

# EFFECT OF CYTOKININ ANALOGUES ON CYTOKININ METABOLISM AND STRESS RESPONSIVE GENES UNDER OSMOTIC STRESS IN WHEAT

SHIVANI NAGAR<sup>1\*</sup>, AJAY ARORA<sup>1</sup>, V. P. SINGH<sup>1</sup>, S. RAMAKRISHNAN<sup>1</sup>, DEEPIKA K. UMESH<sup>1</sup>, SHAILESH KUMAR<sup>1</sup> AND RAVI P. SAINI<sup>2</sup>

<sup>1</sup>Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi - 110 012, INDIA

<sup>2</sup>National Research Centre on Plant Biotechnology, New Delhi - 110 012, INDIA

e-mail: shivaninagar19@gmail.com

## KEYWORDS

Cytokinin  
Osmotic stress  
LEA  
IPT  
CKX genes

Received on :  
31.08.2014

Accepted on :  
07.01.2015

\*Corresponding  
author

## ABSTRACT

Osmotic stress induced by conditions like drought, salinity and heat etc. impairs plant growth and development by affecting plant physiological processes. Present study was conducted in laboratory to understand the role of cytokinin analogues (TDZ and BAP) in osmotic stress amelioration and their relative efficiency in inducing stress tolerance. Twenty days old wheat seedlings were treated 20% PEG-6000 to induce osmotic stress (-0.491 MPa). Exogenous application of cytokinin analogues enhanced endogenous cytokinin levels by up-regulating expression of IPT (isopentenyl transferase) and down-regulating CKX (cytokinin oxidase) gene expression under osmotic stress. Further, increase in relative water content (30%), membrane stability (15%) and nitrogen assimilation was observed along with delayed leaf senescence in cytokinin treated seedlings under osmotic stress. The current finding suggested that application of TDZ was found to be more effective as compared to BAP even at lower concentrations.

## INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the important cereal crop and grown worldwide under various climatic conditions. However, drought and salinity are the most serious threats to wheat production especially in India. Use of low quality water for irrigation also disrupts the osmotic environment of the root zone causing osmotic stress (Emily *et al.*, 2011). Osmotic stress symptoms in plants include stomatal closure and reduced transpiration rates, a decreased water potential of plant tissues, decrease in photosynthesis and growth inhibition, accumulation of abscisic acid (ABA), proline, mannitol, sorbitol, formation of free radical scavenging compounds (ascorbate, glutathione,  $\alpha$ -tocopherol etc.), and synthesis of new proteins and mRNAs. Polyethylene glycols (PEG) are widely used in laboratory for inducing osmotic stress in plants (Tingey and Stockwell, 1977). PEG induces osmotic stress by decreasing the water potential which results in reduced water uptake by the plants causing water deficit stress. Plant growth regulators like auxins, cytokinins (CKs), gibberellic acid, jasmonic acid and brassinosteroids mediate the adaptation of plant growth and development under changing environmental conditions (Wolters and Jurgens, 2009) in which CKs are mainly associated with stress tolerance through delayed leaf senescence. CKs may play important roles in regulating plant responses to water deficit stress. CK

metabolism is disturbed in plants under water deficit stress, mainly its synthesis and transport is inhibited leading to reduced growth, early leaf senescence, reduced nutrient mobilization and sink activity. Plant responses to CKs under stress are often judged from their responses to exogenous application. Application of CKs exogenously can increase the content of endogenous CKs by their uptake and by promotion of CK biosynthesis (Hare *et al.*, 1997; Kaminek *et al.*, 1997). Peleg *et al.* (2011) showed that overexpressed isopentenyl transferase (*ipt*) in bent grass which increased endogenous CK content even under stress conditions that improved stress tolerance. Cytokinin oxidase (CKX) is a cytokinin degrading enzyme and its activity usually decreases under stress in order to maintain level of endogenous cytokinin in plants under stress.

Among proteins, late embryogenesis abundant (LEA) proteins have important role in water deficit stress tolerance (Grelet *et al.*, 2005). Intracellular accumulation of LEA proteins is tightly correlated with acquisition of desiccation tolerance, and data support their capacity to stabilize other proteins and membranes during stress condition. We hypothesised that exogenously applied cytokinin may impart osmotic stress tolerance by inducing cytokinin metabolism and stress responsive genes. With this background, the objective of studying CKs induced water and salinity stress tolerance mechanism as well as its associated proteins like LEA, IPT and

CKX in wheat under osmotic stress was taken.

