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Production of proline and other biochemicals as stress measures in mycorrhizal, biotic and abiotic factors in *Luffa cylindrica*

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Abstract

Luffa is a genus of tropical and subtropical vines in the cucumber (Cucurbitaceae) family. Proline and other biochemical parameters effect was evaluated on bottle gourd (*Luffa cylindrica* (L.) Roem) in control, mycorrhizal and other biotic and abiotic soil components treated plants. Higher proline concentrations reflected in leaves of treated plants as compared with mycorrhizal treated plants. The decrease in shoot length, number of leaves and leaf area were observed in non mycorrhizal and plant treated with other biotic and abiotic stresses. Higher amount of proline was produced in non mycorrhizal and stressed plants in result of reduced availability of water and dry matter translocation to the shoots. The proline contents of *Luffa* leaves showed a vast difference in different treatments. It may be concluded here that proline is an indicator of environmental stresses imposed on plants.

Keywords: *Luffa cylindrica*, proline, biochemical parameters, biotic and abiotic stresses.

Several environmental stresses limit the agriculture production. Abiotic (water availability, temperature and nutrients) stresses affect the growth of higher plants. Among them, drought stress is a major abiotic factor limiting the growth and affecting the production of the plants (Kasuga *et al*., 1999). Beside, abiotic factors, plants under threat of viral, bacterial, fungal pathogens and nematodes. Parasitism is the only negative interaction between the plants and microbes. As parasites, the microbes, like bacteria, fungi, viruses and nematodes caused infections in the host plant leading to the development of disease and loss of commercial value in case the host plant is an agricultural crop.

Abiotic and biotic stresses were endangering plant survival. Plant system grows and develops to its maximum potential only under an optimum range of factors like water, temperature, light. Whenever, there was deficit among these factors, the consequence reduced growth and development (Griffiths & Parry, 2002; Jackson & Ram, 2003); difficult environmental conditions also adversely affect their metabolism, growth and yield.

Plant morphological and physiological characters showed multiple levels in response to abiotic stresses (Munns & Tester, 2008; Witcombe *et al*., 2008). Many inorganic and organic solutes accumulated in plants subjected to osmotic stress where high concentration of proline acted as solute for intercellular osmotic adjustment (Silveira *et al*., 2003). Proline accumulation is a universal response of plants to various stresses. Proline acts as an osmolyte and helps the plants to maintain tissue water potential under all kinds of stresses. Proline, as an osmo-protectant largely confined to the cytoplasm and mostly absent from the vacuole (Zhu *et al*., 1997). Proline protected protein structures against denaturation, stabilizes the integrity of cell membranes and phospholipids, also functions as a hydroxyl radical scavenger, or serves as an energy and nitrogen source. Phytoparasitic nematodes and vesicular arbuscular mycorrhizal (VAM) fungi frequently colonize root tissues, and both types of organisms display the spatial and temporal coincidence which increases the chances of interaction (Beltrano & Ronco, 2008). In plants stresses such as drought, salinity or pathogen attack a number of

metabolites and survival defense mechanisms were activated (Shulaev *et al*., 2008). Higher tolerance observed in mycorrhizal induced plants (Porcel *et al*., 2007). Since, proline accumulation in plants subjected to unfavorable environmental conditions indicated severity of stress. *Luffa* is a genus of tropical and subtropical vines in the cucumber (Cucurbitaceae) family. Therefore, proline and other biochemical parameters effect was evaluated in *Luffa cylindrica* as a measure of the stress in the greenhouse of National Nematological Research Centre (NNRC), University of Karachi, Karachi under natural conditions.

Biotic components

***Pseudomonas stutzeri*:** *Pseudomonas stutzeri* is a gram-negative, rod-shaped, motile and single polar-flagellated soil bacterium. It is a denitrifying bacterium and strain KC of *P. stutzeri* used for bioremediation of carbon tetrachloride. For the present study the bacterial inoculum was obtained from Department of Microbiology, Jinnah University for Women, Karachi, Pakistan.

