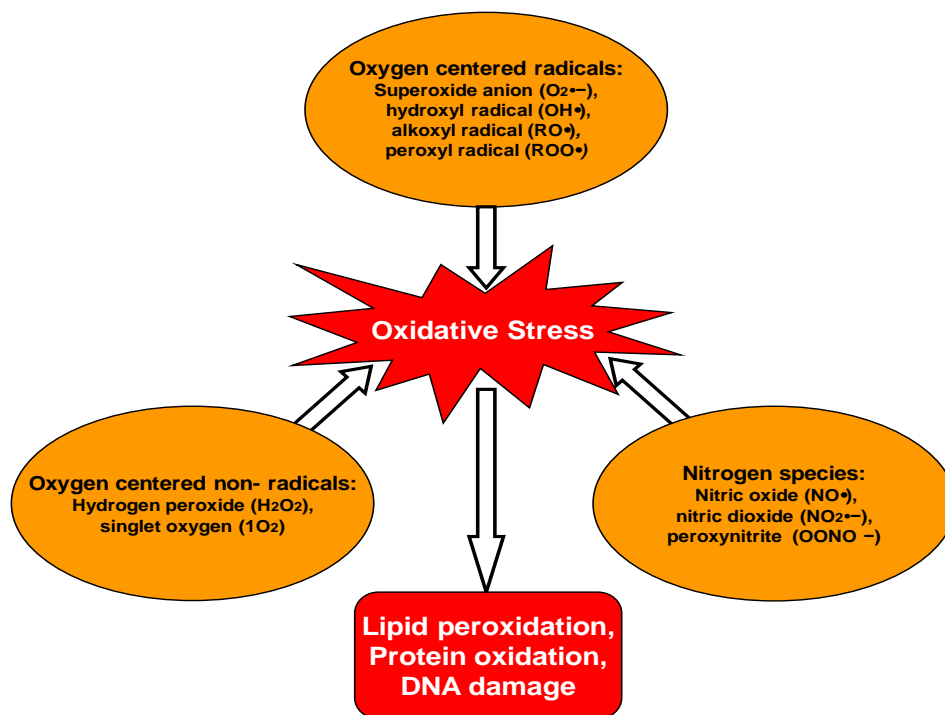
Fig. 2: Contributors of oxidative stress and its effects in plant

To protect plant cells from oxidative damage a steady state between ROS and antioxidant defense (Fig. 3) is required. Plants possess an efficient non-enzymatic (ascorbate, AsA; glutathione, GSH; α-tocopherol; phenolic compounds, alkaloids and non-protein amino acids) and enzymatic (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione reductase, GR; glutathione peroxidase, GPX; glutathione *S*-transferase, GST and peroxidases, POX) antioxidant defense systems which work in concert to control the cascades of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging ROS (Gill and Tuteja 2010; Fig. 3). These antioxidant defense systems are found in almost all cellular compartments (Fig. 3), demonstrating the importance of ROS detoxification for cellular survival. These defenses are not restricted to the intracellular compartment, but are also found in the apoplast to a limited extent (Gill and Tuteja 2010).
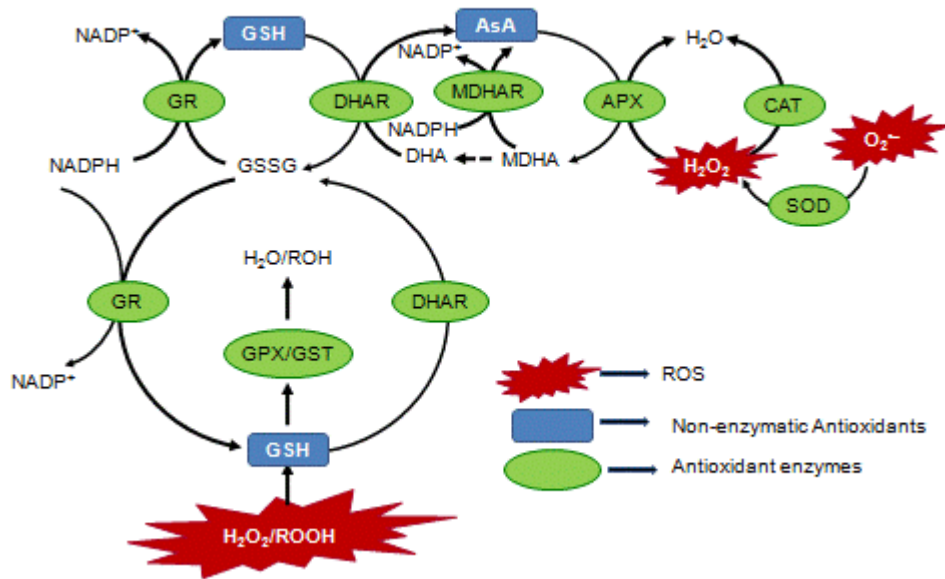
Fig. 3: Antioxidant defense system

