



## Effect of Ascorbic Acid Seed Priming on Agronomic and Physiochemical Traits of *Pisum sativum*

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**Abstract:** Pea (*Pisum sativum* L.) is one of the prime food ingredients of vegetarian meals and fulfills the nutritive commodities of the human individuals in the whole world. It also contains most of the essential nutrients like fibers and proteins. To approach whether the artificial application growth fastening substances like ascorbic acid, sorbitol, mannitol and polyethylene glycol etc. at development stages i.e., seed germination or emerging foliar or inflorescence buds would be valuable or not. The role of ascorbic acid through seed priming in pea (*Pisum sativum* L.) was analyzed in different cultivars of Pea plant i.e., Meteor and Classic. The morphological patterns were found to be enhancing under all levels ascorbic acid treatments. Basic biochemical components were also increased gradually in both varieties (Meteor and Classic) of pea. It was recommended that the application of growth promoting hormones or vitamins before sowing was helpful in crop plants to increase their qualitative and quantitative yields. It would be workable procedure to shield the economical plants against stresses.

**Keywords:** Ascorbic acid; Seed priming; Vegetative, Reproductive and biochemical features of Pea plants.

### 1. INTRODUCTION

Pea is cultivated as an annual legume as it is the part of human food and also of animal forage [1]. Its beans and silage for cattle are main source of proteins and mineral stuffing [2]. A recent survey polled out that pea is the one of the main crop vegetables cultivated in Pakistan fields roundly 10,478 hectares producing an amount of 7192 tons and normal average production of 6.9 tons hec-1 and major involvement of 71% of total production is from Punjab lands [3, 4]. There are many factors that are involved in declining the pea crop yield by affecting their vegetative characteristics and production in Pakistan [5]. Non-living stresses mean those environmental conditions like desiccation, water saturation, heat, cold, alkalinity, ozone accumulation, heavy metals, & depletion and UV radiations which are deleteriously affecting plants vegetative growth, quality of their harvests

like proteins etc. and quantitative yield [6]. Not only plants are suffered but also yield per acre is minimized and level of quality of their valuable chemicals like proteins, lipids and vitamins is declined by stressing concentrations of many factors like deficiency of water, saline soils or infectious pathogens or pests. Salt suffering lessens the aquatic uptake ability of plants and interrupts their biochemical metabolism [7].

Ascorbic acid (AsA) is a growth regulating mediator that encourages biotic developments and possesses antioxidant properties. Thus, it is utilized in very low levels to retain essential metabolic interactions and a normal growth development in plants [8]. AsA is present in every compartment of cell but greater concentrations are being determined in chloroplast. The most abundant uses of AsA were known in photosynthesis as enzyme co-factor and completely effective upon

cell growth. Ascorbate is considered as precursor of tartrate and oxalate in some species [9]. In expanding cells, ascorbate peroxidase of cell wall is determined in higher amounts [10]. AsA possesses a significant role to promote mitotic cell proliferation and in growing phases of plants [9]. Phytohormone facilitated signaling mechanisms are reinforced by AsA during the transition from vegetative stage to the reproductive stage in life cycle and proceeds to growth and developmental final phases [11]. It excites the action of nutritional cyclic metabolic pathways in plants and has a significant working in electron transport system [12]. Exogenous or pre-sowing treatments of AsA or any other bio-molecules i.e., poly ethylene glycol, sorbitol, mannitol, indole acetic acid and GA was explored and termed as 'short cut method' to make plants tolerant particularly wheat crop against certain environmental harsh nesses [13, 14, 15]. It participates as activators of many basic enzymes of large number of biochemical changes [16, 17]. Utilization of AsA in cold time of the year is responsible for the production of unusual promotion in sizes and weights of leaves, stems, flowers, fruits and total crop yield of tomato plants [18]. Foliar implementation of AsA worthily accelerates the increase in amount of N, P and K of wheat (*Triticum vulgare*) leaf and grain in kernels relatively to control [19]. Ascorbic Acid plays a cooperative role in the manufacturing of a deal of phytohormones, as well as in the biosynthesis of ethylene, jasmonic acid, salicylic acid, abscissic acid and gibberallic acid. Therefore, it is emphasized that the AsA not only affecting the bioproduction but also carry out a considerable role in the initiating of plant growth promoting hormones in huge levels during stress situations. Therefore, AsA can be applied to improve the growth and yield of plants (crops) not only qualitatively but also quantitatively and is also of worth considerations to help the plants in interactions with surrounding environmental stresses [20]. The above concerning study revealed that ascorbic acid functions as stress tolerant specifically exposure to heavy metal stress. This reflects the capacity of ascorbic acid as stress tolerant substance and also growth stimulator. Present investigations were commenced to highlight the role and function of ascorbic acid in fluctuations of morphological, reproductive and physiochemical properties in pea plants at their flowering and fruit production stages.