Root-knot nematode: Root-knot nematode *Meloidogyne incognita* is one of the most important plant pathogen found throughout the world. Root-knot nematode larvae (J_2) infect plant roots, causing the development of root-knot that drains the plant's photosynthates and nutrients. Culture of root-knot nematode was maintained on brinjal plants at greenhouse of NNRC.

Vesicular arbuscular mycorrhizal (VAM) fungi: Mycorrhizae are indigenous to soil and plant rhizosphere and potential tools for sustainable agriculture. Mycorrhizal fungi improved plant physiology and soil quality by using the greater surface area. VAM fungal spores were cultured on maize plants at greenhouse of NNRC.

Abiotic components

Sodium chloride (NaCl): Plant growth was severely affected by high NaCl concentrations (Jouyban, 2012). The proline and sugar content was increased, but nitrate reductase activity and chlorophyll decreased in leaves.

Chromium nitrate $Cr(NO_3)_3$: Cr phytotoxicity appeared as inhibition of seed germination or early seedling development, reduction of root growth, leaf chlorosis and depressed biomass (Panda *et al*., 2003).

Materials and Methods

A comparative study was made on production of proline and other biochemicals as a measure of stress in mycorrhizal and other biotic and abiotic soil components in *L. cylindrica*. The plants were grown in green house at National Nematological Research Centre, University of Karachi. Seeds of *Luffa* were sterilized with $HgCl_2$ and then washed with distill water. The sterilized seeds were sown into pots filled with garden soil and irrigated after germination as per requirement of water.

Experimental design: Six sets of plants with six replicates each were placed in earthen pots filled with sandy-clay loam soil. Treatments were supplied 10 days alternately with Hoagland solution. The pots were arranged in a complete randomized block design. After 15 days different treatments were applied separately in each pot.

Treatments: i) C = Control, ii) T_1 = VAM fungi @ 500 spores/pot, iii) T_2 = *Pseudomonas stutzeri* (150 ml), iv) T_3 = nematodes @1000 J_2/pot, v) sodium chloride (150 ppm) and vi) chromium nitrate (150 ppm).

Physical parameters: Plants were taken out at the end of experiment for physical parameters: i) length of plant; ii) area of leaves and iii) number of leaves.

Biochemical analysis

Carbon Profile: Estimation of total carbohydrates (Yemm & Willis 1954), glucose, sucrose and total soluble sugar (Riazi *et al*., 1985).

Nitrogen profile: Estimation of total protein (Lowry *et al*., 1951), amino acid (Moore & Stein, 1948), proline (Bates *et al*., 1973), protease (Prisco *et al*., 1975) and nitrate reductase (NO_3) (Bordon, 1984).

Statistical analysis: Statistical analysis was performed by the procedure followed by Gomez & Gomez (1984).

Results and Discussion

Physical parameters under biotic and abiotic stresses: Physical parameters viz., shoot length, number of leaves and leaf area significantly increased in mycorrhizal plants as compared to control, while under other biotic stresses i.e., bacteria and nematodes and abiotic stresses i.e., salinity and heavy metal showed a drastic decrease in all physical parameters, negative effect of heavy metal was more pronounced than other stresses (Table 1).

Water stress produced by bacteria, nematodes, salinity and heavy metal which induce water levels reduction in plant tissue and photosynthesis, synthesis of protein, N metabolism, leaf elongation and properties of cell membrane (Shangguan *et al*., 2000).

Chromium nitrate: *Luffa* treated with chromium nitrate $Cr(NO_3)_3$ showed drastic effects on growth; decrease in height and leaves size. The leaves also became dark green in color. The shoots were decreased and weak as the concentration of chromium nitrate was increased from 100 to 200 ppm (Table 1). The growth was severely affected by necrosis, chlorosis, curling and burning from edges of leaves. Tomato plants growth was reduced due to the presence of chromium (Moral *et al*., 1995). Cr inhibited shoot growth in lucerne (Barton *et al*., 2000), impaired metabolic activities (Kimbrough *et al*., 1999).

