

Plant Stress

Abbreviation: Plant Stress

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Scope and target readership: *Plant Stress* deals with the network of biotic and abiotic aspects inducing stress in plants.

Plant Stress will consider manuscripts that explore the following topics:

- 1) Environmental stress;
- 2) Modelling stress and stress-reduction;
- 3) Physiological, biochemical, molecular, ecological, genetic and economic aspects of plant stress at the cellular, tissue, organ or whole plant level. Preference will be given to multi-level studies;
- 4) Programmed Cell Death directly related to a stress factor;
- 5) Reactive oxygen species and destructive cellular mechanisms;
- 6) Stress caused by diseases (temperate and tropical) induced by fungi, bacteria, insects, viruses, phytoplasmas and nematodes.

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Cover figures/photos: Top left: Scanning electron microscopy photograph showing the leaf surface of a NaCl + glycinebetaine-treated (above) and control (below) plant (Scalia *et al.*, pp 49-54). Top right: Proposed structure of two major classes of retrotransposons (Mansour, pp 33-39). Bottom: A scheme of imbibing pea seed behavior at germination (Veselova and Veselovsky, pp 71-78).

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Plant Stress

Alma Balestrazzi, Anca Macovei, Claudia Testoni, Elena Raimondi, Mattia Donà, Daniela Carbonera (Italy) Nitric Oxide Biosynthesis in White Poplar (*Populus alba* L.) Suspension Cultures Challenged with Heavy Metals (pp 1-6)

ABSTRACT

Original Research Paper: The present work reports on the generation of nitric oxide (NO) in white poplar (*Populus alba* L., cv. 'Villafranca') cell suspension cultures exposed to copper (150 μM CuCl_2), zinc (2 mM ZnSO_4) and cadmium (200 μM CdSO_4). Since it is currently believed that at least two distinct enzymatic pathways are responsible for NO production in plants, the response of 'Villafranca' cells to heavy metals was monitored using specific inhibitors of the nitrate-dependent pathway and a mammalian inhibitor of the L-arginine-dependent pathway. Production of nitrite (NO_2^-), as a measure of NO released in the culture medium, was quantified using the Griess reaction. Copper treatment resulted into a 3.2-fold enhancement of NO production in white poplar cell cultures. A lower increase (2-fold) was observed with the cadmium treatment. In contrast, NO production did not change in the zinc-treated cells. The use of 100 μM sodium azide and 200 μM sodium tungstate resulted into complete inhibition of NO production while in cells exposed to 500 μM N^G -monomethyl-L-arginine the rate of NO generation was only partially affected. The white poplar cultures exposed to heavy metals showed the morphological hallmarks of both Programmed Cell Death and necrosis, as evidenced by Evans Blue staining. The nuclear morphology was also investigated.

Sikander Pal Choudhary, Renu Bhardwaj, B. D. Gupta, Prabhu Dutt, Priya Arora (India) Effect of 24-Epi brassinolide on Polyamine Titrers, Antioxidative Enzyme Activities, and Seedling Growth of *Raphanus sativus* L. under Copper Stress (pp 7-12)

ABSTRACT

Original Research Paper: In the present investigation, exogenous application of 24-epibrassinolide (24-epiBL) to *Raphanus sativus* L. cv. 'Pusa chetki' seedlings, under copper (Cu) stress showed the synthesis of various polyamines (PAs). Cu metal treatment alone enhanced production of putrescine (Put), spermidine (Spd) and spermine (Spm) significantly over control values. However when metal treatment was supplemented with different concentrations of 24-epiBL, total PA content showed a significant decrease under Cu stress. Put/Cadaverine (Cad) ratio showed maximum rise when seedlings were treated with 10^{-7} M 24-epiBL alone whereas a maximum increase in the Put/Spd ratio was found in 10^{-11} M 24-epiBL treatment alone and the minimum ratio was recorded in Cu and 10^{-9} M 24-epiBL combination treatment. Besides these, 24-epiBL also altered the activities of peroxidase, catalase and superoxide dismutase. The shoot and root growth reduced by Cu metal treatment was restored to normal values by 24-epiBL.