## MATERIALS AND METHODS

In the present study, two experiments, one preliminary and another main experiment were conducted on 20 days old wheat seedlings. Three wheat seedlings were raised in 50mL test tubes with three replications in 20mL of half strength Hoagland solution which was replaced after every three days. They were kept in growth chamber under light conditions of 1000  $\mu\text{molm}^{-2}\text{sec}^{-1}$  and temperature of 25/18°C. Preliminary experiment was conducted to optimize dose of cytokinin using varying levels of benzyl amino purine (BAP; 0.1, 10 and 100 ppm) and thidiazuron (TDZ; 0.1, 10 and 100 ppm) and water spray as control were used for foliar application on 18 days old seedlings for 2 days. For main experiment, the optimized concentrations of TDZ and BAP were employed on 18 days old seedlings for 48 hrs over which osmotic stress was imposed at the last 24 hrs using 20% PEG-6000 (-0.491 Mpa). Third fully opened leaf from top was collected to study various physiological parameters and molecular analysis.

Relative water content (RWC) was estimated by recording the turgid weight of 0.5 g fresh leaf samples by keeping in water for 4 h, followed by drying in hot air oven till constant weight was achieved  $\text{RWC} = [(\text{Fresh wt.} - \text{Dry wt.}) / (\text{Turgid wt.} - \text{Dry wt.})] \times 100$  (Weatherley, 1950). Membrane stability index (MSI) was measured according to method of Sairam *et al.* (1997). 100 mg leaf material was taken in test tubes containing 10 mL of double distilled water. Initial (40°C) and final (100°C) conductivity of the solution was recorded on a conductivity bridge ( $C_1$  and  $C_2$ ). MSI was calculated as:  $\text{MSI} = [1 - (C_1/C_2)] \times 100$ . Chlorophyll (Chl) and carotenoid were estimated by non-maceration method of Hiscox and Israelstam 1979. Leaf samples (0.05g) were incubated in 10 ml of dimethyl sulphoxide (DMSO) at 65°C for 4 h. Absorbance was recorded at 645, 665 and 470 nm and chlorophyll (Aron, 1949) and carotenoid content (Linchtenthaler and Wellburn, 1983) [35] H.K. Linchtenthaler and W.R. Wellburn, Determination of carotenoids and chlorophylls 'a' and 'b' of leaf extracts in different solvents. *Biochem. Soc. Trans.*, 11 (1983), pp. 591–592. were calculated. Nitrate reductase activity (NR) was estimated by using method of Nair and Abrol (1973).

In order to determine the semi quantitative gene expression analysis of stress responsive genes LEA, COR and DHN, and cytokinin metabolism genes IPT2, IPT9, CKX1 and CKX2; isolation of total RNA was carried out by TRIzol<sup>®</sup> reagent

**Table 1: Effect of cytokinins on Relative water content (RWC; %), membrane stability index (MSI; %) and total chlorophyll content on 20 days old wheat seedling**

Treatment	RWC	MSI	Total Chl.
Control	90.1 + 1.5	59.6 + 1.3	11.0 + 0.6
T <sub>1</sub> (0.1)ppm TDZ)	97.5 + 0.7	72.2 + 1.6	14.6 + 0.7
T <sub>2</sub> (10)ppm TDZ)	92.0 + 2.7	63.4 + 1.9	10.4 + 0.1
T <sub>3</sub> (100)ppm TDZ)	93.4 + 2.1	62.5 + 1.7	11.0 + 0.6
T <sub>4</sub> (0.1)ppm TDZ)	99.0 + 0.5	56.7 + 2.3	12.9 + 0.8
T <sub>5</sub> (10)ppm TDZ)	99.0 + 0.3	64.9 + 2.0	12.9 + 0.7
T <sub>6</sub> (100)ppm TDZ)	95.6 + 0.5	61.4 + 1.6	12.5 + 0.1

(Invitrogen, USA) and RNase-free DNase I (Promega, USA) was applied to remove contaminating genomic DNA at 37°C for 1 h. Quality and integrity of total RNA were then determined by running appropriate amount in a formamide denaturing gel, and quantity of total RNA was determined using a Nano Drop™ 1000 spectrophotometer (ThermoFisher Scientific, USA). The first-strand cDNA was synthesized according to the instructions of the cDNA Synthesis Superscript<sup>®</sup> III First-Strand Synthesis System (Invitrogen, USA). Resulting cDNA was stored at -20°C and employed as template for two-step RT-PCR reactions following recommended conditions provided in user's manual.

