



Influence of ascorbic acid seed priming on physico-chemical attributes of pea

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Abstract

Pea (*Pisum sativum* L.) is most important food ingredient of vegetarian meals and fulfills the nutritive value (fibers and proteins) across the whole world. For this, different concentration of ascorbic acid through seed priming (No priming (control), Priming in distilled water, priming in 0.1 mM Ascorbic acid and Priming in 0.2 mM Ascorbic acid) in pea was used in different cultivars of pea plant i.e., Metroer and Classic. The morphological patterns were found to be enhancing under all levels ascorbic acid treatments except control. Basic biochemical components were also increased gradually in both varieties (Meteor and Classic) of pea. It was concluded that the application of growth promoting hormones or vitamins before sowing was helpful in pea plants to increase their qualitative and quantitative yields traits. It would be workable procedure to shield the economical plants against stresses.

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Introduction

Pea is cultivated as an annual legume crop and part of human food and animal forage (McKenzie and Spooner, 1999). Its beans and silage for cattle are main source of proteins and mineral stuffing (Acikgoz *et al.*, 1985). A recent survey polled out that pea is the one of the main vegetable crop 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 (Anon, 1999; Achakzai and Bangulzai, 2006). There are many factorsthat are involved in declining the pea crop yield by affecting their vegetative characteristics and production in Pakistan (Hussain *et al.*, 2002). 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 (Hasanuzzaman, 2013). Salt suffering lessens the aquatic uptake ability of plants and interrupts their biochemical metabolism (Munns, 2002).

Ascorbic acid (AsA) is growth regulating mediator which encourages many biotic developments and have antioxidant properties. Thus, it is utilized in very low levels to retain essential metabolic interactions and normal growth developments in plants (Podh, 1990). 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 (Smirnoff and Wheeler, 2000). In expanding cells, ascorbate peroxidase of cell wall is determined in higher amounts (Nocter and Foyer, 1998) AsA possesses a significant role to promote mitotic cell proliferation and in growing phases of plants (Smirnoff and Wheeler, 2000). 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 (Barth *et al.*,

2006). It excites the action of nutritional cyclic metabolic pathways in plants and has a significant working in electron transport system (Liu *et al.*, 1997). 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 harshnesses (Al-Hakimi and Hamada, 2001; Wahid *et al.*, 2007; Ashraf *et al.*, 2008). It participates as activators of many basic enzymes of large number of biochemical changes (Belanger *et al.*, 1995; Arrigoni and deTullio, 2000). 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 cropyield of tomato plants (El-Greadly, 2002). Foliar implementation of AsA worthily accelerates the increase in amount of N, P and K of wheat leaf and grain in kernels relatively to control (Abdel-Hameed *et al.*, 2004). Ascorbic Acid plays a cooperative role in the manufacturing of a deal of phyto-hormones, 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 bio-production 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. (Khan *et al.*, 2011; El-Mashad and Mohamed, 2011).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 highlighttherole and function of ascorbic acid in fluctuations of morphological, reproductive and physiochemical properties in pea plants at their flowering and fruit production stages.

Materials and methods

Seeds of pea cultivars (meteor and classic) were taken from Ayub Agricultural Research Institute (AARI), Vegetables Section, and 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. Three replicates for each ascorbic acid treatment were established in completely randomized design (CRD) throughout the experiment. Ten days after germination, healthy plants were allowed to grow in plastic vessels. During the experimental period, distilled water was used to maintain moisture contents at field capacity in all the pots. 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 & 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 of fresh plants were calculated. Estimation of biochemical substances were carried out to check the qualitative yield under ascorbic acid treatments. Total free amino acids were calculated as prescribed by Hamilton and Van Slyke (1943). Total soluble protein contents were quantified by using method of Bradford (1976). Soluble sugars were investigated through the method of Dubois *et al.*, (1956).

Statistical analysis

Analysis of variance (ANOVA) was based on two factorial completely randomized design (CRD). The significant or non-significant differences among

various factors were determined with the help of Analysis of Variance. The statistical analysis of present experiment was determined by the use of computer software COSTAT (CoHort Software2003, Monterey, California). For graphically presentation of data, MS-Excel was used in the experiment.

Results

Growth attributes

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 better improvements in plants whose seeds were soaked with 0.1mM and 0.2mM ascorbic acid. However, all the above mentioned growth parameters were measured good in classic compared to meteor by using distilled water and ascorbic acid (0.2mM) as treatment (Fig.1).

Yield attributes

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).

Nutritional attributes

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. 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.

