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Qualitative analysis of Phytohormones in brassinosteroid treated tomato plants during nematode pathogenesis

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Abstract : Phytohormones are the signalling molecules that are produced in extremely low concentrations in plants. They regulate physiological and cellular processes both locally and at far. Under stress, in addition to the physico-chemical modulations, slight alterations in the indigenous levels of these hormones, thereby, activate plant's defence responses. Therefore, in the present study, qualitative analysis of phytohormones was carried out in brassinosteroid treated tomato plants during nematode infection. Qualitative analysis of phytohormones was carried out in whole plant material. Surface sterilized seeds of tomato cultivars Pusa Ruby (susceptible) and PNR-7 (resistant) were treated with different concentrations of 28-Homobrassinolide. Comparisons were made between Control and 10^{-9} M HBI in Pusa Ruby. While in PNR-7, comparisons were made between Control and 10^{-7} M HBI treated plants. Analysis was carried out 120hrs and 72hrs post-nematode inoculation in Pusa Ruby and PNR-7 respectively. Results ascertained the presence of three hormones viz. Putrescine, Salicylic acid and Jasmonic acid in both the treatments in cultivars. Thus, the study indicated the role of these hormones in plants under stress.

Key Words: Qualitative analysis, Phytohormones, Brassinosteroids, Pusa Ruby, PNR-7, *M. incognita*.

INTRODUCTION

Phytohormones serve as chemical messengers, by which the activity of certain organs is coordinated with that of others. These are generally produced in one place and are then transported to other place for the manifestation of their effect¹. Unlike animals, plants lack specialised organs that produces and stores hormones, but, like animal hormones, plant hormones profoundly influence physiological processes such as growth, development and differentiation of cells and tissues and also brought about changes in plants at transcriptional level at very low concentrations. Perhaps, the field of plant hormones is now at the stage of its most frequent growth. In addition to its generalized role in boosting plant growth, phytohormones have been suggested playing important roles in stress responses and adaptation^{2,3}. Any alterations

in the indigenous levels of these hormones, activate plant's defence responses in addition to the physico-chemical modulations.

Plants encounter various biotic stresses. Of these, plant-parasitic nematodes are among the major pathogens that affect the worldwide agricultural production leading to a global loss of over \$125 billion per annum⁴. In India, Rs 21,068.73 million crop losses have been reported due to nematode parasitism⁵. The most advanced plant-parasitic nematodes are biotrophic sedentary endoparasites, which invade and migrate through the root before initiating specialised feeding cells and becoming sedentary. Among these are the root-knot nematodes (e.g. *Meloidogynes* species) and the cyst nematodes (e.g. *Heterodera* and *Globodera* species). *Meloidogynes* species are generally the most promiscuous and polyphagous with respect to host range. They infest plants and results into malfunctioning in root system, reduced shoot growth and biomass accumulation, nutritional deficiencies in the foliage, chlorosis, temporary wilting, reduced

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photosynthesis and suppressed yields by severely affecting plant-water relations^{6,7,8,9,10}.

Currently, investigations are being carried out on the interactions of different hormones. Cross-talk between phytohormones such as IAA, SA, ABA, JA, BRs, polyamines etc. revealed their role in stress management. Each of these hormones generates and transmits distinct defence signals and they influence each other through a complex network of harmonious and inimical interactions^{11,12,13} affirming the plant to efficiently comply its defence reaction depending on the type of attacker encountered. Therefore, in the present study, qualitative analyses of phytohormones were carried out in treated and non-treated susceptible and resistant tomato plants during nematode pathogenesis.

MATERIALS AND METHODS

Surface sterilized seeds of tomato (*Lycopersicon esculentum* Mill.) cultivars viz. Pusa Ruby (susceptible) and PNR-7 (resistant) were treated with the prepared concentrations of HBI (10^{-9} and 10^{-7} M respectively). Treated seeds were then germinated in sterilized petri-plates lined with moistened Whatman sheet. Petri-plates were placed in B.O.D incubator at $24 \pm 2^\circ\text{C}$, relative humidity between 60-75% and photoperiod of 14L/10D. After germination, seedlings were inoculated with infective juveniles (@ $5J_2$ /seedling) of *M. incognita*. Estimations were carried out 120hrs and 72hrs respectively post-nematode inoculation in susceptible and resistant cultivar.