C. Abdul Jaleel (United Arab Emirates), M. Iqbal (Pakistan), R. Panneerselvam (India) Triadimefon Protects Blackgram (*Vigna mungo* L. Hepper) Plants from Sodium Chloride Stress (pp 13-16)

ABSTRACT

Original Research Paper: The effects of triadimefon (TDM), a triazole compound, on NaCl-stressed blackgram (*Vigna mungo* (L.) Hepper) plants were studied to understand the ameliorative effect of this triazole derivative. Seeds were sown in plastic pots and irrigated with groundwater up to 35 days after sowing (DAS) to field capacity. Later plants were irrigated with groundwater as control and others were treated with 100 mM NaCl, 100 mM NaCl + 20 mg l^{-1} TDM and 20 mg l^{-1} TDM. The samples were collected randomly at 40 and 80 DAS. Salinity treatment decreased the protein content and increased the amino acid, proline, glycine betaine (GB), ascorbate peroxidase (APX) and catalase (CAT) activities in blackgram compared with the control. The addition of NaCl with TDM showed an increase in protein, APX and CAT activities and decreased proline, GB content when compared with NaCl-stressed plants. TDM treatment increased all parameters compared with the control. However, TDM-mediated salinity tolerance could be attributed to the increased activities of APX and CAT when compared to NaCl-stressed plants.

Rupinder Kaur, Renu Bhardwaj, Ashwani K. Thukral (India) Uptake of Heavy Metals, and Antioxidative Enzymes in *Brassica juncea* L. Seedlings as Affected by Zn in Binary Combinations with Other Heavy Metals (pp 17-25)

ABSTRACT

Original Research Paper: The present study attempts to understand the uptake of heavy metals and stress tolerance in *Brassica juncea* L. seedlings under the effect of Zn in binary combinations with Cr, Ni, Co and Cu through the production of

antioxidative enzymes - superoxide dismutase (SOD), guaiacol peroxidase (GPX), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR). It was observed that the order of uptake of heavy metals by the seedling in single metal solutions was Zn > Cu > Co > Cr > Ni. Zn in binary combination with other heavy metals, mutually decreased the uptake of each other, the maximum decrease being 66.1% in the uptake of Cr in (Cr100+Zn100) binary solution. All the metals, whether applied singly or in combinations, significantly increased the activities of antioxidative enzymes, except for CAT. Zn was the most effective metal in increasing the activities of antioxidative enzymes. At 100 mg l⁻¹, it increased the activities of GR, GPX and APX by 101%, 64%, and 42% respectively, whereas maximum SOD activity (16 mM UA mg⁻¹ protein) was induced by 100 mg l⁻¹ Cr. Of all the binary combinations, Zn+Co and Zn+Ni were most effective in increasing the activities of GPX and GR, respectively, whereas Zn+Cu and Zn+Cr increased the activities of APX and SOD, respectively. Binary interaction models revealed that Cr, Ni, Co and Cu act antagonistic to Zn to increase the activity of GR, whereas for GPX, APX and SOD, these metals in binary combinations with Zn, were mutually antagonistic, thereby causing a negative interactive effect. Cr, Co and Cu were mutually antagonistic to Zn for catalase activity, whereas interaction between Ni and Zn was synergistic for this enzyme.