Sodium chloride: Sodium chloride (NaCl) salt showed effects on the leaves. Leaves become soften thin and short sized. Plant treated with salt did not attain full length as compared to the control plants (Table 1). The rate of flowering was very low, a flower arises after three month as only a flower observed in 100 and 150 ppm of NaCl each and no flowering at 200 ppm of NaCl. Salinity reduced sorghum growth and biomass (Ibrahim, 2004) and leaf area about 86% (Ali *et al*., 2009). Salinity negatively affects growth and production of various crops such as tomato (Tantawy, 2007) and sweet pepper (Abdel-Mawgoud, 2002). Its negative effect comes from the osmotic effects of various ions, mainly Na and Cl on plant water uptake which leads to a decrease in growth and productivity (Abdel-Mawgoud, 2002).

VAM fungi: Significant increase in the growth rate of plants observed while treated with VAM soil as compared to control (Table 1). This result proved the efficiency of VAM fungi as a growth promoting factor for plants. It highlighted the nutrients which were required to enhance the plants growth, increased rate of flowering observed as compared to control plants and VAM fungi enhanced the growth. Khaliq *et al*., (2001) revealed that the shoot biomass of peppermint (*Mentha piperita)* increased when inoculated with *Glomus fasciculatum* (145.3%), *G. aggregatum* (131.1%) and *G. mosseae* (87.8%) in comparison to control. *Aloe vera* plant inoculated with two VAM fungi (*G. clarum* and *Gigaspora decipiens*) resulted in increased shoot N and P concentration and shoot fresh weight or growth than uninoculated plants (Tawaraya *et al*., 2007).

***Pseudomonas stutzeri*:** Relatively less growth rate observed as compared to control in the *L. cylindrica* plants treated with *Pseudomonas stutzeri* inoculums per week, no flowering period was observed as compared to control and other growth promoting biotic components (Table 1). *Pseudomonas stutzeri* caused brown spot disease in oyster (*Pleurotus ostreatus*) and button mushroom (*Agaricus bisporus*) as reported by Dawoud & Eweis (2006).

Nematodes: Light colored and short sized leaves were observed after treating *L. cylindrica* plants with inoculums of nematodes. Root damage from the nematode results in stunted and chlorosis plants. Decrease in the growth rate of *L. cylindrica* plant and the rate of flowering was also very slow; only two flowers were grown after three months. It was due to the nematode population which badly affected the roots, the main transport channel of nutrients and growth regulators for the growth of plants (Table 1). In bean and tomato leaves, photosynthetic rates reduced due to infection of root-knot (Melakeberhan *et al*., 1984). Reduced nutrient and water uptake by the damage of roots resulted in weak and low-yielding plants (Abad *et al*., 2003).

Table 1. Effects of biotic and abiotic components of soil on physical parameters of *Luffa cylindrica*.

Treatments		Number of leaves	Length of shoot (cm)	Leaf area (cm^2)
C	Control	114.5 b	727.16 b	138.33 c
T_1	VAM fungi	121.66 a	756.33 a	149 a
T_2	*Pseudomonas stutzeri* (150 ml)	79.33 c	261.33 d	142.4 b
T_3	Nematodes	65.66 d	325.66 c	107.5 d
T_4	Sodium chloride (150 ppm)	66.33 d	202.33 e	47.33 e
T_5	Chromium nitrate (150 ppm)	25.6 e	76.16 f	42.66 f

Mean with similar letters in each column not significantly different at $P \leq 0.05$.

Biochemical parameters

Sugars and carbohydrates: The amount of carbohydrate in control was at par with mycorhhizal plants, while it decreased in other biotic and abiotic stresses. Total soluble sugars in plants treated with salinity and heavy metal increased considerably over control and mycorrhizal plants, but decreased in biotic stresses; more in nematodes than *Pseudomonas stutzeri* (Table 2).