Qualitative analysis of phytohormones was carried out in whole plant material in both the cultivars¹⁴. For the analysis, 1gm of fresh plant material was homogenised in 8ml of extraction solvent (methanol: water:: 80:20; v/v). The homogenate was vortexed and centrifuged at 10,000rpm for 15min at 4°C . Supernatant was collected and the extraction was repeated thrice. The supernatants were combined and 0.4ml of it was diluted with 1ml methanol. Then 20 μl of the diluted supernatant was used for phytohormone analysis using LC/MS. The presence of indigenous phytohormones were observed by using Agilent 1100 LC coupled with Bruker mass spectrometer model Esquire 3000. For the analysis of extract, LC/MS system was operated at both +ve and -ve mode with a constant flow rate of 0.2 $\mu\text{l}/\text{min}$ using C18

column. The samples were run for a total of 20min at a column temperature of 40°C . Mobile phase A and B consisted of Water (0.5 % Formic acid) and Methanol respectively. Comparisons were made between Control and 10^{-9} M HBI (most effective concentration) 120hrs (effective time interval) post-nematode inoculation in Pusa Ruby and Control and 10^{-7} M HBI (most effective concentration) after 72hrs of post-nematode inoculation (effective time interval) in PNR-7. The above selected HBI concentrations and time intervals are as per the other studies conducted (data unpublished).

RESULTS AND DISCUSSION

Results deciphered the presence of three phytohormones i.e. Putrescine (Mol. wt. 88.15; Fig.1, 4), Salicylic acid (Mol. wt. 138.21; Fig.2, 3) and Jasmonic acid (Mol. wt. 210.27; Fig.2, 3) in both the treatments in susceptible cultivar. Similarly in resistant cultivar also, analysis revealed the presence of Putrescine (Fig.5, 7), Salicylic acid (Fig.6, 9) and Jasmonic acid (Fig.6, 8) in both the treatments.

Phytohormone signalling in response to herbivory mediates changes in the plants¹². Number of investigations asserted the role of JA and SA related gene expression levels during induced responses. Investigation on feeding of green peach aphid (*Myzus persicae*) on *Arabidopsis* showed induced transcription of two genes associated with SA-dependent responses to pathogens (PR-1 and BGL2) 10 and 23 fold respectively¹⁵. While a two-fold increase occurred in mRNA levels of PDF1.2, encoding defensin (a peptide) involved in the jasmonate (JA)/ethylene-dependent response pathway. Similarly, cross-communicating signalling pathways between SA, JA and ethylene (ET) were analysed in *Arabidopsis*¹⁶. Here, the plants were attacked by a set of microbial pathogens and herbivorous insects with different modes of attack. Results revealed the primary role of SA, JA, and ET in the orchestration of the plant's defence response. Similar investigations have also been reported by other researchers^{17,18,19,20}.

Characterization of the role of SA, JA, ET and ABA-mediated systemic defence signalling in rice and their importance in root defence against migratory nematode *Hirschmanniella oryzae* was conducted²¹. The study demonstrated prerequisite requirement of intact ET, JA

Kaur et al.: Qualitative analysis of Phytohormones in brassinosteroid treated tomato plants during nematode pathogenesis

and SA biosynthesis pathway while negative role of ABA was revealed. Involvement of BR pathway in rice root susceptibility to root pathogen, *Pythiumgraminicola* in suppressing SA and GA mediated signal transduction pathways during disease resistance have also been demonstrated²². Also, studies on balance between BR and JA pathway which is an effective regulator of the outcome of the rice-*M. graminicola* interaction were conducted¹³. Results showed a negative cross-talk between BR and JA pathway. JA-related gene transcripts were strongly down-regulated in low brassinolide concentrations 24hrs but when brassinolide concentrations were high, JA genes were up-regulated.

Therefore, it can be concluded that phytohormones

form an integral part of plant's defence system. Any modulation in the levels of hormone activates various signalling pathways, which, curbs the damage caused by the environmental stress factors. In the present study also, phytohormones have been detected, thereby, revealing their putative role in host plant during pathogen invasion.