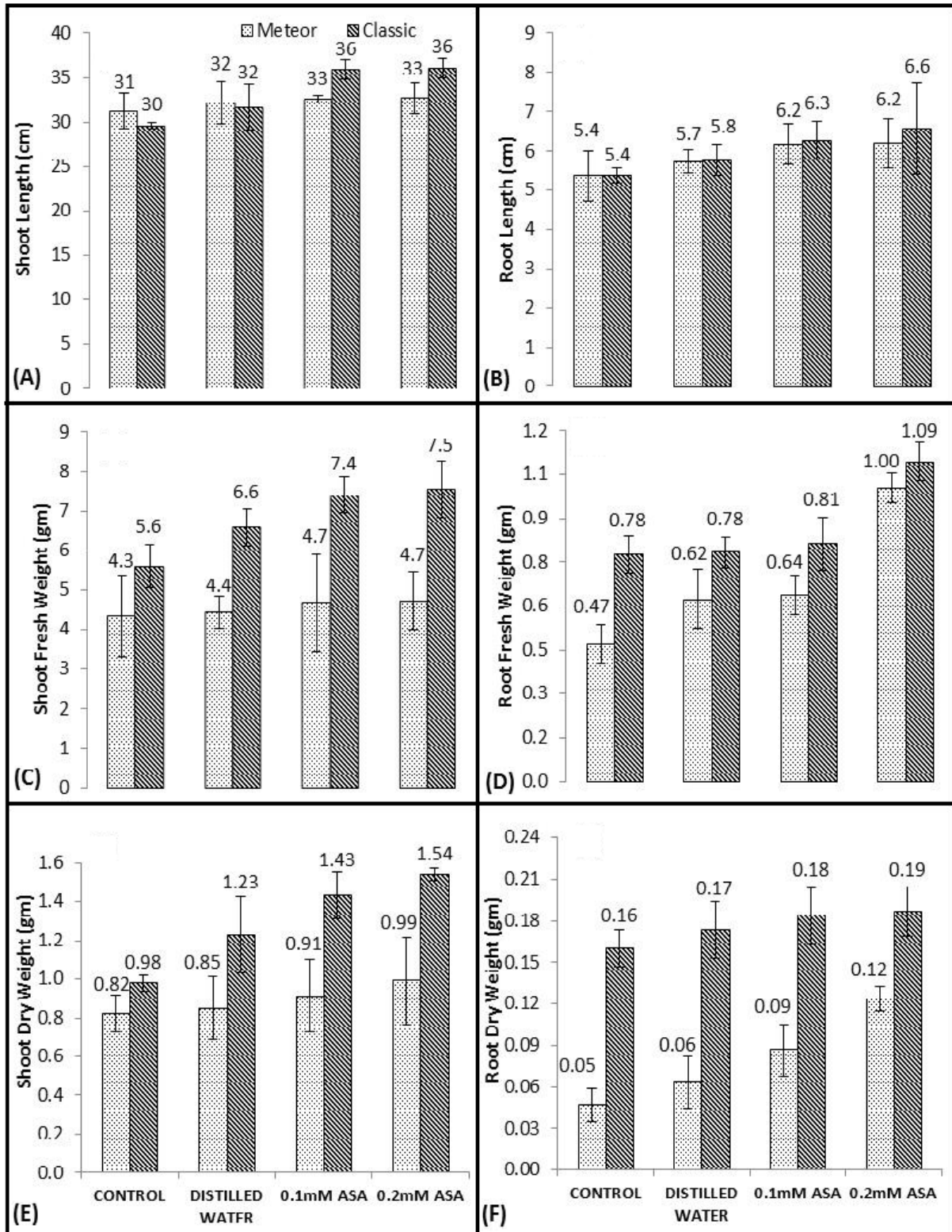


Fig. 1. Enhancing effects on different growth attributes of *Pisum sativum* L. by seed priming with various levels of Ascorbic acid (AsA).

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).

Discussion

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 (Smeets *et al.*, 2005; Clemens, 2006).

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 growth and yield (Figure 1 and 2) 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 (Afzal *et al.*, 2005).

