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Alterations in proline accumulation in *Abelmoschus esculentus* Moench. following infection with *Meloidogyne incognita*

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Abstract
Plants accumulate proline as defence molecules against biotic as well as abiotic stresses. In the present investigation infection of okra plants by *Meloidogyne incognita* resulted in the accumulation of proline in infected plants than healthy uninfected plants. Proline accumulation was maintained high in infected plants, with maximum increase between 3rd and 4th week post inoculation, most probably due to egg production. Moreover the roots of infected plants accumulated higher concentration of free proline than leaves. Similarly the concentration of free proline was higher in galls bearing eggmasses than ungalled portions of the infected roots.

Keywords: Proline, *Abelmoschus esculentus* Moench, *Meloidogyne incognita*, Plants

1. Introduction
Plants are exposed to a myriad of biotic stresses such as harmful rhizospheric microorganisms like nematodes, fungus, bacteria and other insects. Biotic stress is one of the major stresses resulting in economic loss and reduced crop productivity (Mazid *et al*. 2011) [9]. Root-knot nematodes, *Meloidogyne* spp. are considered to be the most significant nematode pests to crop production, causing multi-billion dollar losses to world agriculture annually. Root knot nematodes are sedentary, endoparasitic in nature causing disruption of vascular system thereby reducing translocation of water and nutrients from roots to leaves (Abad *et al*. 2003) [1], subjecting the plant to water stress and reduced photosynthesis. Water stressed plants have been reported to accumulate osmo-protectant, hydroxyl radical scavenger amino acid proline as an adaptive response to mitigate the water stress (Smirnoff and Cumbes, 1989) [18]. Galls in tomato induced by root knot nematode contain higher amounts of proline, protein and nucleic acids (Owens and Specht, 1966) [13]. Similarly *Radopholus similis* (Cobb) Thorne parasitized grapefruit seedlings (Hanks and Feldman, 1963) [5] and *Longidorus africanus* Merny infected *Bidens tripartita* L. (Epstien and Cohn, 1971) [4] amassed high concentrations of free amino acid proline. Proline accumulation has been advocated as a parameter of selection for stress tolerance (Yancy *et al*., 1982) [22]. In the present study an attempt was made to find the changes in the distribution of proline in okra following infection with root knot nematode, *Meloidogyne incognita*.

2. Materials and Methods
Infected eggplants were collected from Badauli Fateh Khan Area of Aligarh (Uttar Pradesh)-India and root knot nematode was identified as *M. incognita* (Kofied and White) Chitwood through analysis of perennial pattern as described by Taylor and Netscher (1974) [21]. A single eggmass was used to raise pure culture of nematode in the greenhouse on eggplants (*Solanum melongena* L.) in earthen pots. The infested roots were thoroughly washed free of the dirt and debris with running tap water. Eggmasses picked from the infected roots were gently washed with distilled water and placed in 0.5% sodium hypochlorite solution to dissolve the gelatinous matrix. The solution shaken for 4 minutes was then rinsed with distilled water on a sieve having 26µm pores and the eggs were incubated for 3-5 days using modified Baermann funnel technique to obtain infective second stage juveniles (J_2) for pot study.
The seeds of okra cv. 'Varsha Uphar' were sown singly in earthen pots (25 cm in diameter) filled with 2 kg of steam sterilized soil (7 clay: 2 loam: 1 farm yard manure). Regular sprinkling of water was done to ensure the proper germination of seeds.

Fifteen days after germination, each plant was inoculated with 2000 juveniles of *M. incognita* with five replicates per treatment. Pots were arranged randomly on the glasshouse benches where temperatures fluctuated between 25 °C and 30 °C. One week post inoculation, the roots were washed free of soil. Root and leaf samples of five plants from each group were harvested every 7 days for proline determination. Leaves and roots were cut into small sections and pooled. Random samples were then taken for determination of free proline. Variation in proline concentration in infected okra plants over an extended period of 6 weeks was studied. Uninfected plants were used as controls. Distribution of proline was also determined in galls having eggmasses on their surface and ungalled portions of the infected roots. All the samples were wrapped in aluminium foil, frozen immediately in liquid N, and stored at -20 °C until assayed. Another experiment on parallel lines was done side by side and the results of both the experiments were pooled.

2.1 Proline extraction and estimation.

Proline content was estimated following Bates *et al*. (1973) [3] method. The sample (leaf and root) tissue (1.0 g) was crushed in 10 ml of aqueous sulpho-salicylic acid (3%) using mortar and pestle and centrifuged at 11000 g for 10 min. The supernatant was taken out, and the volume was maintained to 10 ml with sulphosalicylic acid. The reaction was started by taking 2 ml of the extract in a test tube having 2 ml acid ninhydrin (prepared by warming 1.25 g of ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6 mol. orthophosphoric acid with agitation until dissolved) and 2 ml glacial acetic acid. Above mixture was boiled in water bath at 100°C for 30 min until a brick red colour developed. The reaction was terminated by cooling in an ice bath, and the reaction mixture was extracted with 4 ml of toluene. The extract obtained was transferred to a separating funnel, and the upper layer was collected after mixing vigorously for 15–20 seconds. The chromophore containing toluene was aspirated from the aqueous phase and warmed to room temperature, and the absorbance was read at 520 nm using toluene for a blank. The concentration of proline in samples was determined according to the standard curve plotted with known concentrations of L-proline.