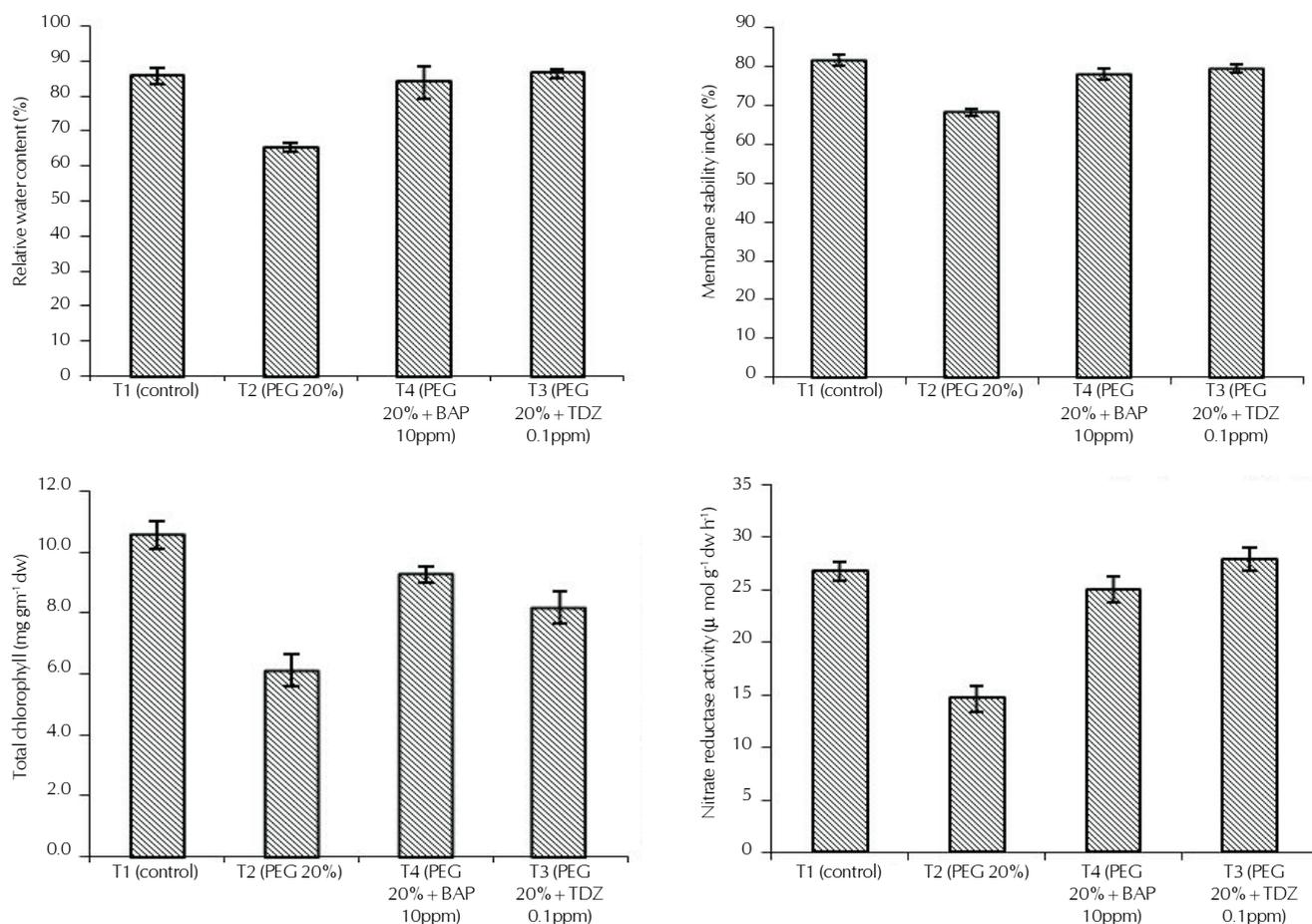
Nucleotide sequences for genes were obtained from National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). The Basic Local Alignment Search Tool (<http://www.ncbi.nlm.nih.gov/BLAST/>) was used to identify the homologs of genes. For RT-PCR expression analysis the following oligonucleotide primers were designed manually, and oligo quality (to avoid primer dimer, self dimer, etc.), GC % and Tm were analyzed by using Oligoanalyzer 3.0 tool (<http://www.idtdna.com/analyzer/Applications/OligoAnalyzer>, Intergrated DNA Technologies, Coralville, IA 52241, USA).