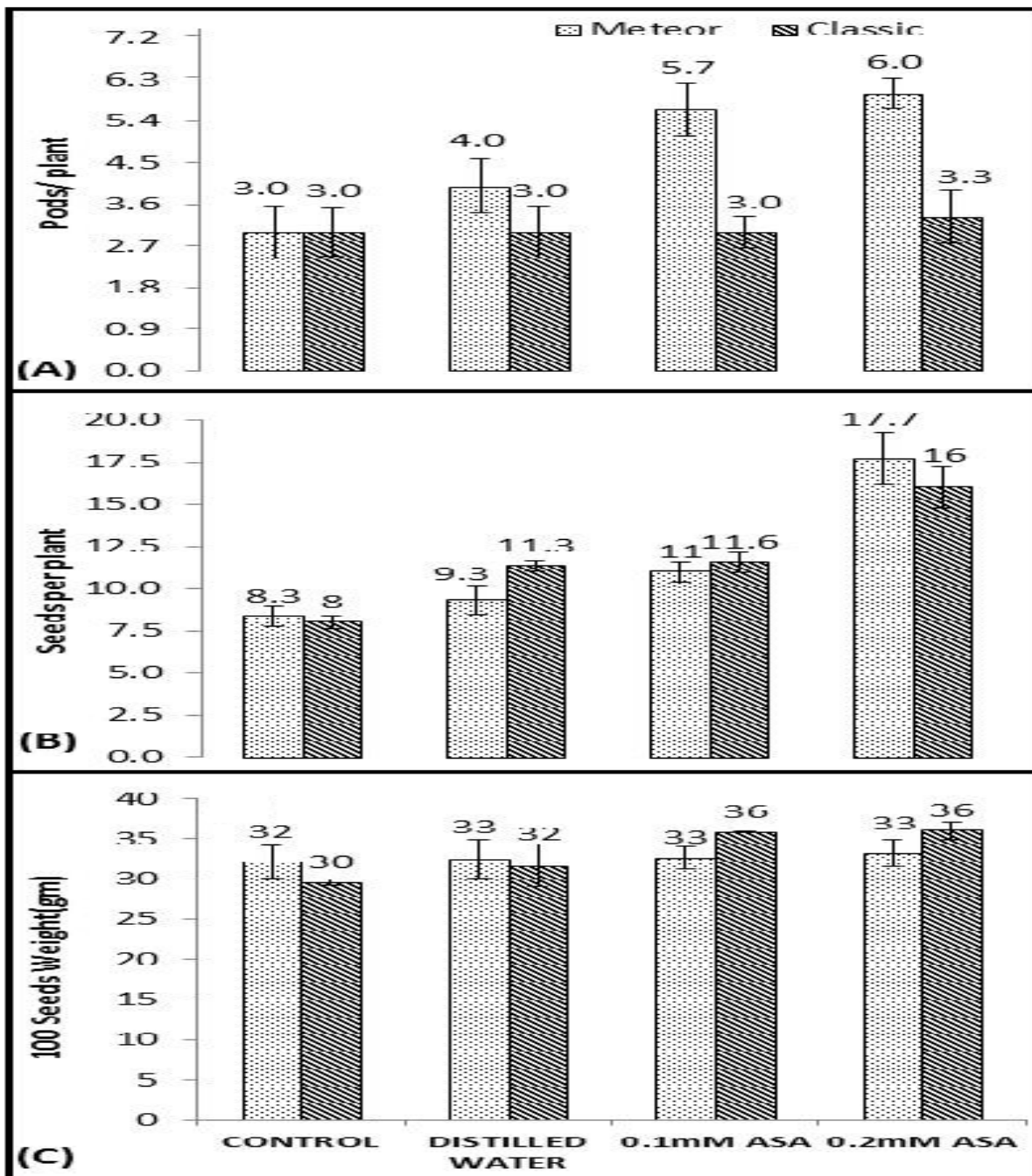


Fig. 2. Enhancing effects on yield attributes of *Pisum sativum* L. by seed priming with various levels of Ascorbic acid (AsA).

In addition, free amino acids, total soluble protein and total sugars were decreased in Meteors compared to pea Classic (Figure 3). Ascorbate acts as antioxidant which increased in tolerant variety. According to Nair *et al.* (2008) there was a decrease in ascorbate content in vulnerable condition in varieties of cowpea when the levels of water stress were increased. Increased ascorbate accumulation was studied by Jaleel (2007) 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 by Zengen and Munzuroglu (2005).