Methylglyoxal (MG) is a toxic compound that builds up under different abiotic stress (Wang et al., 2009; Desai et al., 2010). In plants, MG can be detected under normal growth conditions, but it accumulates at higher levels under various environmental stresses. There are several reports indicating the overproduction of MG under abiotic stress (Yadav et al. 2008). In plants, MG is detoxified mainly via the glyoxalase system (Fig. 4), which comprises two enzymes, glyoxalase I (Gly I) and glyoxalase II (Gly II). Gly I converts MG to *S*-D-lactoylglutathione, utilizing GSH, while Gly II converts *S*-D-lactoylglutathione to D-lactic acid. During the latter reaction, GSH is regenerated. Both ROS and MG can cause oxidative stress and cause substantial damage through peroxidation of lipids, oxidation of protein and inhibit inactivation of enzymes. These may clog up membrane structure and function, cause ion leakage, deterioration of ultrastructural molecules and organelles, DNA damage or even plant death (Hasanuzzaman et al. 2012c). Considering the sternness of ROS and MG, protection of plant cell from their adverse affects are considered as noteworthy strategy for conferring tolerance against the abiotic stresses and plants have acquired an efficient antioxidant defense system constituting of antioxidant enzymes and non-enzymatic components and MG detoxification system those together effort to protect plant cells from oxidative damage (Yadav et al. 2005b; Gill and Tuteja 2010, Hasanuzzaman et al. 2012c).
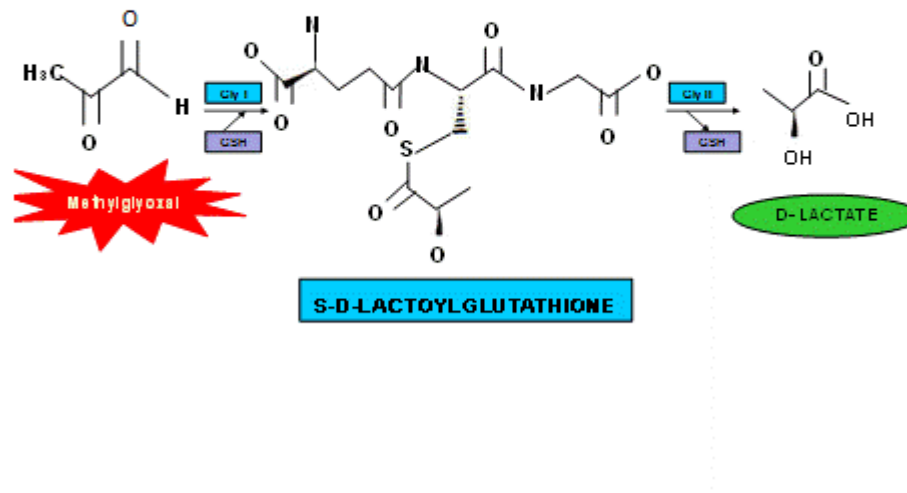
Fig. 4: Glyoxalase system

Antioxidant system and glyoxalase system are vital to reduce ROS and MG generation and are necessary to for enhancing tolerance against oxidative stress (El-Shabrawi et al. 2010; Hasanuzzaman et al. 2011b). Numerous research findings reported that induction and regulation of the antioxidant and glyoxalase pathway enzymes increase oxidative stress tolerance. Thus, enhancement of one or several components of antioxidant and glyoxalase system or over-expression of those enzymes conferred different abiotic stress tolerances by reducing oxidative damages in several crops (Yadav et al. 2005b; Gill and Tuteja 2010; Hasanuzzaman et al. 2012c; Nahar et al. 2015a, b, c).

Since starting crop or plant culture practices, human being is always trying to improve the productivity. Moreover, with the altered plant growth and environmental conditions and increasing environmental pollution with rising population increasing plant productivity has become one of the incredible challenges (Thakur et al. 2010). Increasing plant productivity is generally complex process as regulated by multiple genes. Plant stress tolerance is also a multigenic and quantitative in nature (Collins et al. 2008). Immense challenge subsists in understanding the key molecular mechanisms underlying stress tolerance. Sauntering through this path towards development of high yielding crop varieties is extremely tough but not impossible which have been already proved for many crop or plant species (Collins et al. 2008). Traditional plant breeding approaches have been marginally successful in improving the tolerances to these stresses as many of the molecular mechanisms underlying abiotic

stress tolerances in plants are being unrevealed still now (Sanchez et al. 2011). Various approaches are being used to observe the responses towards better plant performances in recent times. Use of exogenous protectants for abiotic stress tolerances is one of them (Hasanuzzaman et al. 2012c).