G rard Ledoigt, Karine Chevalier, Eric Debiton, Bernard Griffaut (France) Secretion of Specific Cytotoxic Products by Plant Storage Organs after Wound-Desiccation Stress (pp 26-32)

ABSTRACT

Original Research Paper: After a wound-desiccation stress, plant tissues can secrete products that have specific cytotoxicity against melanoma B16 cells. Healthy plant or animal cells were not susceptible to these products. Secretion of the active product varied with the plant species and the plant organs. The secretion of tubers of yam (*Dioscorea cayenensis* Lam), cassava (*Manihot esculenta*), Jerusalem artichoke (*Helianthus tuberosus*), ginger (*Zingiber officinale*) and potato (*Solanum tuberosum* L.) showed activity against murine melanoma B16 cells, but not against immortalized fibroblast L929 cells. A decrease of 20 to 30% of the B16 clonogenicity was observed, while the treated L929 fibroblast cells did not show any significant difference with the control cell colonies. In contrast, the secreted products of roots of beet (*Beta vulgaris*) and tubers of sweet potato (*Ipomoea batatas*) only showed slight cytotoxicity. Secretion of the active product was associated with the storage tissues. The physiology of the plant, such as the light growth conditions (for hypocotyls of sunflower, *Helianthus annuus* L., and tomato, *Lycopersicon esculentum* Mill), or dormancy (for Jerusalem artichoke tubers, *Helianthus tuberosus* L.), was involved in the secretion process of an active product. The active agent was associated with a protein complex. The secretion of the cytotoxic products against melanoma B16 cells, thus, can be performed through a specific stress on the storage organs of plants usually cultivated.

Ahmed Alzohairy Mansour (Egypt) Water Deficit Induction of *Copia* and *Gypsy* Genomic Retrotransposons (pp 33-39)

ABSTRACT

Original Research Paper: LTR-retrotransposons and other mobile DNA elements respond directly or indirectly to a wide variety of stresses by increasing or decreasing their copy numbers. This effect is specific for different retrotransposons and stresses. A potential osmotically-stressed action has been ascribed to sorbitol, but *in vivo* evidence of this action and its genomic impact remains elusive. However, sorbitol is still widely used to mimic the effects of drought and water deficit in plants. In the present investigation, the effect of sorbitol treatments was compared, in both *Copia* and *Gypsy* groups, of genomic retrotransposons with drought in barley. Transcriptional analysis showed that sorbitol exerted a strong influence upon *Copia* elements group after 4, 21 and 32 hours of sorbitol treatment. Transcription of the *BARE-1* retrotransposon family from the *Copia* group was actively induced *in vivo* by sorbitol treatment. When *BARE-1*-specific primers were used to amplify universal *Copia* cDNA products, this revealed unique and strong DNA bands at the same time points of *Copia* elements group which it belong. However, immunoblotting of *BARE-1* GAG protein-specific antibody showed no specific increase after treatments at the same time intervals. These results suggest that sorbitol has the capacity to increase the transcription activity of *Copia* elements, especially *BARE-1* retrotransposon.

Mejda Daami-Remadi, Hedia Ben Oun, Ahmed Souissi, Mohsen Mansour, Hayfa Jabnoun-Khiareddine, Bouzid Nasraoui (Tunisia) Effects of Saline Irrigation Water on Verticillium Wilt Severity and Tomato Growth (pp 40-48)

ABSTRACT

Original Research Paper: The present study describes the combined interactive effect of water salinity and *Verticillium dahliae* on tomato, and quantifies the effect of this abiotic stress on Verticillium wilt (VW) severity, plant growth and subsequent yield