Table 2. Effects of biotic and abiotic components of soil on carbon metabolism of *Luffa cylindrica*.

Treatments		Carbohydrates (g/ml)	Glucose (g/ml)	Sucrose (g/ml)	Total soluble sugars (g/ml)
C	Control	272.26 a	82.5 c	240.84 b	125.49 c
T_1	VAM fungi	270.84 b	95.36 a	247.16 a	116.76 d
T_2	*Pseudomonas stutzeri* (150 ml)	209.53 c	56.36 e	183.64 c	32.54 f
T_3	Nematodes	187.1 e	72.85 d	169.8 d	80.5 e
T_4	Sodium chloride (150 ppm)	210 c	86.61 b	159.9 e	223.43 a
T_5	Chromium nitrate (150 ppm)	200.5 d	46.4 f	132.68 f	189.26 b

Mean with similar letters in each column not significantly different at $P \leq 0.05$.

Environmental stresses like, cold, drought and salinity greatly alter carbohydrate metabolism (Kaur *et al*., 2000) and interaction of sugar signaling pathways occurs with stress pathways to modulate metabolism. Under abiotic stresses sugars play an important indirect role during plant growth and development to regulate carbohydrate metabolism. In all stresses nematode infected plants have low amount of total carbohydrates. Highly infected host tissues have decreased amount of total sugar due to the excess utilization of sugars by the organisms for their growth and sustainability (Sheen & Anderson, 1974). Decrease in carbohydrate content in the highly infected plant can be correlated with the increase in two important

enzymes of carbohydrate metabolism (glucose 6 phosphate dehydrogenase and 6 phosphogluconate dehydrogenase) in nematode infected soyabean (Salt *et al*., 1988; Gupta *et al*., 2010).

High concentration of carbohydrates related to high infections in plants (Horsfall & Diamond, 1957). Increase of 11.4% in reducing sugars of highly infected plants than partially infected due to reduced leaf area and malformation of leaves in infected plants resulting in less productivity (Shree & Umesh, 1989). Total sugar content was much higher in the treated seedlings.

Salinity and water stress induced soluble sugar accumulation (Binzel *et al*., 1989; Wang & Stutte, 1992; Kameli & Losel, 1995). Anjum (2008) also reported that in leaves of *Cleopatra mandarin* and both leaves and roots of *Troyer citrange* in seedling stage concentrations of sugars i.e., sucrose, glucose and fructose decreased with increase in salinity level in the irrigation water. However, reduction in carbohydrates concentrations has been related to tissue re-hydration during stress recovery (Lacerda *et al*., 2005). Silva *et al*., (2003) reported that the accumulation of soluble carbohydrates were significantly related to salt tolerance in relation to leaf osmotic adjustment and soluble carbohydrate contents of leaves were significantly correlated with the acclimatization to salt stress.

Proteins: There was no significant change in amount of protein in VAM, bacteria and nematode treated plants but it decreased in salinity and heavy metal treated plants (Table 3).

In *Capsicum annuum*, significant gain in fruit protein content and increase in plant height and dry weight observed after VAM fungi inoculation (Samanta & Verma, 2006). The protein content underwent a non-significant reduction in the leaves of mung bean after inoculation with the nematode (Naeem *et al*., 2009). Similarly, Oka *et al*., (1997) found an early infection in tomato plants susceptible to *M. javanica* did not change the soluble protein composition of their leaves as compared with uninfected plants. The synthesis of a number of specific polypeptides found decreased upon water loss in the leaves (Barnett & Nylor, 1966). Soluble protein levels in bermuda grass found decrease with increasing water stress. During severe water stress, photosynthesis, starch accumulation, and protein synthesis inhibited to some degree.