Polyamines (PAs) are low molecular mass aliphatic amines and organic polycations found in a wide range of organisms from bacteria to plants and animals (Alcázar et al. 2006a). They were first reported more than 300 years ago in human spermatozoa (van Leeuwenhoek 1678). The biosynthesis of PAs in plants involves several reactions involving various enzymes. The biosynthesis pathway (Fig. 5) of polyamine is directly overlapping with arginine and ethylene and arginine being the precursor of both PAs and nitric oxide (NO).
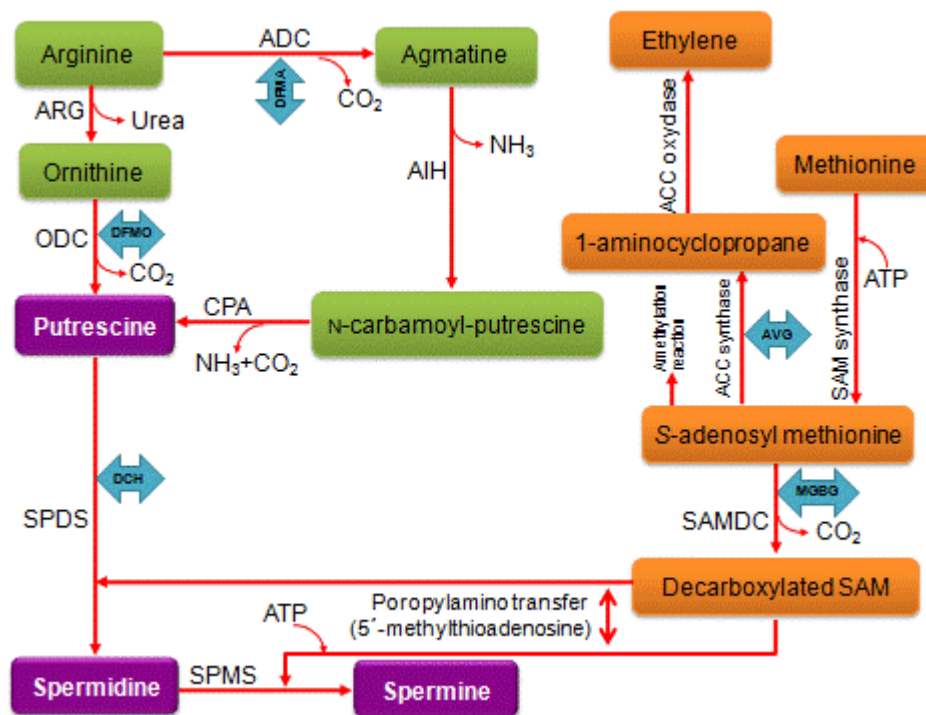

Fig. 5: Biosynthesis pathway of polyamine. Abbreviations: ACC− Acetyl-CoA carboxylase; ADC− arginine decarboxylase; AIH− agmatine iminohydroxylase; ARG− arginase; ATP− Adenosine-5'-triphosphate; AVG− aminoethoxyvinylglycine; CPA− *N*-carbamoylputrescine amidohydrolase; DCH− dicyclohexylamine; DFMA− difluoromethyl arginine; DFMO− difluoromethyl ornithine; MGBG− methylglyoxal bis (guanalhydrazone); ODC− ornithine decarboxylase; SAMDC− *S*-adenosylmethionine decarboxylase; SPDS− spermidine synthase; SPMS− spermine synthase

The levels of PAs in plant cells depend on transport, degradation and conjugation. The major and free

PAs in plants are diamine putrescine (Put), triamine spermidine (Spd) and tetraamine spermine (Spm). They are involved in various processes (Fig. 6) such as cell proliferation, somatic embryogenesis, seed germination, growth, morphogenesis, differentiation, development of flowers and fruits and programmed cell death (Gill and Tuteja 2010; Hussain et al. 2011).
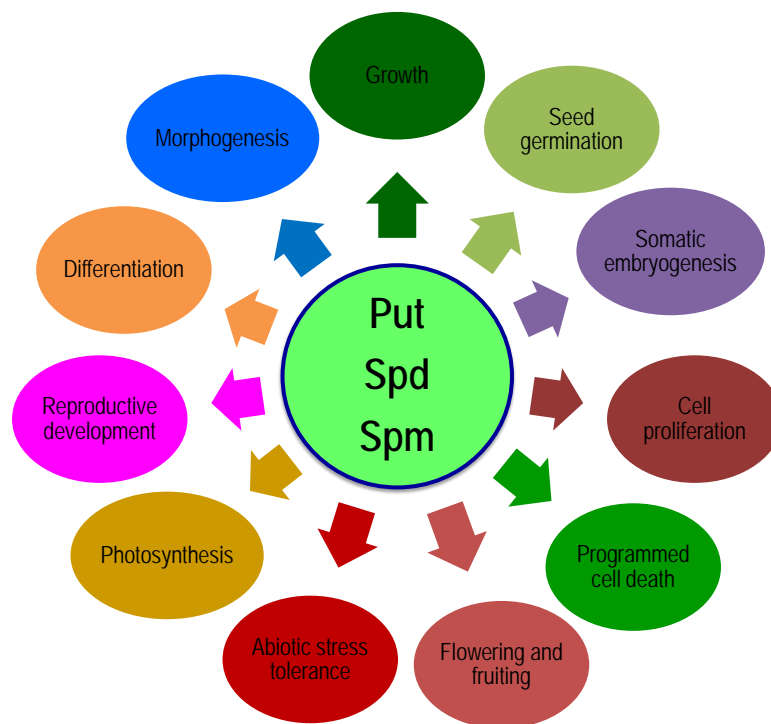