## 2. MATERIALS AND METHODS

Seeds of pea cultivars meteor and classic were taken from Ayub Agricultural Research Institute (AARI) Vegetables Section, Faisalabad, Pakistan. Seed priming with four concentrations of ascorbic acid were made as; No priming (control), Priming in distilled water, priming in 0.1mM Ascorbic acid, Priming in 0.2mM Ascorbic acid. Seeds were kept in dark place for overnight. Plastic vessels were filled with soil by making equal volume of soil in each pot and nine seeds were sown in each pot. 03 replicates for each ascorbic acid treatment were established in completely randomized design throughout the experiment. After 120 days of applying treatment, sample of fresh plants were reaped. Samples were stored at -20°C. Vegetative parameters like length of shoot in centimeter, length of root in centimeter, fresh root weight in gram, shoot fresh weight in gram and dry shoot and dry root weights in gram were measured at all levels of ascorbic acid. Shoot and root dry weights in grams were determined following the drying the sample plants in oven at 70°C for 6-days.

Reproductive measures e.g. pods per plant, seeds per plant and mass of 100 seeds per plant (g) of fresh plants were calculated. Estimation of bio chemical substances was carried out to check the qualitative yield under ascorbic acid treatments. Total free amino acids were calculated [21]. The amounts of reducing sugar residues will be determined according [22]. Total soluble protein contents were quantified by using method of Bradford [23]. Soluble sugars were investigated through the method of [24]. The significant or non-significant differences among various factors were determined with the help of one way analysis of variance (ANOVA). The statistical analysis of present experiment was determined by the use of computer software COSTAT (CoHort Software2003, Monterey, California).

## 3. RESULTS AND DISCUSSION

Vegetative growth attributes viz.; length of shoot, length of root, shoot and root fresh weights, shoot dry weight and root dry weight in both varieties of pea plants showed exclusive and astonishing improvements in plants whose seeds were soaked with 0.1mM and 0.2 mM ascorbic acid. However,

all the above-mentioned growth parameters were measured well in classic compared to meteor by using distilled water and ascorbic acid (0.2mM) as treatment (Fig.1).

The treatment of seeds with distilled water and ascorbic acids (0.1mM and 0.2mM) were also influencing positively on reproductive characters i.e., pods per plant, seeds per plants weight (grams) of 100 seeds in both meteor and classic varieties (Fig. 2).

In meteor and classic pea plant varieties the contents of free amino acids were stepped up with respect to soaking treatments of seeds with distilled water and ascorbic acids (0.1mM and 0.2mM) which boosted the free amino acids level in both meteor and classic varieties. However, the much concentration of free amino acids was harvested

in classic than meteor when using distilled water and ascorbic acid (0.2mM) as treatment. It also amplified the reducing sugars level in both meteor and classic varieties. However, much concentrations of reducing sugars were observed in classic than meteor when using distilled water and ascorbic acid (0.2mM) as treatment. Total soluble proteins were also found to be maximizing at all levels of treatments but much quantity was observed in classic variety relatively to meteor. In meteor and classic pea plant varieties, the experimental soaking treatment of seeds with distilled water and ascorbic acid (0.1mM and 0.2mM) also synchronized the biochemical reactions in pea which increased the total soluble sugars concentrations. However, the enhanced observations of total soluble sugars content were recorded in classic with respect to meteor when using distilled water and Ascorbic Acid (0.2mM) as treatment (Fig. 3).