Name	Sequence
IPT 9 F	GATGAAGCTGTCTCTCAG
IPT 9 R	CTAGTCCACCACCATCGATC
CKX 2 F	AGGAGGAGGAGGTGTTCTAC
CKX 2 R	GGATCGCCTTGGGATCATA
TaDHN F	CATCGATGAGAACGGTGAGGT
TaDHN R	TTGTCCATGATCTTGCCCAGT
TaCOR F	CCCGGCGGCCACAAGAA
TaCOR R	GCCCAGCAAATAACCAATACA
Actin F	AATACAGTGTCTGATCGGTG
Actin R	GGTACACATCTTCTACAGAACAC

## RESULTS

### Optimization of BAP and TDZ dosage

The effect of varying doses of benzyl amino purine (BAP) (0.1, 10 & 100 ppm) and thidiazuron (TDZ) (0.1, 10 and 100 ppm) and water spray as control were studied on 20 days old wheat seedlings to optimize the dose of TDZ and BAP for further studying the osmotic stress tolerance (Table 1). On application of TDZ and BAP there was significant increase in RWC, MSI and total chlorophyll content in seedlings after 24 hours of treatment as compared to their control seedlings in all the three concentration of cytokinin analogues. Among three concentrations effect of 0.1ppm TDZ application was maximum on all the three parameters studied, similarly best outcome for BAP was obtained at 10 ppm concentration. RWC was shown to have an increment of 8 and 9 % on application of 0.1 ppm of TDZ and 10 ppm of BAP respectively as compared to their controls. In case of MSI increase of 20 and 9 % was observed on application of 0.1 ppm of TDZ and 10 ppm of BAP respectively, whereas increase of 30 and 16 % was seen for total chlorophyll content. Based on these results we selected 0.1 ppm of TDZ and 10 ppm of BAP for studying the effect of cytokinin under osmotic stress.



**Figure 1: Effect of cytokinins on (a) relative water content (b) membrane stability index (c) total chlorophyll content and (d) nitrate reductase activity on 20 days wheat seedling under osmotic stress (PEG 20 %).**

### Effect of osmotic stress on physiological traits

Effect of osmotic stress was studied on wheat seedlings after 24 hrs of 20 % PEG treatment (Fig. 1). RWC and MSI of PEG treated seedlings were decreased by 23 and 16 % respectively. Upon application of selected concentration of TDZ and BAP in osmotically stressed plants, there was increase of 32 and 28 % in RWC and 14 and 17 % in MSI respectively as compared to their water sprayed controls. Osmotic stress accelerated the rate of chlorophyll and carotenoid degradation which was decreased by 40 and 18 % respectively in stressed seedlings. But application of TDZ and BAP were able to maintain the level of chlorophyll and carotenoid contents in osmotically stressed plants. Effect of CK application was relatively higher on chlorophyll content.

Nitrate reductase is first enzyme of N metabolism and it reduced by 50 % of its level as compared to control seedling under osmotic stress. Application of CKs was able to restore the NR activity in wheat seedlings under stress among which the ameliorative effect of TDZ was relatively higher than BAP.

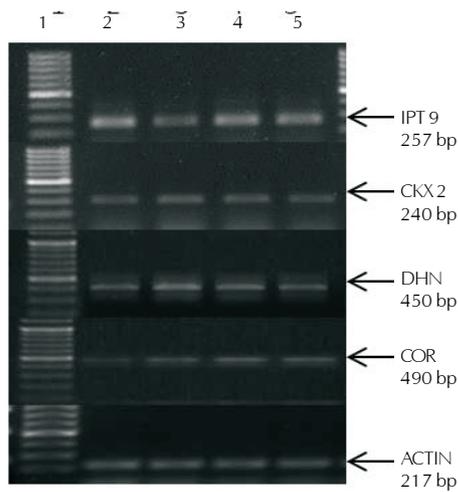
### Effect of osmotic stress on stress responsive and cytokinin metabolism genes