Fig. 6: Multiferous roles of PAs in plants' developmental processes and abiotic stress tolerances

These PAs also play important roles in plants' responses to abiotic stress. Previous findings demonstrating that an accumulation of the three main PAs occurs under many types of abiotic stresses (Hussain et al. 2011; Nahar et al. 2015 d-f, 2016a, b). Several plant studies have also revealed that increased levels of PAs through biosynthesis or exogenous application confers tolerance to salinity (Kuznetsov and Shevyakova 2010), drought (Yamaguchi et al. 2007; Yang et al. 2007), heavy metals (Groppa and Benavides 2008) and extreme temperature (Cheng et al. 2009). Due to their cationic nature at physiological pH, they can interact with negatively charged macromolecules such as membrane phospholipids, DNA and proteins. Thus, PAs are also involved in the stabilisation of the membrane or DNA structure and in protein conformation. In addition, they regulate the activity of many enzymes such as antioxidant enzymes and H^+-ATPase under stressful conditions (Duan et al. 2008).
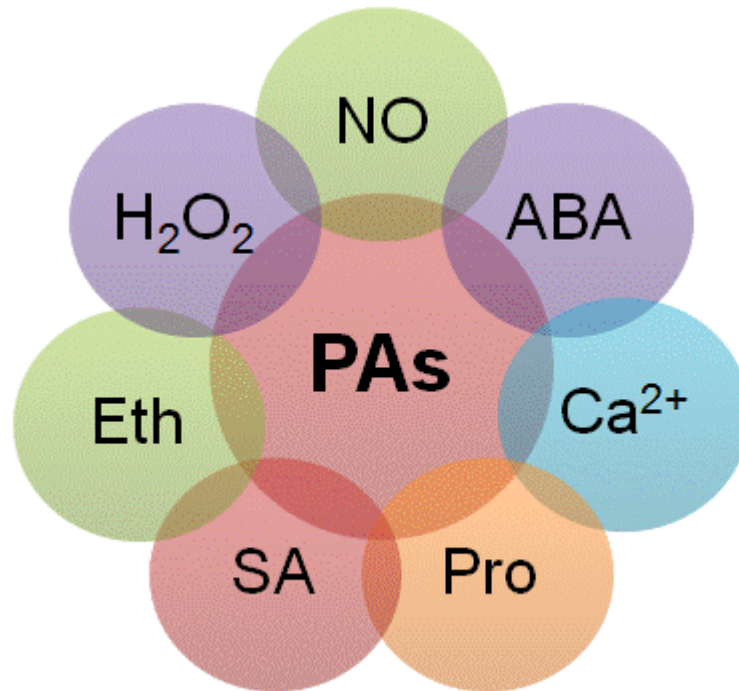
Fig. 7: Polyamines and other associated molecules interacted during various plant processes

Polyamines also interact with other phytohormones such as abscisic acid (ABA), ethlyne (Eth), auxin, cytokinin (CTK) and with some signalling molecules such as nitric oxide (NO) and hydrogen peroxide (H_2O_2) in the regulation of stress responses (Fig. 7). Numerous reports have indicated that enhanced levels of endogenous PAs upregulate the antioxidant defence system in plants, thereby mitigating abiotic stress-induced oxidative stress. In plants, exogenous PAs have also been found to have positive effects under various kinds of environmental stresses (Hasanuzzaman et al. 2014). Moreover, several transgenic approaches have shown enhanced stress tolerance in plants that overexpress genes that regulate PA biosynthetic enzymes (Alcázar et al. 2010a; Wang et al. 2011a,b).