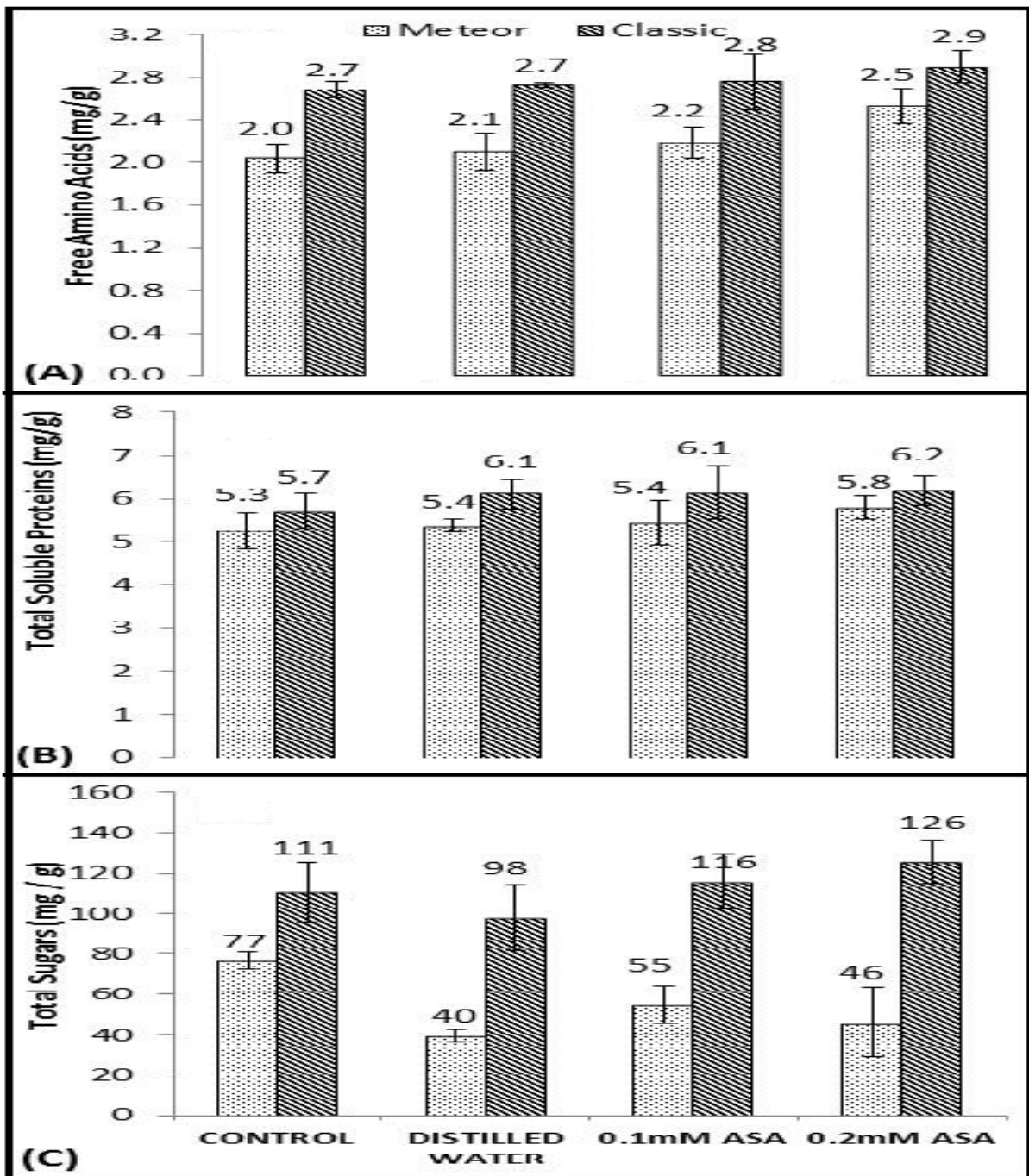


Fig. 3. Enhancing effects on nutritional attributes of *Pisum sativum* L. by seed priming with various levels of Ascorbic acid (AsA).

Conclusion

It was observed that vegetative and yield attributes were improved under the influence of all levels of ascorbic acid. A great rising in biochemical like free amino acids, total soluble proteins 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 by seed priming application would help 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.