Expression of stress responsive genes (Fig. 2) *i.e.*, COR and DHN were upregulated in PEG treated seedlings as compared

to their controls. COR gene expression did not show any change upon application of TDZ and BAP. Expression of cytokinin metabolism genes (Fig. 2) *i.e.*, biosynthesis gene IPT 9 was downregulated and cytokinin degradation gene CKX2 was upregulated under osmotic stress. Exogenous application of TDZ and BAP upregulated the expression of IPT 9 genes in stressed seedlings and brought the level equal to non stressed seedling. Expression of IPT gene was relatively higher in BAP treated seedlings as compared to TDZ under osmotic stress. CKX 2 expression got downregulated slightly by application of cytokinin analogues and in TDZ treated seedling relatively more decrease in CKX 2 gene was reported under stress.

## DISCUSSION

Most climate-change studies indicate an expansion of dry land areas and degraded land on our planet. Water scarcity, high temperatures and salinity create the most significant limitations to crop productivity (Boyer, 1982). Most abiotic stresses induce osmotic stress in plant which adversely affects plant growth and development. Presently, there is a greater research interest in identifying and employing bioregulatory molecules, which can be used to achieve the stress tolerance in crops under field conditions without compromising the yield. In the present



**Figure 2: Effect of cytokinins on RT-PCR expression analysis of IPT 9, CKX 2, DHN and COR gene under different treatment in 20 days old seedlings. Lane 1- 100 bp DNA ladder Lane 2- Control (C), Lane 3- PEG 20% , Lane 4- PEG 20% + 10 ppm BAP Lane 5- PEG 20% + 0.1 ppm TDZ**

investigation, an attempt has been made to study the mitigating effect of the synthetic cytokinins (benzylaminopuine and thidiazuron) on physiological attributes, stress responsive genes and cytokinin metabolism in wheat seedlings under osmotic stress condition.

RWC is an important indicator of water deficit stress in leaves (Sairam *et al.*, 1997) and MSI gives a picture of cell membrane stability. Stress exposed plants were reported to have reduced RWC of their leaves (Singh *et al.*, 2014). During stress, the plasma membrane and the membranes of other organelles lose their permeability due to molecular modifications of the lipid bilayer, initially promoted by oxidative processes (Hopkins *et al.*, 2007). We have also reported decrease in RWC and MSI in wheat seedlings under osmotic stress (Fig. 1). Foliar application of cytokinin analogues during water stress maintained higher RWC and MSI values than the water sprayed osmotic stressed seedlings. Nagar *et al.*, 2014 also reported increase in RWC on application of benzyl adenine during water stress in wheat (). Cytokinins are well recognized for enhancing the antioxidant activities, which scavenge the ROS production and ultimately reduce the membrane damage and improve the cell membrane thermostability (CMT) (Sayd *et al.*, 2010). Furthermore, TDZ has also been reported to stimulate endogenous cytokinin biosynthesis (Guo *et al.*, 2000) and thus probably improved the membrane stability which supports our results of increase in MSI on application of TDZ. LEA proteins are involved in membrane stabilization. Evidence from *in vitro* experiments, where members of LEA2 proteins family (dehydrins) were found to be associated to anionic phospholipid vesicles (Koag *et al.*, 2009). In plants, a number of reports indicate that over-expression of LEA proteins confers tolerance to a variety of water deficit treatments (Eriksson and Harryson, 2011; Duan and Cai, 2012). We have also reported increase in expression of LEA family proteins *viz.* LEA, COR and DHN under osmotic stress in wheat seedling but cytokinin application brought no significant change in expression of

DHN and COR gene (Fig. 2).

Exogenous application of cytokinin was able to upregulate the expression of IPT 9 and downregulated CKX2 which enhanced the level of endogenous cytokinin levels in wheat seedlings and induced osmotic stress tolerance (Fig. 2). The IPT overexpressing transgenic line of *Agrostis stolonifera* has previously been reported to exhibit superior drought tolerance compared with the wild type, when plants were subjected to soil drying by withholding irrigation (Merewitz *et al.*, 2011). Increased level of cytokinin was able to increase cell membrane thermostability, delays leaf senescence and enhanced the processes of N- assimilation which overall contributed to osmotic stress tolerance. It may be assumed that enough CK was produced to overcome degradation of the free forms of CK by cytokinin oxidases, which are up-regulated in the drought response in most plant organs (Vyroubalova *et al.*, 2009). Cytokinins are likely to increase the synthesis of accessory pigments to protect the reaction centers of light and oxygen evolution complex (Wingler *et al.*, 2004; Vlekova *et al.*, 2006). A similar trend was reported by Zavaleta-Mancera *et al.*, (2007) in fragments of wheat leaves incubated 6 d in BAP in dark.