In contrast to number of publications on the plant responses and tolerance to abiotic stress, there is a remarkable lack of knowledge on the coordinated effect of PAs on non-enzymatic and enzymatic regulation in response to abiotic stresses. Considering all aspects, studies were undertaken with the following objectives:

1) To investigate the physiological and biochemical responses of mung bean seedlings under different abiotic stresses
2) To investigate the possible biochemical mechanisms of PAs- induced abiotic stress tolerance in

mung bean seedlings

3) To investigate coordinated interaction between the antioxidant defense system and glyoxalase system in conferring abiotic stress tolerance in plants

To fulfil these objectives, several experiments were conducted and the findings are described in six different titles.

1) Polyamines confer salt tolerance in mung bean (*Vigna radiata* L.) by reducing sodium uptake, improving nutrient homeostasis, antioxidant defense and methylglyoxal detoxification systems

2) Insights into spermine-induced combined high temperature and drought tolerance in mung bean: osmoregulation and roles of antioxidant and glyoxalase system

3) Exogenous spermidine alleviates low temperature injury in mung bean (*Vigna radiata* L.) seedlings by modulating ascorbate-glutathione and glyoxalase pathway

4) Physiological and biochemical mechanism of spermine-induced cadmium tolerance in mung bean (*Vigna radiata* L.) plant

5) Polyamine and nitricoxide crosstalk: Antagonistic effects on cadmium toxicity in mung bean plants through upregulating the metal detoxification, antioxidant defense and methylglyoxal detoxification systems

6) Mechanism of polyamine-induced aluminium toxicity tolerance in mung bean plants: A study on antioxidant defense and methylglyoxal detoxification systems

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Experiment 1

Polyamines confer salt tolerance in mung bean (*Vigna radiata* L.) by reducing sodium uptake, improving nutrient homeostasis, antioxidant defense, and methylglyoxal detoxification systems

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Materials and Methods

Plant material, growth condition, and treatments

Mung bean (*Vigna radiata* L. cv. BARI Mung-2) seedlings were grown in petri dishes under the conditions: light, 350 μmol photon m^{-2} s^{-1}; temperature, 25±2°C; relative humidity, 65–70%; 10,000-fold diluted Hyponex solution (Hyponex, Japan) was applied as nutrient. Six-day-old seedlings (three sets) were exposed to salt stress (NaCl, 200 mM). Three sets of 5-day-old seedlings were grown with Put (0.2 mM), Spd (0.2 mM) and Spm (0.2 mM) solution as pre-treatment for 24 h. These pre-treated seedlings were then exposed to the same level of salt stress on day six. Control seedlings were grown with Hyponex solution. Another three sets of seedlings were grown with Put, Spd and Spm without any stress. Data were taken after 48 h. There were three replicates per treatment.

Determination of Physiological and Biological parameters

Different physiological and biochemical parameters including Na content, nutrient contents (such as, K, Ca, Mg and Zn) contents, dry weight (DW), leaf relative water content (RWC) of leaf, chlorophyll (chl) content, proline (Pro) content, MDA content, H_2O_2 content, $O_2^{\bullet-}$ generation rate, histochemical detection of ROS (H_2O_2 and $O_2^{\bullet-}$) in leaf, lipoxygenase (LOX) activity, non-enzymatic antioxidants (ASA and GSH content), enzymatic antioxidants (activities of APX, MDHAR, DHAR, GR, SOD, CAT, GPX and GST), MG content glyoxalase system components (activities of Gly I and Gly II), endogenous free PAs contents were assayed according to the standard methods.

Statistical analysis

All data obtained were subjected to analysis of variance (ANOVA) and the mean differences were compared by Tukey's HSD (honest significant difference) test using XLSTAT v. 2015.1.01 software. Differences at $P \leq 0.05$ were considered significant.

Results and summary

The physiological roles of PAs (Put, Spd and Spm) were investigated for their ability to confer salt tolerance (200 mM NaCl, 48 h) in mung bean seedlings (*Vigna radiata* L. cv. BARI Mung-2). Salt stress resulted in Na toxicity, decreased K, Ca, Mg, and Zn contents in roots and shoots, and disrupted antioxidant defense system which caused oxidative damage as indicated by increased lipid

peroxidation, H_2O_2 content, $O_2^{\bullet-}$ generation rate, and lipoxygenase activity. Salinity-induced MG toxicity was also clearly evident. Salinity decreased leaf chlorophyll (chl) and leaf relative water content (RWC). Supplementation of salt affected seedlings with exogenous PAs enhanced the contents of GSH and AsA, increased activities of antioxidant enzymes (DHAR, GR, CAT, and GPX) and glyoxalase enzyme (glyoxalase II), which reduced salt-induced oxidative stress and MG toxicity, respectively. Exogenous PAs reduced cellular Na content and maintained nutrient homeostasis and modulated endogenous PAs levels in salt affected mung bean seedlings. The overall salt tolerance was reflected in improved tissue water and chl content, and better seedling growth.