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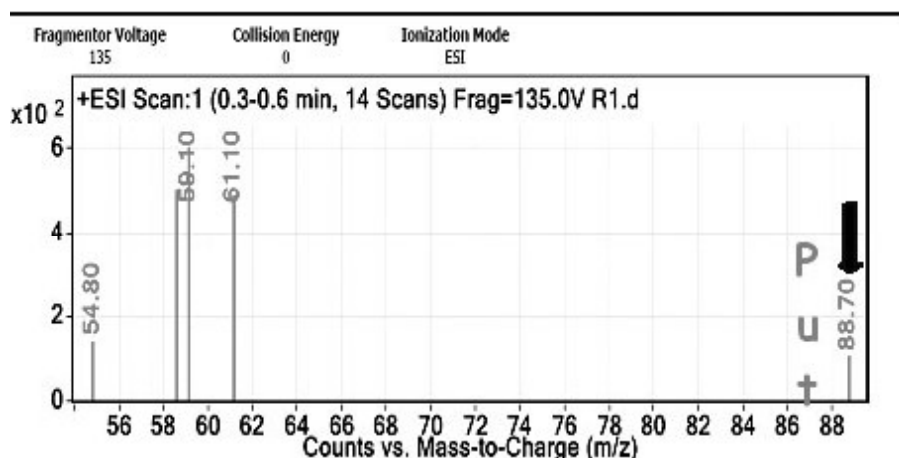


Fig.1. Chromatogram showing LC/MS analysis of Putrescine in Pusa Ruby after nematode inoculation

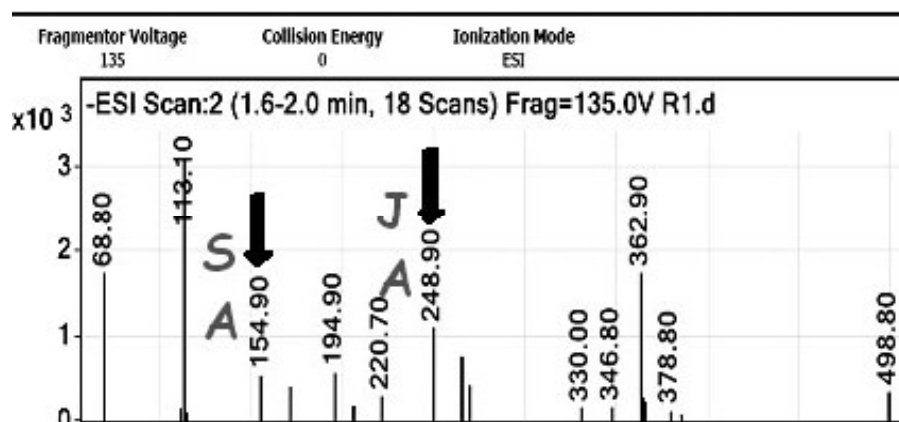


Fig.2. Chromatogram showing LC/MS analysis of Salicylic acid and Jasmonic acid in Pusa Ruby after nematode inoculation

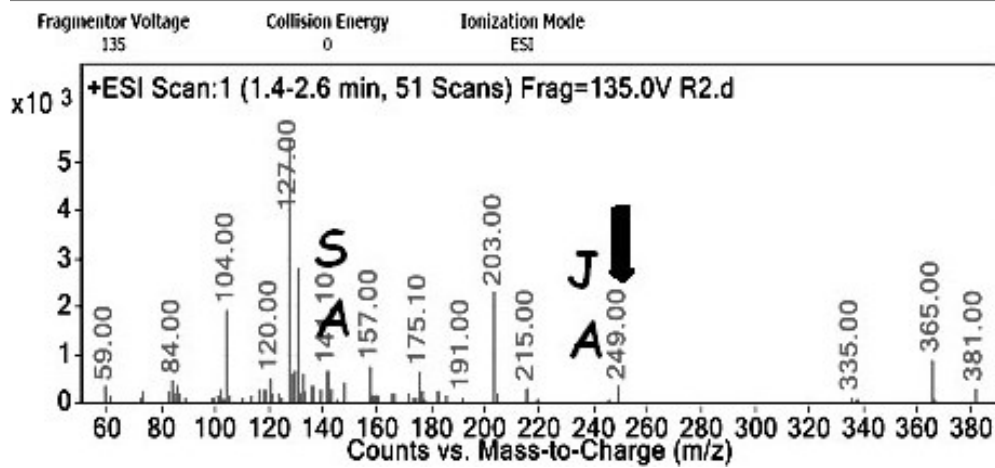


Fig.3. Chromatogram showing LC/MS analysis of Salicylic acid and Jasmonic acid in HBI treated Pusa Ruby after nematode inoculation