Leaf senescence is characterized by Chl and carotenoid loss (leaf yellowing) which is accelerated under stress. In this study we showed that, the Chl and carotenoid content of control plants was higher than stressed plants and application of cytokinins in stressed ones delayed the process of leaf senescence. Application of TDZ was more effective than BAP even at lower concentrations in delaying the process of leaf senescence. Drought stress reduces the chlorophyll content (Patel and Hemantranjan, 2013). Similarly, Kraus (1995) reported that exogenous cytokinins could retard senescence in intact bean (*Phaseolus vulgaris*) leaves. CKs accelerate the regeneration and the *de novo* formation of chloroplasts by regulating membrane formation and the synthesis of components of the electron transport system (Pospisilova *et al.*, 2000). In transgenic plants overexpressing *IPT* gene transcriptional activation of several genes coding for enzymes involved in chlorophyll biosynthesis was enhanced e.g. porphobilinogen deaminase and protochlorophyllide oxidoreductase under water stress (Reviro *et al.*, 2007).

In many experiments, it has been found that wheat leaf N concentration decreases with increasing soil drought (Sinclair *et al.*, 2000). Water deficit stress can also have adverse effects on NR activity (Burman *et al.*, 2004), which is involved in nitrogen metabolism. Similarly we reported decrease in NR activity and total nitrogen content in osmotically stressed plant as compared to control plants (Fig. 1). NR activity and total nitrogen content was restored nearly to the level of control non stressed plants on application of BAP and TDZ in stressed seedlings. Reguera *et al.* (2013) also reported that PSARK::IPT overexpressing plants were able to maintain primary N assimilation without the activation of reassimilation processes by increasing  $\text{NO}_3^-$  uptake together with higher NR and nitrite reductase activities.

We conclude that exogenous application of cytokinins can induce osmotic stress tolerance as cytokinin treated wheat seedlings were having higher endogenous CK level which gave overall plant tolerance to water stress. The effects of

cytokinin treatment on improving plant tolerance to water stress were reflected by upregulation of expression of IPT and stress tolerance genes with an enhancement in relative water content, membrane stability, delaying leaf senescence and enhanced N- assimilation was reported in wheat seedlings. Exogenous application of TDZ was more effective under osmotic stress as compared to BAP even at lower doses.

## ACKNOWLEDGEMENT

The authors express their thankfulness to Indian Council of Agricultural Research, New Delhi, India for funding this research.