Experiment 2
Insights into spermine-induced combined high temperature and drought tolerance in mung bean: osmoregulation and roles of antioxidant and glyoxalase system

Materials and Methods
Plant material, growth condition, and treatments

Healthy and uniform seeds of mung bean (*Vigna radiata* L. cv. BARI Mung-2) were immersed in to 70% ethanol for five minutes and then washed thoroughly with distilled water. Seeds were sown in Petri dish containing six layers of blotting paper with 10 ml of distilled water and placed in the dark germinator for three days. Germinated seedlings were then grown in new petri dishes under controlled conditions (light, 350 μmol photon m^{-2} s^{-1}; temperature, 25±2°C; relative humidity, 65–70%) where 10,000-fold diluted Hyponex solution (Hyponex, Japan) was applied as nutrient. Before starting the main experiment we have conducted several trial experiment where different concentrations of Spm was used against HT and/or drought stress. Based on preliminary test 0.2 mM Spm was selected among different concentrations of Spm as using that concentration we got the best result considering the protective effect against HT and/or drought stress. A set of 5-day-old seedlings were pretreated with 0.2 mM Spm and grown for 24 h. These pretreated seedlings and another set of six-day-old seedlings were either grown without stress or exposed to high temperature (HT, 40°C) and drought [induced by 5% polyethyleneglycol 6, 000 (PEG)] stress both individually and in combined. Control seedlings were grown only with Hyponex solution only. Another set of seedlings were grown with Spm without any stress. The performance of seedlings was observed and data were recorded after 48 h. The experiment was repeated three times under the same condition.

Determination of Physiological and Biological parameters

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In addition to the physiological and Biological parameters determined in Experiment 1, the plant water status parameters (water saturation deficit, water retention capacity, water uptake capacity), carotenoid content, plant height and root length, leaf area were determined according to the standard method.

Statistical analysis

All obtained data were subjected to analysis of variance (ANOVA) and the mean differences were compared by Fisher's least significant difference (LSD) test using XLSTAT v. 2015.1.01 software . Differences at P≤ 0.05 were considered significant.

Results and summary

High temperature and drought stress often occur simultaneously, and due to global climate change, this kind of phenomenon occurs more frequently and severely, which exerts devastating effects on plants. Polyamines play crucial roles in conferring abiotic stress tolerance in plants. Present study investigated how exogenous pretreatment of Spm (0.2 mM) enhances mung bean (*Vigna radiata* L. cv. BARI Mung-2) seedlings tolerance to high temperature (HT, 40 °C) and drought [induced by 5 % polyethyleneglycol (PEG)] stress individually and in combination. Spm pretreatment reduced ROS production including H_2O_2 and $O_2^{\bullet-}$, LOX activity, and membrane lipid peroxidation (indicated by malondialdehyde, MDA) under HT and/or drought stress. Histochemical staining of leaves with diaminobenzidine and nitro blue tetrazolium chloride also confirmed that Spm-pretreated seedlings accumulated less H_2O_2 and $O_2^{\bullet-}$ under HTand/or drought stress. Spermine pretreatment maintained the AsA and GSH levels high, and upregulated the activities of SOD, CAT, GPX, DHAR, and GR which were vital for imparting ROS-induced oxidative stress tolerance under HT and/or drought stress. The cytotoxic compound MG was overproduced due to HT and/or drought, but exogenous Spm pretreatment reduced MG toxicity enhancing the glyoxalase system. Spermine pretreatment modulated endogenous PA levels. Osmoregulation and restoration of plant water status were other major contributions of Spm under HT and/or drought stress. Preventing photosynthetic pigments and improving seedling growth parameters, Spm further confirmed its influential roles in HT and/or drought tolerance.

Experiment 3

Exogenous spermidine alleviates low temperature injury in mung bean (*Vigna radiata* L.) seedlings by modulating ascorbate-glutathione and glyoxalase pathway

Materials and Methods

Plant material, growth condition, and treatments

Seeds of mung bean (*Vigna radiata* L. cv. BARI Mung-3) plants were placed in a petri dish containing six layer of moist filter paper and kept in germinator in dark place for three days. Then, germinated seedlings were transferred into growth chamber under controlled conditions (light, 350 μmol photon m^{-2} s^{-1}; temperature, 25±2°C; relative humidity, 65–70%); 10,000-fold diluted Hyponex solution (Hyponex, Osaka, Japan) was applied as nutrient. Two sets of four-day-old seedlings were pretreated with Spd (0.25 mM) for 24 h. These pre-treated seedlings were then exposed to 6 °C temperature at the fifth day. Other two sets of seedlings were exposed to 6 °C temperature (without Spd pretreatment). Control seedlings were grown with Hyponex solution. Another two sets of seedlings were grown with Spd without any stress. All treatments were considered for 2 and 3 days and after that data on different parameters were taken following the standard methodology.