loss. The effects of six saline levels added to Potato Dextrose Agar (PDA) medium on the pathogen mycelial growth *in vitro* at 25°C was shown to be insignificant at $P \leq 0.05$ but reduced mycelium density and increased microsclerotia production, compared to the unamended controls, were recorded with highest NaCl doses such as 8 and 10 g/l. Increasing the salinity stress (2 to 10 g of NaCl/l) to inoculated cv. 'Ventura' tomato plants enhanced the severity of VW disease and resulted in a significant increase in leaf damage index (LDI) recorded from 35 to 62 days post-planting (DPP). Moreover, LDI noted on plants under highest salt stress (8 and 10 g of NaCl/l) was more than four times higher than the LDI recorded at 0 g/l. Watering plants (inoculated or not with *V. dahliae*) with saline water, from 15 DPP until the end of the assay (i.e., 62 DPP), negatively affected plant growth: height decreased with increasing salinity. A similar effect was noted after inoculation with *V. dahliae*. The aerial part fresh and dry weights (FW and DW, respectively) were generally lower as the salinization level increased; these parameters were reduced by 52-57 and 33-43% with higher salt treatments tested (8 and 10 g of NaCl/l) compared to the non-saline water (0 g/l), respectively. The decrease in root FW and DW ranged between 12-73 and 9-57% when salinity levels increased from 2 to 10 g of NaCl/l, compared to the control plants irrigated with non-saline water. The fruit FW was also adversely affected by the highest NaCl doses tested (8 and 10 g/l); it was reduced by 38 and 72% when compared to the non-saline treatment.

Rosalinda Scalia, Elisabetta Oddo, Filippo Saiano, Francesca Grisafi (Italy) Effect of Salinity on *Puccinellia distans* (L.) Parl. Treated with NaCl and Foliarly Applied Glycinebetaine (pp 49-54)

ABSTRACT

Original Research Paper: Turfgrasses general appearance is much affected by environmental stresses because the species used for this purpose are particularly exigent in terms of technical inputs and water need. In the Mediterranean area, sometimes irrigation is provided by using waste water which may contain high concentrations of dissolved salts which can cause salt stress injury and poor turf quality. *Puccinellia distans* (L.) Parl. is a halophyte cool season grass that seems to have a high salinity tolerance when cultivated in sodic soils or in NaCl-rich hydroponic cultures. We investigated the response of *P. distans* to salinity in a soil culture in a controlled growth environment. The effect of different concentrations of NaCl (85, 275 and 600 mM) on shoot and root growth and chloride content was assessed. After determining the resistance of *P. distans* to the highest salinity level, we tested the efficiency of the osmoprotectant glycinebetaine (GB) in reducing salt stress effects by measuring some physiological parameters. The results showed a very good adaptability of *P. distans* to salinity conditions. When irrigated with salt solution at the highest concentration (600 mM), plants showed a reduction in growth rate and biomass production that seemed to be relieved by GB application. With GB application, leaf relative water content and biomass production were similar to the control. However, GB application did not result in a generally better turf quality compared to untreated plants, so it did not give a relevant advantage in reducing technical and labor inputs with respect to the maintenance of a *P. distans* turf.

Manzer H. Siddiqui (India/Saudi Arabia), Firoz Mohammad, Mohd. Nasir Khan, Mohd. Naeem, Mohd. Masroor A. Khan (India) Differential Response of Salt-Sensitive and Salt-Tolerant *Brassica juncea* L. Genotypes to N Application: Enhancement of N-Metabolism and Anti-Oxidative Properties in the Salt-Tolerant Type (pp 55-63)

ABSTRACT

Original Research Paper: Different strategies in response to the application of nutrients are required to overcome the adverse effects of NaCl stress. The objective of the present study was to determine if different added levels of nitrogen (N) in growth medium could alleviate the adverse effects of salt stress on plant N-metabolism and the antioxidative system. Two-week-old plants of salt-sensitive (cv. 'Chuutki') and salt-tolerant (cv. 'Radha') genotypes of *Brassica juncea* L. Czern. & Coss. were treated with: (i) 0 mM NaCl + 0 mg N kg⁻¹ sand (control), (ii) 90 mM NaCl + 30 mg N kg⁻¹ sand, (iii) 90 mM NaCl + 60 mg N kg⁻¹ sand, (iv) 90 mM NaCl + 90 mg N kg⁻¹ sand and (v) 90 mM NaCl + 120 mg N kg⁻¹ sand. Under salinity stress, the salt-tolerant genotype exhibited maximum value for growth attributes [shoot length (SL) plant⁻¹, area (A) leaf⁻¹, and leaf area index (LAI)], sulphur assimilation enzyme [ATP-sulphurylase (ATP-S)], N-assimilation enzymes [nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS) and glutamate synthase (GOGAT)], and antioxidative enzymes [superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR)] and content of antioxidant [glutathione (GSH) and ascorbate (ASC)] at 60 mg N kg⁻¹ sand than the salt-sensitive genotype. These results suggest that the salt-tolerant genotype may have better N-metabolism, antioxidative system and protection from reactive oxygen species (ROS) with N application under salt stress.