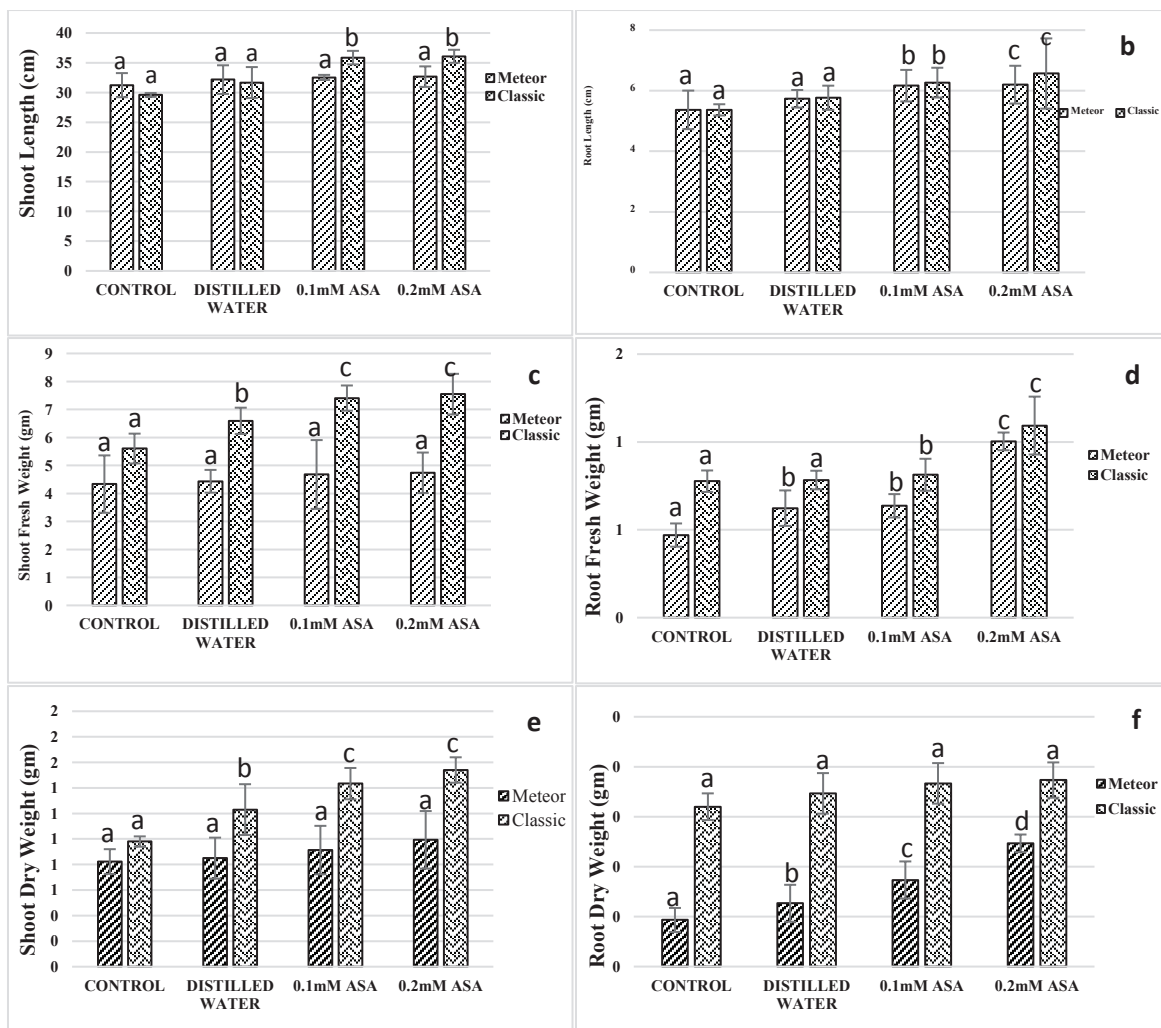
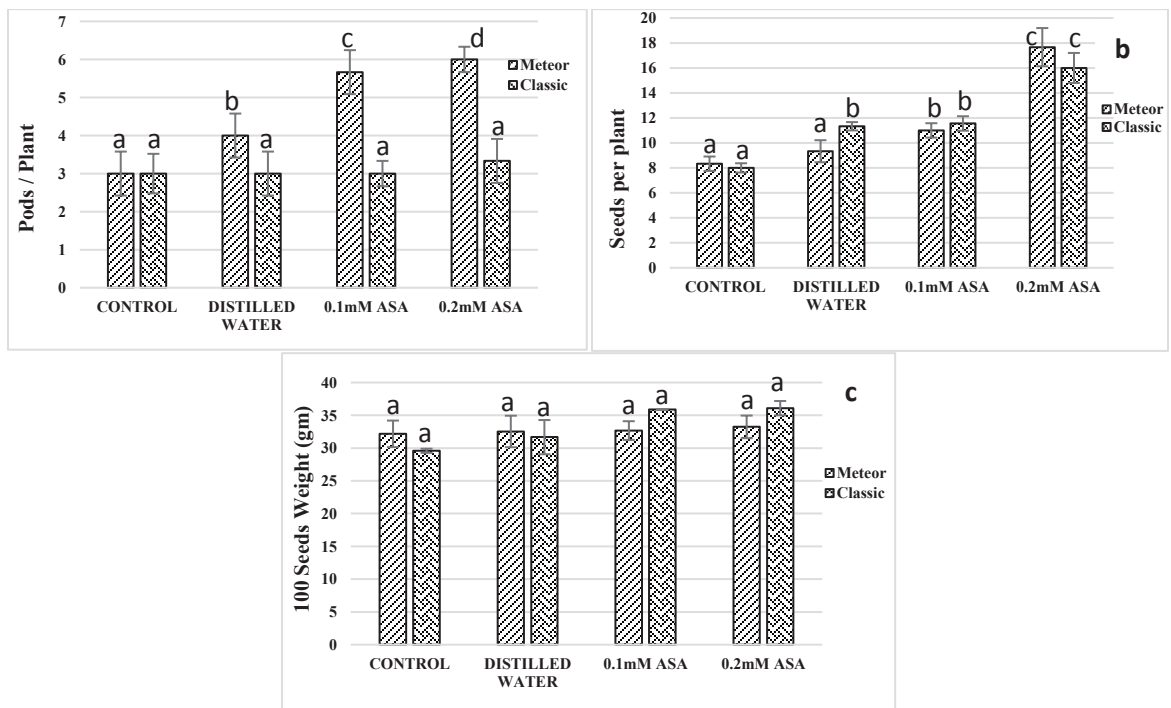