## REFERENCES

- Arnon, D. I. 1949.** Copper enzymes in intact chloroplast, polyphenoxide in *Beta vulgaris*. *Plant Physiol.* **24**: 1-15
- Boyer, G. L. and Zeevart, J. A. D. 1982.** Isolation and quantification of  $\beta$ -D glucopyranosyl abscisate from leaves of Xanthium and spinach. *Plant Physiol.* **70**: 227-231.
- Burman, U., Garg, B. K. and Kathju, S. 2004.** Interactive effects of thiourea and phosphorus on cluster bean under water stress. *Biol. Plant.* **48**: 61-65.
- Duan, J. and Cai, W. 2012.** OsLEA3-2, an abiotic stress induced gene of rice plays a key role in salt and drought tolerance. *PLoS ONE*,
- Eriksson, S. K. and Harryson, P. 2011.** "Dehydrins: molecular biology, structure and function," in *Plant Desiccation Tolerance*, eds pp. 289-305.
- Grelet, J., Benamar, A., Teyssier, E., Avelange-Macherel, M. H., Grunwald, D. and Macherel, D. 2005.** Identification in pea seed mitochondria of a late-embryogenesis abundant protein able to protect enzymes from drying. *Plant Physiol.* **137**: 157-167.
- Guo Li, A., Sheng Hou, Y., Wall, G. W., Trent, A., Kimball, B. A. and Pinter, P. J. 2000.** Free-air CO<sub>2</sub> enrichment and drought stress effects on grain filling rate and duration in spring wheat. *Crop Sci.* **40**: 1263-1270.
- Hare, P. D., Cress, W. A. and Van Staden, J. 1997.** The involvement of cytokinins in plant responses to environmental stress. *Plant Growth Regul.* **23**: 79-103.
- Hiscox, J. D. and Israelstam, G. F. 1979.** A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.* **57**: 1332-1334.
- Hopkins, M., Taylor, C., Liu, Z., Ma, F., Mc Namara, L., Wang, T. W. and Thompson, J. E. 2007.** Regulation and execution of molecular disassembly and catabolism during senescence. *New Phytol.* **175**: 201-214.
- Jones, J. B. 1988.** Soil testing and plant analysis: procedure and use. Technical bulletin 109. *Food and Fertilizer technology centre*. p.14.
- Kaminek, M., Motyka, V. and Vankova, R. 1997 a.** Regulation of cytokinin content in plant cells. *Physiol. Plant.* **101**: 689-700.
- Kaminek, M., Zazimalova, E., Brezinova, A. and Motyka, V. 1997b.** Control of cytokinin biosynthesis and metabolism. In: *Biochemistry and Molecular Biology of Plant Hormones*. Eds. Hooykaas, P.J.J., Hall, M.A., Libbenga, K. R. Elsevier, Amsterdam. pp. 141-160.
- Koag, M. C., Wilkens, S., Fenton, R. D., Resnik, J., Vo, E., and Close, T. J. 2009.** The K-segment of maize DHN1 mediates binding to anionic phospholipid vesicles and concomitant structural changes. *Plant Physiol.* **150**: 1503-1514.
- Kraus, T. E., McKersie, B. D. and Fletcher, R. A. 1995.** Paclobutrazol induced tolerance of wheat leaves to paraquat may involve increased antioxidant enzyme activity. *J. Plant Physiol.* **145**: 570-576.
- Lichtenthaler, H. K. and Wellburn, A. R. 1983.** Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions.* **11**: 591-592.
- Martinez, D. E., Bartoli, C. G., Grbic, V. and Guamet, J. J. 2007.** Vacuolar cysteine proteases of wheat (*Triticum aestivum* L.) are common to leaf senescence induced by different factors. *J. Exp. Bot.* **58**: 1099-1107.
- Merewitz, E. B., Gianfagna, T. and Huang, B. 2011.** Photosynthesis, water use, and root viability under water stress as affected by expression of SAG12-ipt controlling cytokinin synthesis in *Agrostis stolonifera*. *J. Experimental Botany.* **62**: 383-395.
- Nagar, S., Ramakrishnan, S., Singh, V. P., Singh, G. P., Dhakar, R., D. K., Umesh, D. K. and Arora, A. 2014.** Cytokinin enhanced biomass and yield in wheat by improving N-metabolism under water limited environment. *Ind. J. Plant Physiol.* DOI 10.1007/s40502-014-0134-3.
- Nair, T. V. R. and Abrol, Y. P. 1973.** Nitrate reductase activity in developing wheat ears. *Experientia.* **29**: 1480-1491.
- Patel, P. K. and Hemantaranjan, A. 2013.** Differential sensitivity of chickpea genotypes to salicylic acid and drought stress during pre-anthesis: effects on total chlorophyll, phenolics, seed protein and protein profiling. *The Ecoscan.* **8(2)**: 569-574.
- Peleg, Z., Reguera, M., Tumimbang, E., Walia, H. And Blumwald, E. 2011.** Cytokinin mediated source/sink modifications improve drought tolerance and increase grain yield in rice under water-stress. *Plant Biotechnol. J.* **9**: 747-758.
- Pervaiz, Z., Hussain, K., Gill, S. S. H. and Sheikh, A. A. 2003.** Iron requirement of Barani wheat. *Int. J. Agri. Biol.* **5(4)**: 608-610.
- Pospisilova J., Synkova, H. and Rulcova, J. 2000.** Cytokinins and water stress. *Biologia Plantarum.* **43**: 321-328.
- Reguera, M., Peleg, Z., Abdel-Tawab, Y. M., Tumimbang, E. B., Delatorre, C. A. and Blumwald, E. 2013.** Stress-Induced Cytokinin Synthesis Increases Drought Tolerance through the Coordinated Regulation of Carbon and Nitrogen Assimilation in Rice. *Plant Physiol.* **163**: 1609-1622.
- Rivero, R. M., Kojima, M., Gepstein, A., Sakakibara, H., Mittler, R., Gepstein, S. and Blumwald, E. 2007.** Delayed leaf senescence induces extreme drought tolerance in a flowering plant. *Proc. Natl. Acad. Sci., USA* **104**: 19631-19636.
- Sairam, R. K., Deshmukh, P. S. and Shukla, D. S. 1997.** Tolerance to drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. *J. Agron. Crop. Sci.* **178**: 171-177.
- Sayd, S. S., Taie, A. A. H. and Taha, L. S. 2010.** Micropropagation, antioxidant activity, total phenolics and flavonoids content of *Gardenia jasminoides* ellis as affected by growth regulators. *Int. J. Acad. Res.* **2**: 184-191.
- Sinclair, T. R., Pinter, P. J., Kimball, B. A., Adamsen, F. J., LaMorte, R. L., Thompson, T., Leavitt, S. and Mattias, A. 2000.** Leaf nitrogen concentration of wheat subjected to elevated (CO<sub>2</sub>) and either water or N deficits. *Agr. Ecosyst. Environ.* **79**: 53-60.
- Singh, A. K., Singh S. K., Garg, H. S., Kumar R., 1 and Choudhary R. 2014.** Assessment of relationships and variability of morphophysiological characters in bread wheat (*Triticum aestivum* L.) under drought stress and irrigated conditions. *The Ecoscan.* **9(2)**: 473-484.
- Singh, D. V., Srivastava, G. C. and Abdin, M. Z. 2001.** Amelioration of negative effects of water stress in *Cassia angustifolia* by benzyladenine and/or ascorbic acid. *Biol. Plant.* **44**: 141-143.
- Tingey, D. T. and Stockwell, C. 1977.** Semipermeable membrane system for subjecting plants to water stress. *Plant Physiol.* **60(1)**: 58-60.
- Tunio, S. D., Korejo, M. N., Jarwar, A. D. and Waggan, M. R. 2006.**