Proline: It was observed that amount of proline was decreased in mycorrhizal plants than control, while amount of proline increased in *Pseudomonas stutzeri*, nematodes, Sodium chloride and Chromium nitrate indicating that no stress produced in plants treated with VAM fungi. In both kind of stresses, biotic i.e., bacteria and nematodes and abiotic i.e., salinity and heavy metal a large amount of proline produced which indicates a severe stress produced by abiotic factors than biotic (Table 3).

Proline accumulation is a common metabolic response of higher plants to water deficits and salinity stress (Taylor, 1999). Very high accumulation of cellular proline due to increased synthesis and decreased degradation under a variety of stress conditions such as salt and drought has been reported in many plant species (Delauney *et al*., 1993). Proline protected membranes of plants and proteins against the adverse effects of high concentrations of salts and temperature extremes (Santarius, 1992; Santoro *et al*., 1992). Proline may also function as a hydroxyl radical scavenger (Smirnoff & Cumbes, 1989). The proline accumulated in response to water stress or salinity stress in plants is primarily localized in the cytosol (Ketchum *et al*., 1991).

Proline content increased remarkably in the *Luffa* plants under all biotic and abiotic stresses hence, no stress produced in mycorrhizal plants with addition of VAM fungi. Nelson & Achar (2001) reported that VAM fungi on *Brassica oleraceae* for the first time and observed increased plant biomass, growth and phosphorus uptake.

In field trials, Afek *et al*., (1991) found that growth response, yield and fresh weight in cotton (*Gossypium hirsutum*), onion (*Allium cepa*) and pepper (*Capsicum annuum*) were highest in VAM fungi colonized plants.

Amino acids: Amino acid metabolism was greatly affected by both biotic and abiotic factors. Mycorrhizal plants showed less amount of amino acid than control. Other treatments also had lower amino acid level than mycorrhizal treatment and control (Table 3).

Protease: The amount of protease greatly increased in VAM treated plants than control while it increased gradually in bacteria heavy metal and nematodes than mycorrhizal plants and found maximum in salinity (Table 3). Amount of protein, free amino acids and enzyme protease were not affected by both biotic and abiotic stresses.

Nitrate reductase: Control and mycorrhizal plants have more or less equal amount of nitrate reductase (NR) enzyme, while amount of NR was decreased in all other treatments. Plants treated with bacteria had least amount of NR (Table 3). In water deprivation, maximal foliar extractable NR activity has been found to decrease in some cases (Correia *et al*., 2005). Photosynthesis regulated nitrate reduction by modulating NR activity (Kaiser & Brendle-Behnisch, 1991).

Table 3. Effects of biotic and abiotic components of soil on nitrogen metabolism of *Luffa cylindrica*.

Treatments		Protein (g/ml)	Amino acids (g/ml)	Proline (g/ml)	Protease (g/ml)	Nitrate reductase (g/ml)
C	Control	51.9 c	17.6 a	117.5 e	17.29 f	210.6 b
T_1	VAM fungi	60.9 a	11.7 b	94.3 f	61.5 e	223.8 a
T_2	*Pseudomonas stutzeri* (150 ml)	55.3 b	08.6 c	253.2 d	63.61 d	83.7 f
T_3	Nematodes	50.3 d	05.6 d	297.6 c	74.26 b	146.7 c
T_4	Sodium chloride (150 ppm)	08.9 f	05.5 e	426.7 a	80.23 a	113.8 e
T_5	Chromium nitrate (150 ppm)	30.8 e	04.4 f	389.5 b	66.75 c	133.9 d

Mean with similar letters in each column not significantly different at $P \leq 0.05$.

Conclusion

Results of this study comprising of physical and biochemical parameters clearly indicated the positive effects of VAM fungi on growth of *Luffa*. VAM fungi have a low, or negligible, saprophytic ability and can apparently produce viable propagules only upon the biotrophic colonization of a susceptible host root. VAM fungi did not create any kind of stress in plants.

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