Determination of Physiological and Biological parameters

Physiological and Biological parameters were assayed following the previously discussed methods.

Statistical analysis

All data obtained were subjected to analysis of variance (ANOVA) and the mean differences were compared by a Duncan's multiple range test (DMRT) using XLSTAT v.2010 software. Differences at $p < 0.05$ were considered significant.

Results and summary

The role of exogenous Spd in alleviating low temperature (LT) stress in mung bean (*Vigna radiata* L. cv. BARI Mung-3) seedlings has been investigated. Low temperature stress modulated the non-enzymatic and enzymatic components of ascorbate-glutathione (AsA-GSH) cycle, increased H2O2 content and lipid peroxidation, which indicate oxidative damage of seedlings. Low temperature reduced the leaf RWC and destroyed leaf chlorophyll, which inhibited seedlings growth. Exogenous pretreatment of Spd in LT-affected seedlings significantly increased the contents of non-enzymatic antioxidants of AsA-GSH cycle, which include AsA and GSH. Exogenous Spd decreased DHA, increased AsA/DHA ratio, decreased GSSG and increased GSH/GSSG ratio under LT stress. Activities of AsA-GSH cycle enzymes such as APX, MDHAR, DHAR and GR increased after Spd pretreatment in LT affected seedlings. Thus, the oxidative stress was reduced. Protective effects of Spd are also reflected from reduction of MG toxicity by improving glyoxalase cycle components, and by maintaining osmoregulation, water status and improved seedlings growth. The present study reveals

the vital roles of AsA-GSH and glyoxalase cycle in alleviating LT injury.

Experiment 4

Physiological and biochemical mechanism of spermine-induced cadmium stress tolerance in mung bean (*Vigna radiata* L.) plant

Materials and Methods

Plant material, growth condition, and treatments

Mung bean (*Vigna radiata* L. cv. BARI Mung-2) seedlings were grown in petri dishes under controlled conditions (light, 350 μmol photon m^{-2} s^{-1}; temperature, 25±2°C; relative humidity, 65–70%); 10,000-fold diluted Hyponex solution (Hyponex, Japan) was applied as nutrient. Seven-day-old, two different sets of seedlings were exposed to Cd (CdCl$_2$, 1.0 mM and 1.5 mM which have been considered as mild and severe stress, respectively). Two sets of 6-day-old seedlings were grown with Spm (0.25 mM) as pre-treatment for 24 h. These pre-treated seedlings were then exposed to the same levels of Cd on day seven. Control seedlings were grown with Hyponex solution. Another set of seedlings were grown with Spm without any stress. Data were taken after 48 h. The experiment was conducted with a completely randomized design. The experiment was replicated three times.

Determination of Physiological and Biological parameters

Along with the previously discussed parameters, Cd content, biological concentration factor, translocation factor, and biological accumulation coefficient were determined following the standard method.

Statistical analysis

The statistical analysis was similar with that of Experiment 2.

Results and summary

The role of exogenous Spm (0.25 mM) in reducing Cd uptake and alleviating Cd toxicity (containing 1mM and 1.5 mM CdCl$_2$ in the growing media) effects was studied in mung bean (*Vigna radiata* L. cv. BARI Mung-2) plant. Exogenously applied Spm reduced Cd content, accumulation and translocation in different plant parts. Increasing phytochelatin content exogenous Spm reduced of Cd accumulation and translocation. Spermine application reduced the Cd-induced oxidative damage which was reflected from the reduction of H_2O_2 content, $O_2^{\bullet-}$ generation rate, LOX activity and lipid peroxidation level, and also reflected from the reduction of spots of H_2O_2 and $O_2^{\bullet-}$ from mung bean leaves (compared to

control treatment). Spm pretreatment increased non-enzymatic andtioxidants contents (ascorbate, AsA and glutathione, GSH) and activities of antioxidant enzymes (SOD, CAT, GST, MDHAR, DHAR and GR) which reduced oxidative stress. The cytotoxicity of MG also reduced by exogenous Spm because it enhanced glyoxalase system enzymes and components. Through osmoregulation Spm maintained a better water status of Cd affected mung bean seedlings. Spermine prevented the chl damage and increased its content. Exogenous Spm also modulated the endogenous free PAs level which might have the roles in improving physiological processes including antioxidant capacity, osmoregulation, Cd and MG detoxification capacity. The overall Spm induced tolerance of mung bean seedlings to Cd toxicity was reflected through improved growth of mung bean seedlings.