Studies on indigenous and exotic weed competition in wheat. *Pak. J. Agri. Biol.* **5(4)**: 1-8.

**Vlekova, A., Spundova, M., Kotabova, E., Novotny, R., Doleal, K. and Naus, J. 2006.** Protective cytokinin action switches to damaging during senescence of detached wheat leaves in continuous light. *Physiol. Plantarum.* **126**: 257-267.

**Vyroubalova, S., Vaclavíkova, K., Tureckova, V., Novak, O., Smečilova, M., Hluska T., Ohnoutkova, L., Frebort, I. and Galuszka, P. 2009.** Characterization of new maize genes putatively involved in cytokinin metabolism and their expression during osmotic stress in relation to cytokinin levels. *Plant Physiology.* **151**: 433-447.

**Weatherley, P. E. 1950.** Studies in the water relations of the cotton plant I. The field measurements of water deficit in leaves. *New Phytol.* **48**: 81-97

**Wingler, A., Purdy, S., MacLean, J. A. and Pourtau, N. 2006.** The role of sugar in integrating environmental signals during the regulation

of leaf senescence. *J. Exp. Bot.* **57**: 391-399.

**Wolters, H. and Jurgens, G. 2009.** Survival of the flexible: hormonal growth control and adaptation in plant development. *Nat. Genet.*, **10**: 305-317.

**Xu, Y., Tian, J., Gianfagna, T. and Huang, B. 2009.** Effects of SAG12-ipt expression on cytokinin production, growth and senescence of creeping bentgrass (*A. stolonifera* L.) under heat stress. *Plant Growth Regulation.* **57**: 281-291.

**Yang, J., Zhang, J., Wang, Z., Zhu, Q. and Liu, L. 2002.** Abscisic acid and cytokinins in the root exudates and leaves and their relationship to senescence and remobilization of carbon reserves in rice subjected to water stress during grain filling. *Planta.* **215**: 645-652.

**Zavaleta-Mancera, H. A., Thomas, B. J., Thomas, H. and Scott, I. M. 1999.** Regreening of senescent *Nicotiana* leaves. II. Redifferentiation of plastids. *J. Exp. Bot.* **50**: 1683-1689.