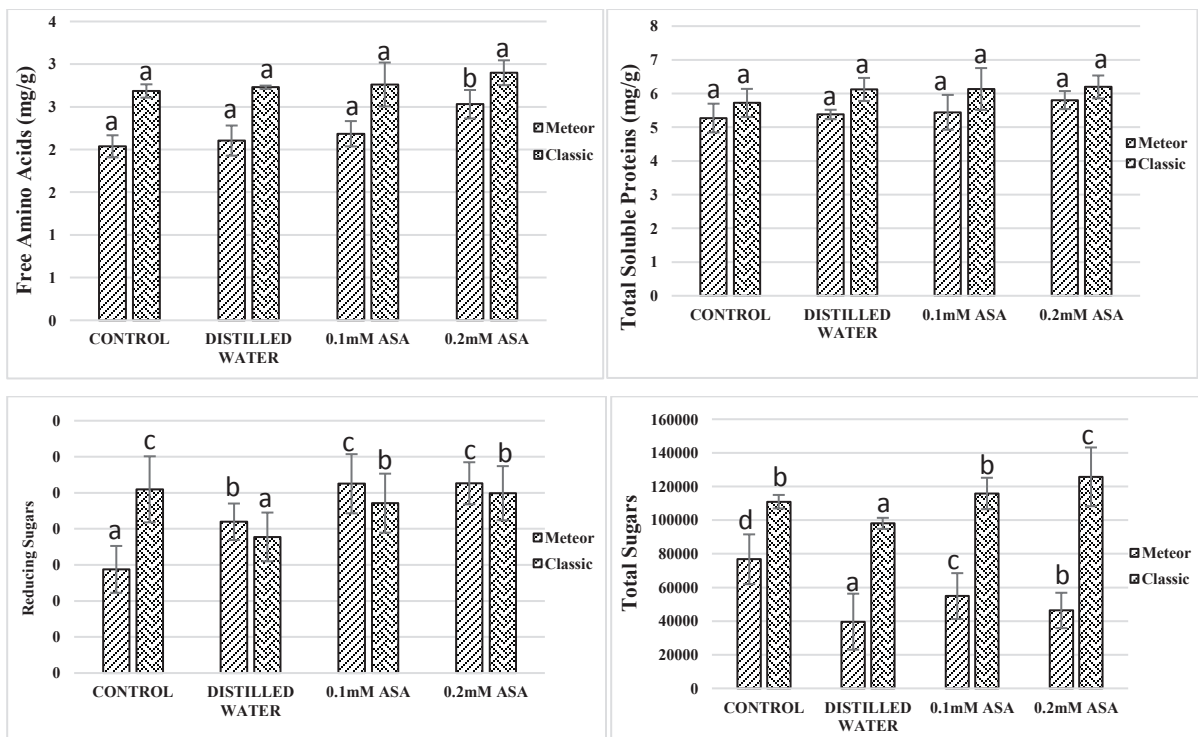