References

- Abdel-Hameed AM, Sarhan SH, Abdel-Salam HZ.** 2004. Evaluation of some organic acid as foliar application on growth, yield and some nutrient contents of wheat. The Journal of Agricultural Sciences **20**, 2476-2481.
- Acikgoz E, Katkat V, Omeroglu S, Okan B.** 1985. Mineral elements and amino acid concentrations in field pea and common vetch herbage and seeds. Journal of Agronomy and Crop Sciences **55**, 179-185.
- Achakzai AKK, Bangulzai MI.** 2006. Effect of various levels of nitrogen fertilizer on the yield and yield attributes of pea (*Pisum sativum* L.) cultivars. Pakistan Journal of Botany **38**, 331-340.
- Afzal I, Basra SMA, Ahmad N, Farooq M.** 2005. 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.
- Ahmad K, Ejaz A, Azam M, Khan IZ, Ashraf M, Al-Qurainy F, Fardous A, Gondal S, Bayat RA, Valeem EE.** 2011. Lead, cadmium and chromium contents of canola irrigated with sewage water. Pakistan Journal of Botany **43**, 1403-1410.
- Arrigoni O, de Tullio MC.** 2000. The role of ascorbic acid in cell metabolism between gene-directed functions and unpredictable chemical reactions. Journal of Plant Physiology **157**, 481-488.
- Al-Hakimi AM, Hamada AM.** 2001. Counteraction of salinity stress on wheat plants by grain soaking in ascorbic acid, thiamin or sodium salicylate. Biologia Plantarum **44**, 253-261.
- Anonymous.** 1999. Fruit, Vegetables and Condiments Statistics of Pakistan. Government of Pakistan, Ministry of Food, Agriculture and Livestock, Economics Wing, Islamabad.
- Ashraf M, Athar HR, Harris PJC, Kwon TR.** 2008. Some prospective strategies for improving crop salt tolerance. Advances in Agronomy **97**, 115-127.
- Barth C, Tullio MD, Conklin PL.** 2006. The role of Ascorbic Acid in the control of flowering time and the onset of senescence. Journal of Experimental Botany **57**, 1657-1665.
- Belanger FC, Leustek AT, Chu-BoYang Kriz L.** 1995. Evidence for the thiamine biosynthetic pathway in higher-plant plastids and its developmental regulation. Plant Molecular Biology **29**, 809-821.
- Bradford MM.** 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry **72**, 248-254.
- Clemens S.** 2006. Toxic metal accumulation, response to exposure and mechanism of tolerance in plants. Journal of Biochemistry **88**, 1707-1719.
- Dubois M, Gilles KA, Hamilton JK, Rebers P, Smith F.** 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry **28**, 350-356.
- El-Greadly NHM.** 2002. Effect of foliar application of ascorbic acid, ethrel and their combinations on

growth, yield and endogenous hormones in cucumber plants. *The Journal of Agricultural Sciences* **27**, 5269-5281.

El-Mashad AA, Mohamed HI. 2011. Brassinolide alleviates salt stress and increases antioxidant activity of cowpea plants (*Vigna sinensis*). *Protoplasma* **7**, 222-234.

Hamilton PB, Van Slyke DD. 1943. The gasometric determination of free amino acids in blood filtrates by the ninhydrin-carbon dioxide method. *Journal of Biological Chemistry* **150**, 231-250.

Hasanuzzaman M, Nahar K, Fujita M. 2013. 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.

Hussain SI, Mahmood T, Khokhar KM, Laghari MH, Bhatti MH. 2002. Screening of pea germ plasma for yield and resistance towards powdery mildew. *Asian Journal of Plant Science* **1**, 230-231.

Jaleel CA, Gopi R, Sankar B. 2007. 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.

Liu W, Hu WY, Hao JJ, Chen G. 1997. 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.

McKenzie DB, Spooner D. 1999. White Lupin: An alternative to pea in oat-legume forage mixtures grown in New Foundland. *Canadian Journal of Plant Sciences* **79**, 43-47.

Munns R. 2002. Comparative physiology of salt and water stress. *Plant, Cell and Environment* **25**, 239-250.

Nair AS, Abraham TK, Jaya DS. 2008. 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.

Noctor G, Foyer CH. 1998. Ascorbate and glutathione: Keeping active oxygen under control. *Plant Molecular Biology* **49**, 249-279.

Podh, H. 1990. Cellular functions of ascorbic acid. *Biochemistry and Cell Biology* **68**, 1166-1173.

Smeets K, Cypers A, Lamrechts A, Semane B, Hoet P, Laere AV, Vangronsveld J. 2005. Induction of oxidative stress and antioxidative mechanisms in *Phaseolus vulgaris* after Cd application. *Plant Physiology and Biochemistry* **43**, 437-444.

Smirnoff N, Wheeler LG. 2000. Ascorbic Acid in plants: Biosynthesis & function. *Critical Reviews in Biochemistry and Molecular Biology* **35**, 291-314.

Wahid A, Perveen M, Gelani S, Basra SMA. 2007. Pretreatment of seed with H₂O₂ improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. *Journal of Plant Physiology* **164**, 283-294.

Zengin KF, Munzuroglu O. 2005. Effects of some heavy metals on content of chlorophyll, proline & some antioxidant chemicals in bean (*Phaseolus vulgaris* L.) seedlings. *Acta Biologica Cracoviensia Series Botanica* **41**, 157-164.