3. Statistical analysis

The data obtained for proline over an extended period of six weeks was subjected to one way analysis of variance (ANOVA) using SPSS 17.00 software (SPSS Inc., Chicago, IL, USA) and graphs were prepared using Excel.

4. Results and Discussion

The results depicted in figure clearly indicate a rise in free proline concentration in *M. incognita* infected okra plants as compared to healthy ones. Proline accumulation in infected roots was maintained high over a period of 6 weeks, with highest level of proline getting amassed between 3rd and 4th week, most probably at the time of egg production. The concentration of proline initially in the leaves of infected okra plants was high but following second week it started declining almost to about that of healthy plants. In the infected roots, the galled portions having eggmasses contained higher proline concentration as compared to non-galled portions of the root (fig.).

Accumulation of free proline in plants under biotic and abiotic stresses is well documented (Sharma *et al*., 1980; Sharma *et al*., 1990; Kameli and Losal, 1993; Sharma and Trivedi, 1996; Mohanty *et al*., 1999; Jaleel *et al*., 2007; Manivannan *et al* 2007; Szabados and Savouré, 2010; Manoharan *et al*., 2010; Bañuelos *et al*., 2012; Sreedevi *et al*., 2013; Slama *et al*., 2015) [15, 14, 7, 16, 12, 6, 8, 20, 10, 2, 19, 17]. Proline is an important amino acid and there is a positive correlation between its accumulation and plant stress. Besides acting as osmoprotectent, proline plays three key roles during stress i.e., it acts as a signalling molecule, an antioxidant defence molecule and as a metal chelator. Over production of proline during stressful conditions imparts stress tolerance to plants by maintaining osmotic balance of the cell; preventing electrolyte leakage by stabilising the cellular membranes and preventing oxidative burst in plants by bringing concentrations of reactive oxygen species within the normal range. The proline accumulation in okra plants infected with *M. incognita* occurs due to water stress resulting due to obturation of water conducting xylem conduits impeding translocation of water and nutrients from roots to shoots when giant cells and root galls are formed in response to nematode infection. However, root knot nematode infected plants accumulated more proline in roots as compared to tops. The high variation in the accumulation of proline in roots than shoots may be due to high metabolic activities associated with the formation of giant cells, gall and egg formation that require higher quantities of energy which in turn is supplied by free proline produced and translocated from leaves to the site of nematode activity (Meon *et al*., 1978) [11].

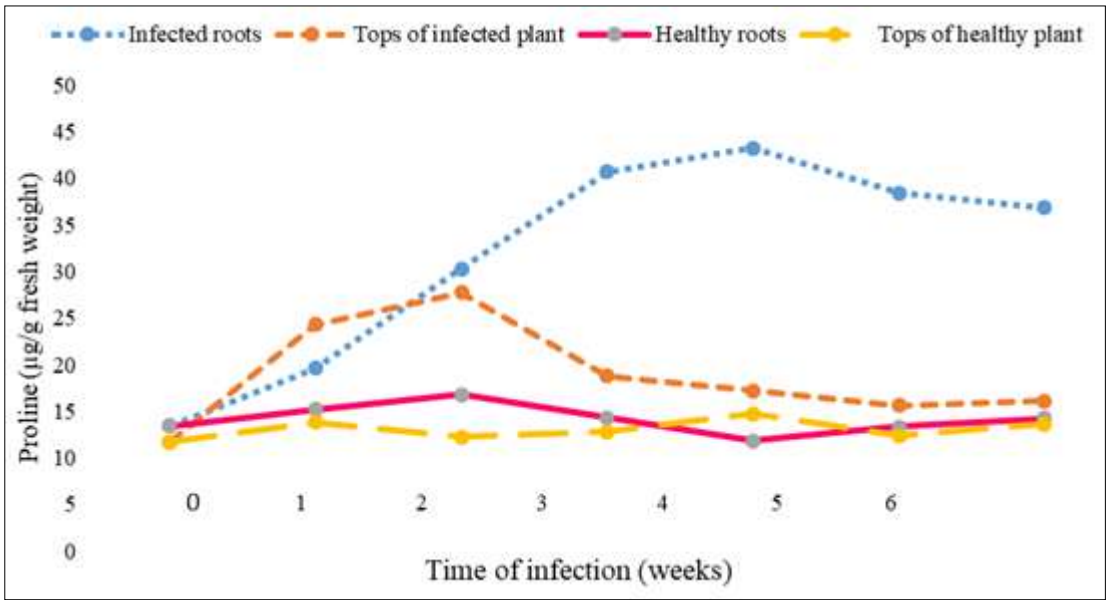

Fig 1: Depicting alteration in proline concentration in healthy and *Meloidogyne incognita* infested okra plants.The values are pooled from two parallel experiments.

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