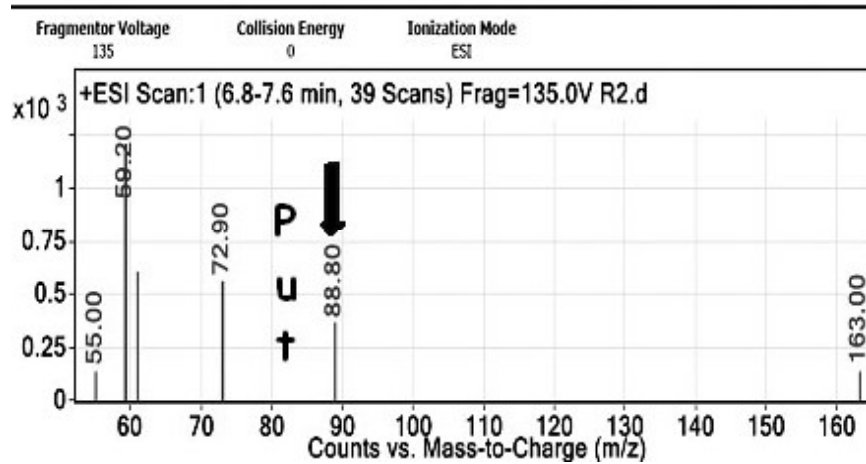


Fig.4. Chromatogram showing LC/MS analysis of Putrescine in HBI treated Pusa Ruby after nematode inoculation

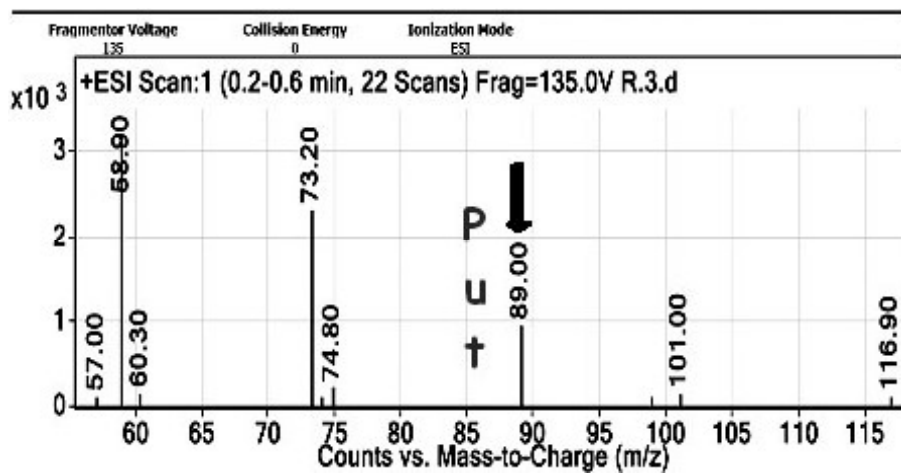


Fig.5. Chromatogram showing LC/MS analysis of Putrescine in PNR-7 after nematode inoculation

Kaur *et al.*: Qualitative analysis of Phytohormones in brassinosteroid treated tomato plants during nematode pathogenesis

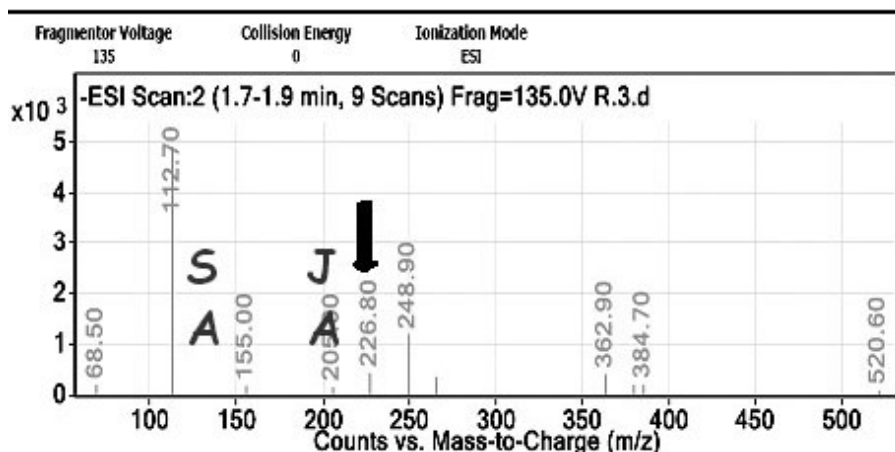


Fig.6. Chromatogram showing LC/MS analysis of Salicylic acid and Jasmonic acid in PNR-7 after nematode inoculation