Ajay Kumar Singh, Avinash Mishra, Arvind Shukla (India) Assessment of Salt Tolerance in *Vigna mungo* Revealed by

ABSTRACT

Original Research Paper: Twenty eight accessions of blackgram (*Vigna mungo* L. Hepper) were screened against salt stress under controlled conditions using five different parameters (germination percentage, plumule length, radicle length, plumule-radicle length ratio and dry matter weight) at four different salt concentrations (0.00 EC, 4.65 EC, 11.25 EC and 16.00 EC). Selected genotypes were further analyzed to assess salt stress-associated biochemical and RAPD markers. A band of peroxidase isoenzyme (Rm 0.38) was observed in tolerant genotypes at all four salt concentrations while in susceptible genotypes at high salt concentration (11.25 and 16.00 EC) only. A peroxidase band (Rm 0.50) was also observed in tolerant genotypes at 11.25 EC with faint intensity. Two bands of malate dehydrogenase (Rm 0.12 and 0.14) were observed under salt stress only. The RAPD banding pattern showed high polymorphism with several unique loci which can help in identification and discrimination of tolerant and susceptible genotypes. A 300 bp band, identified as a RAPD marker for discriminating tolerant from susceptible genotypes, was amplified by four primers (G 08, H02, H 06 and H 08) in the tolerant group only. The study results in categorization and listing of germplasm that can be explored while breeding for saline stress in blackgram.

Tatiana V. Veselova, Vladimir A. Veselovsky (Russia) Post-hypoxic Oxidative Stress in Aging Pea Seeds: I. Hypoxia Development during Imbibition (pp 71-78)

ABSTRACT

Original Research Paper: During seed transition from quiescence to metabolic activity and subsequent germination, orthodox seeds become sensitive to stress. Aging seeds become particularly sensitive to stress conditions and during storage, seeds undergo aging that deteriorates their quality. Each seed lot contains strong seeds, weak seeds, and dead seeds, but dry seed quality cannot be assessed prior to germination. Nevertheless, air-dry pea seeds differing in germinability can be divided into three fractions using the method of room temperature phosphorescence (RTP): fraction I, alive high-quality seeds which will produce normal seedlings; fraction II, alive but weak seeds which will produce morphologically abnormal seedlings or will not germinate at all; and fraction III, dead seeds. The imbibition patterns of fraction I and fraction II seeds were compared during germination. The water uptake during imbibition was higher in the fraction II seeds than in those of fraction I. A higher respiration rate and greater limitation of oxygen diffusion by seed coats in fraction II seeds induced oxygen deficiency, which can be considered as hypoxic conditions for embryo. In order to assess the hypoxia level, we developed a non-intrusive luminescent method based on endogenous porphyrin phosphorescence of seeds. Result showed that hypoxia did not appear in fraction I seeds. In fraction II pea seeds, the hypoxia starts to develop after 12-16 h imbibition. When oxygen deficiency enhanced, alive seeds did not germinate as they died due to suffocation during imbibition. If the level of oxygen deficiency was not high (<50 arbitrary units (aU) of porphyrin phosphorescence) and radicles protruded, the emerging seedlings exhibit various morphological defects. Since hypoxia did not impair DNA replication (2C-4C) prior to radicle protrusion, it did not directly contribute to morphological abnormalities in seedlings.