Fig. 1. Enhancing effects on (a): shoot length, (b): root length, (c): shoot fresh weight (d): root fresh weight, (e): shoot dry weight, (f): root dry weight of *P. sativum* by seed priming with Ascorbic acid.



**Fig. 2.** Enhancing effects on (a): pods per plant, (b): seeds per plant, (c): 100 seeds weight of *P. sativum* by seed priming with various levels of Ascorbic acid.



**Fig. 3.** Enhancing effects on (a): free amino acid, (b): total soluble proteins, (c): reducing sugars, (d): total sugars of *P. sativum* by seed priming with various levels of Ascorbic acid

Kitchen vegetables in fields are prone to abiotic stresses like salinity, waterlogging, mineral deficiency and heavy metal stresses through irrigation of sewage water or sewage wastes dumped into agricultural fields. Some of them adversely affected the growth, physiology and also biochemical processes of plants [25, 26]. During this experimental inquiry, the seeds of two cultivars of pea (meteor and classic) were exposed to four different levels of AsA treatments.

The research exhibited the effects of AsA seed treatment on the different agronomic parameters at the reproductive phase i.e., root and shoot length, root and shoot fresh biomass, shoot and grams of dry root, fruits per plant, beans per plant and mass of 100 seeds per plant were found to be enhanced with the levels of AsA treatments of seeds. It had been reported that supplemented ascorbic acid mitigated the negative effects of abiotic stress [27]. In addition, total protein, total free amino acids and total soluble sugars were decreased in Meteors compared to pea Classic. Ascorbate acts as antioxidant which increased in tolerant variety. There was a decrease in ascorbate content in vulnerable condition in varieties of cowpea when the levels of water stress were increased [28].