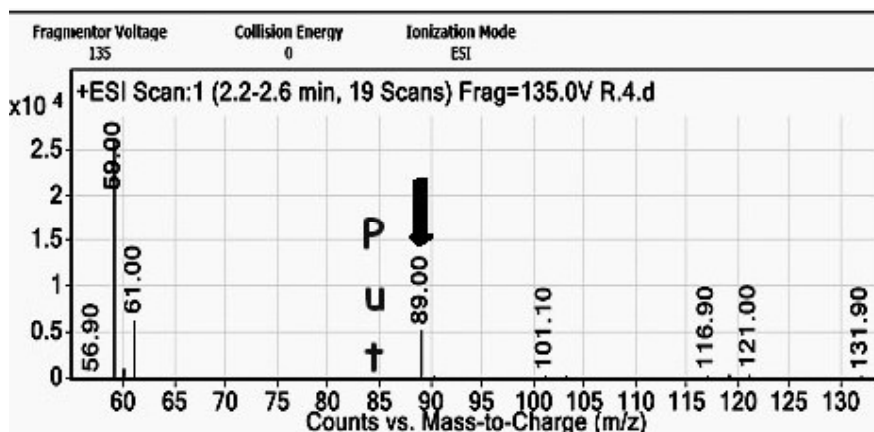


Fig.7. Chromatogram showing LC/MS analysis of Putrescine in HBI treated PNR-7 after nematode inoculation

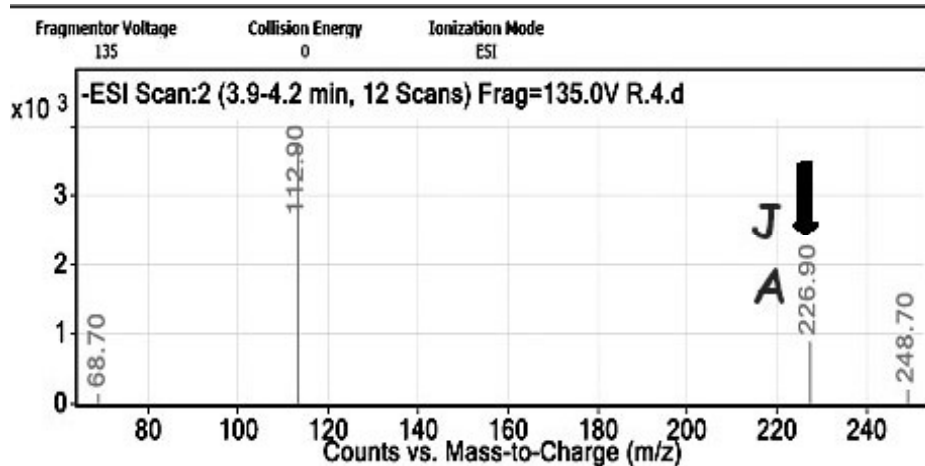


Fig.8. Chromatogram showing LC/MS analysis of Jasmonic acid in HBI treated PNR-7 after nematode inoculation

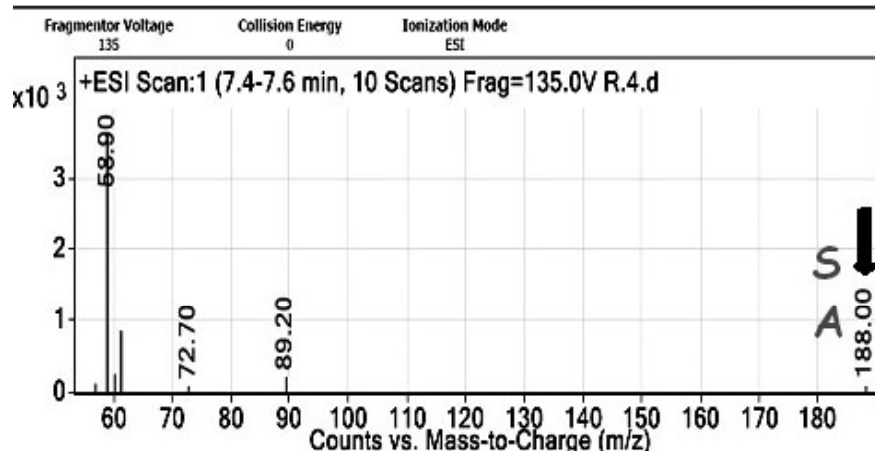


Fig.9. Chromatogram showing LC/MS analysis of Salicylic acid in HBI treated PNR-7 after nematode inoculation

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