Experiment 5

Polyamine and nitric oxide crosstalk: Antagonistic effects on cadmium toxicity in mung bean plants through upregulating the metal detoxification, antioxidant defense and methylglyoxal detoxification systems

Materials and Methods

Plant material, growth condition, and treatments

Six-day-old mung bean (*Vigna radiata* cv. BARI Mung-2) seedlings (grown in light, 350 μmol photon $m^{-2} s^{-1}$; temperature, $25\pm2°C$; RH, 65–70%; hyponex was used as nutrient) were applied with different treatments for 48 h. One set was exposed to Cd ($CdCl_2$, 1.5 mM). Three sets of 5-day-old seedlings were grown with Put (0.2 mM), NO (applied as sodium nitroprusside, NO donor; SNP, 1 mM), and combination of Put with NO as pre-treatment (24 h). Pre-treated seedlings were exposed to Cd on the sixth day. Another three sets of seedlings were grown with Put and/or NO without stress. Control plants were grown with nutrient (hyponex) solution.

Determination of Physiological and Biological parameters

In addition to the previously obseved parameters, NO content was measured following standard method.

Statistical analysis

The statistical analysis was similar to the Experiment 3.

Results and summary

Cadmium (Cd) contamination is a serious agricultural and environmental hazard. The study

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investigates cross-protection roles of Put (0.2mM) and nitricoxide (sodium nitroprusside; SNP, 1mM) in conferring Cd (CdCl$_2$, 1.5mM) tolerance in mung bean (*Vigna radiata* L. cv.BARI Mung-2) seedlings. Cadmium stress in creased root and shoot Cd content, reduced growth, destroyed chlorophyll (chl), modulated proline (Pro) and reduced leaf relative water content (RWC), increased oxidative damage [lipid peroxidation, H$_2$O$_2$ content, O$_2$$^-$ generationrate, lipoxygenase (LOX) activity], methylglyoxal (MG) toxicity. Put and/or SNP reduced Cd uptake, increasd phytochelatin (PC) content, reduced oxidative damage enhancing non-enzymatic antioxidants (AsA and GSH) and activities of enzymes (SOD, CAT, APX, DHAR, GR, GST, and GPX). Exogenous Put and/or SNP modulated endogenous polyamines, PAs (Put, Spd and Spm), and NO; improved glyoxalase system in detoxifying MG and improved physiology and growth where combined application showed better effects which designates possible crosstalk between NO and PAs to confer Cd-toxicity tolerance.

Experiment 6
Mechanism of polyamine-induced aluminium toxicity tolerance in mung bean plants: A study on antioxidant defense and methylglyoxal detoxification systems

Materials and Methods

Plant material, growth condition, and treatments

Uniform and healthy seeds of mung bean (*Vigna radiata* L. cv. BARI Mung-2) were surface-sterilized with 70% ethanol followed by washing several times. The seeds were then placed in Petri plates (9 cm) containing 6 layers of filter paper moistened with 10 ml of distilled water. Seeds were placed in dark germination chamber for germination. After three days, seedlings were transferred and grown under controlled condition in growth chamber (light, 350 μmol photon m^{-2} s^{-1}; temperature, 25±2°C; relative humidity, 65–70%). A 10,000-fold diluted Hyponex solution (Hyponex, Japan) was applied as nutrient. Control seedlings were grown with Hyponex solution only. Seven-day-old seedlings were exposed to Al stress (AlCl$_3$, 0.5 mM, 48 h and 72 h) alone and in combination with Spd (0.3 mM).

Determination of Physiological and Biological parameters

Physiological and Biological parameters were measured according to the previously discussed method.

Statistical analysis

The statistical analysis was similar with that of Experiment 2.

Results and summary

Present study investigates the roles of exogenously applied Spd (0.3 mM) in alleviating Al (AlCl$_3$, 0.5

mM, 48 h and 72 h) induced injury in mung bean seedlings (*Vigna radiata* L. cv. BARI Mung-2). Aluminium toxicity induced oxidative damage overproducing ROS (H_2O_2 and $O_2^{\bullet-}$), increasing lipoxygenase activity and membrane lipid peroxidation. The toxic compound MG also overproduced under Al stress. In order to circumvent Al-induced oxidative stress, enzymatic and non-enzymatic antioxidant pathways were activated by exogenous Spd application. Exogenous Spd increased AsA and GSH content, AsA/DHA, GSH/GSSG, activity of APX, DHAR, GR and CAT which reduced ROS production and oxidative stress under Al stress. Spd-induced improvement of GSH pool and Gly II activity alleviated injurious effects of MG. Exogenous Spd positively modulated the endogenous PAs level. Regulating the osmoprotectant molecule proline, Spd improved plant water status under Al stress. Exogenous Spd was potent to prevent breakdown of Al-induced photosynthetic pigment and to improve growth performances under Al stress.

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