Increased ascorbate accumulation was studied by Jaleel et al. [29] in *Withania somnifera* under water stress. Improvements in the accumulation of ascorbic acid and increase in GR regulated ascorbate-glutathione cycle which was considered as effective mechanism of ROS detoxification. Increased total soluble protein, and total free amino acids molecules accumulation was also recorded in bean plants [30].

#### 4. CONCLUSION

The main focus of the present study was to find out potentials of seed priming with ascorbic acid on the quantitative and qualitative characteristics of pea plants. It was observed that vegetative attributes like shoot and root length, fresh and dry shoot and root weights were promoted under the influence of all levels of ascorbic acid. Such preliminary treatments also enhanced greatly the reproductive characters like pods per plants, seeds per plants and weight of 100 seeds in both cultivars of pea. A great increase in biochemical like free amino

acids, total soluble proteins, reducing sugars and total sugars in fresh leaves were found in response to ascorbic acid treatments. It was recommended that applications of growth supporting chemicals like ascorbic acid, sorbitol, mannitol etc. through different routes whether it is seed priming or foliar applications would help plants like pea plants not only to enhance their qualitative and quantitative yields but also endows the capability of combating against abiotic stresses, natural or human generated pollutants viz., droughts, mineral deficiencies in soils, waterlogging, salinity, global warming, heavy metal smog and acid rains etc.

#### 5. REFERENCES

1. McKenzie, D.B. & D. Spooner. White Lupin: An alternative to pea in oat-legume forage mixtures grown in New Foundland. *Canadian Journal of Plant Science* 79: 43-47 (1999).
2. Acikgoz, E., V. Katkat, S. Omeroglu, & B. Okan. Mineral elements and amino acid concentrations in field pea and common vetch herbage and seeds. *Journal of Agronomy and Crop Science* 55: 179-185(1985).
3. Anonymous. (1999). Fruit, Vegetables and Condiments Statistics of Pakistan. Government of Pakistan, Ministry of Food, Agriculture and Livestock, Economics Wing, Islamabad.
4. Achakzai, A.K.K. & M. I. Bangulzai, Effect of various levels of nitrogen fertilizer on the yield and yield attributes of pea (*Pisum sativum* L.) cultivars. *Pakistan Journal of Botany* 38(2): 331(2006).
5. Hussain, S.I., T. Mahmood, K. M. Khokhar, M. H. Laghari & M. H. Bhatti. Screening of pea germ plasm for yield and resistance towards powdery mildew. *Asian Journal of Plant Science* 1(3): 230-231(2002).
6. Hasanuzzaman, M., K. Nahar & M. Fujita. Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In: *Ecophysiology and responses of plants under salt stress* (pp. 25-87). Springer New York (2013).
7. Munns, R. Comparative physiology of salt and water stress. *Plant Cell and Environment* 25(2): 239-250 (2002).
8. Podh, H. Cellular functions of ascorbic acid. *Biochemistry and Cell Biology* 68: 1166-1173(1990).
9. Smirnoff, N. & L. G. Wheeler. Ascorbic Acid in plants: Biosynthesis & function. *Critical Review in Biochemistry and Molecular Biology* 35: 291-

- 314(2000).
10. Noctor, G. & C. H. Foyer. Ascorbate and glutathione: Keeping active oxygen under control. *Plant Molecular Biology* 49: 249-279 (1998).
  11. Barth, C., M. D. Tullio & P. L. Conklin. The role of Ascorbic Acid in the control of flowering time and the onset of senescence. *Journal of Experimental Botany* 57: 1657-1665 (2006).
  12. Liu, W., W. Y. Hu, J. J. Hao & G. Chen. The relationship between ascorbic acid and changes of several physiological and biochemical indexes in isolated wheat leaves under NaCl stress. *Plant Physiology* 33: 423-425(1997).
  13. Ashraf, M., H. R. Athar, P. J. C. Harris & T. R. Kwon, Some prospective strategies for improving crop salt tolerance. *Advances in Agronomy* 97:115-127 (2008).
  14. Al-Hakimi, A.M. & A. M. Hamada Counteraction of salinity stress on wheat plants by grain soaking in ascorbic acid, thiamin or sodium salicylate. *Biological Plantar* 44: 253-261(2001).
  15. Wahid, A., M. Perveen, S. Gelani & S. M. A. Basra. Pretreatment of seed with H<sub>2</sub>O<sub>2</sub> improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. *Journal of Plant Physiology* 164: 283-294 (2007).
  16. Belanger, F.C., A. T. Leustek, Chu-BoYang, & L. Kriz. Evidence for the thiamine biosynthetic pathway in higher-plant plastids and its developmental regulation. *Plant and Molecular Biology* 29: 809-821(1995).
  17. Arrigoni, O. & M. C. de Tullio. The role of ascorbic acid in cell metabolism between gene-directed functions and unpredictable chemical reactions. *Journal of Plant Physiology* 157:481-488 (2000).
  18. El-Greadly, N.H.M. Effect of foliar application of ascorbic acid, ethrel and their combinations on growth, yield and endogenous hormones in cucumber plants. *The Journal of Agricultural Science* 27: 5269-5281(2002).
  19. Abdel-Hameed, A.M., S. H. Sarhan & H. Z. Abdel-Salam. Evaluation of some organic acid as foliar application on growth, yield and some nutrient contents of wheat. *The Journal of Agricultural Science* 20: 2476-2481(2004).
  20. El-Mashad, A.A. & H. I. Mohamed. Brassino lide alleviates salt stress and increases antioxidant activity of cowpea plants (*Vigna sinensis*). *Journal of Stress Physiology and Biochemistry* 7:222-234(2011).
  21. Hamilton, P.B. & D. D. Van Slyke. The gasometric determination of free amino acids in blood filtrates by the ninhydrin-carbon dioxide method. *Journal of Biological Chemistry* 150(1): 231-250 (1943).
  22. Henson, C.A. & J. M. Stone. Variation in  $\alpha$ -amylase and  $\alpha$ -amylase inhibitor activities in barley malts. *Journal of Cereal Science* 8(1): 39-46 (1988).
  23. Bradford, M.M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Annals of Biochemistry* 72(1): 248-254 (1976).
  24. Dubois, M., K. A. Gilles, J. K. Hamilton, P. Rebers & F. Smith. Colorimetric method for determination of sugars and related substances. *Annals of Chemistry* 28(3): 350-356 (1956).
  25. Smeets, K., A. Cypers, A. Lamrechts, B. Semane, P. Hoet, A.V. Laere & J. Vangronsveld. Induction of oxidative stress and anti-oxidative mechanisms in *Phaseolus vulgaris* after Cd application. *Plant Cell Physiology and Biochemistry* 43: 437-444(2005).
  26. Clemens, S. Toxic metal accumulation, response to exposure and mechanism of tolerance in plants. *Journal of Biochemistry* 88: 1707-1719 (2006).
  27. Afzal, I., S. M. A. Basra, N. Ahmad & M. Farooq. Optimization of hormonal priming techniques for the alleviation of salinity stress in wheat (*Triticum aestivum* L.). Pontifical Catholic University of Rio Grande do Sul. 17:95-109 (2005).
  28. Nair, A.S., T. K. Abraham & D. S. Jaya. Studies on the changes in lipid peroxidation and antioxidants in drought stress induced cowpea (*Vigna unguiculata* L.) varieties. *Journal of Environmental Biology* 29: 689-691(2008).
  29. Jaleel, C.A., R. Gopi & B. Sankar. Studies on germination, seedling vigour, lipid peroxidation, and proline metabolism in *Catharanthus roseus* seedlings under salt stress. *South African Journal of Botany* 73: 190-195 (2007).
  30. Zengin, K.F. & O. Munzuroglu. Effects of some heavy metals on content of chlorophyll, proline & some antioxidant chemicals in bean (*Phaseolus vulgaris* L.) seedlings. *Acta Biologica Cracoviensis Series Botanica* 41: 157-164 (2005).