Nematodes are deceptively simple microorganisms belonging to the phylum Nematoda. These are distributed in every type of habitat ranging from land to ocean. These massively deleterious organisms cause huge social and economic impact on worldwide agricultural crops. Among the most economically important nematodes are the endoparasitic species; the root-knot nematodes, *Meloidogyne* spp. (Moens *et al*., 2009). These biotrophic pathogens induce the formation of complex feeding structures within their host and completely rely upon the nutrient sites of the host (Smant and Jones, 2011). These profound changes in the morphology of host cell are intriguing scientifically and to a large extent are like the black box (Sijmons, 1993). Furthermore, nematode biotrophy in plants induces morphometric, metabolic and genomic alterations, thereby, modifying the overall physiology of plants.

In response to nematode pathogenesis, a range of defence responses are activated in plants. Some of these defences are being suppressed by the invading pathogens while others are enhanced to avoid the direct contact with the host’s defence responses. Under non-stressed conditions, there exists a delicate balance between the reactive oxygen species (ROS) produced during metabolic activities in the host plant and its defence system. However, with nematode invasion, this balance is disturbed directly targeting these pathogens (Fig.58). These over generated ROS undergo a series of oxidation/reduction reactions (known as the Halliwell-Asada pathway) (Arora *et al*., 2002; Gratao *et al*., 2006). Also, it helps the plants in strengthening their cell wall and activating other signalling pathways (Waetzig *et al*., 1999; Teale *et al*., 2008) including the activation of various antioxidative enzymes and activation of secondary metabolites (non-enzymatic antioxidants) (Skórzyńska-Polit *et al*., 2010; Sharma *et al*., 2011a). The varied antioxidative enzymes includes catalase, peroxidases, superoxide dismutase, glutathione reductase etc. (Ruley *et al*. 2004; Simonovicova *et al*., 2004) and the activation of some secondary metabolites includes phenols, tocopherols, carotenoids etc. (Schutzendubel and Polle, 2002; Vardhini and Rao, 2003; Ozdemir *et al*., 2004; Sharma and Dubey 2005). In addition, some phytohormones have also been categorized
including auxins, cytokinins, gibberellins, abscisic acid, ethylene, brassinosteroids, salicylic acid and jasmonic acid that play key role in signalling pathways implicating oxidative stress (Sakhabutdinova et al., 2003; Choudhary and Panda, 2004; Bari and Jones, 2009; Fariduddin et al., 2014). Of these hormones, BRs have emerged as a budding candidate for overcoming various stresses in plants (Anuradha and Rao, 2007; Romanutti et al., 2007; Bajguz and Hayat, 2009). The activation or the suppression of these defences modifies the physiology of plants thereby curbing the growth and development of the pathogen inside the host (Fig.59).

5.1. EFFECT OF NEMATODE INOCULATION ON SUSCEPTIBLE AND RESISTANT VARIETIES OF TOMATO

In the present study, stress protective properties of HBl were observed in susceptible and resistant cultivars of tomato during nematode pathogenesis.

5.1.1. Morphological Parameters

The primary symptoms of nematode infection in plants are highly dramatic. These include both belowground and aboveground modifications. Some of the aboveground symptoms include stunted growth, loss of leaves, poor fruit set, small sized fruits, wilting during midday and nutrient deficiency symptoms such as chlorosis which may turn necrotic. While, belowground symptoms includes lesions of necrotic tissues, discoloration of roots, rhizomes, forking in tap roots, excessive root proliferation and the presence of root-knots (Mitkowski and Abawi, 2011; Fig.60).

Different parameters taken into consideration in the current study included percentage germination, total plant height, root length, shoot length, total plant biomass, root weight and shoot weight. The results inferred that in susceptible cultivar, overall growth and development of plant was reduced during nematode pathogenesis. In contrast, in resistant cultivar, not much effect of nematode infection was seen on the morphology of plants. These observations are in relation to the older studies carried out by Opoku-Asiama and Yeboah (2003) where significant effect was seen in the yield of susceptible cultivars in terms of reduced plant height, root length, root weight, shoot weight while the tolerant plants were able to maintain their growth parameters, leading to relatively lesser suppression in the overall yield during root-knot nematode infection.
Fig. 58. Balance between AOX (antioxidant defence system) and ROS (reactive oxygen species) in plants under no stress and stress.

Fig. 59. Generation of stress in plants and activation of antioxidative system during nematode invasion.

Fig. 60. Morphological modifications in plants during plant-parasitic nematode infection.
Udo et al. (2005) evaluated the pathogenicity of *M. incognita* on the Nigerian pepper lines individually inoculated with *M. incognita* eggs. The study accorded that susceptible lines were severely affected in terms of reduced root and shoot growth when compared with the control. But, the resistant plants had significantly fewer galls and egg masses per root system and were least affected.

In 2009, Ahmed et al. reported deteriorating effect of *M. javanica* inoculated on mung bean plants. Nematode pathogenesis significantly affected the growth of susceptible plants in terms of shoot weight that showed significant decrease after nematode infection. But, a significant increase was observed in root weight. Reports have also documented the effect of *M. incognita* on growth and yield of resistant and susceptible tomato varieties under controlled growth chamber conditions. The nematode invasion in roots 7 days after inoculation was drastically reduced in resistant cultivars (Hesar Lalit and PBNR-7) compared to susceptible (Pusa Ruby) one. Further, nematode population suppression has been reported to increase the plant growth and yield of resistant vs. susceptible cultivar (Grace et al. 2009). Similar results have been revealed in investigations on changes in growth parameters in blackgram mutant lines induced by different mutagenic treatments towards *M. incognita*. It was found that nematode inoculation reduced plant height, root length, root and shoot dry weight as compared to uninoculated control (Mahalik and Routray, 2009). In a study by Azam et al., 2011, growth and yield of tomato inoculated with different inoculum levels of *M. incognita* have also been analysed and it was found that tomato plants inoculated with highest inoculum levels (3000 J2) showed maximum reductions in growth and yield of plants. Similar results have also been reported by other studies as given by Kokalis-Burelle et al., 2009; Hussain et al., 2011; Jaiswal et al., 2011 and Osman et al., 2012.

### 5.1.2. Effect of HBl Treatment on Morphological Parameters

In the present study, it has been asserted that pre-sowing of tomato seeds in HBl has improved the seed vigour and hampered the root-knot nematode penetration in both the cultivars. Therefore, suggesting the positive impact of HBl on development of plants. These results are in accordance with the previous studies conducted by Ohri et al. (2004) in *R. sativus* where it was reported that root length decreases with nematode
invasion but enhances with brassinolide (a brassinosteroid) treatment. Recently, Nahar et al. (2013) examined the role of BR in rice innate immunity during *M. graminicola*. Exogenous epibrassinolide (EBL) supply at low concentrations (0.1 and 1 µM) favoured subsequent infection with *M. graminicola* as compared with the non-treated plants implying a role for BR in rice susceptibility to this nematode. However, exogenously applying high concentrations of brassinolide (5 and 10 µM) resulted in significantly lower susceptibility of rice plants to *M. graminicola* as compared to non-treated plants. Thereby, explaining the fact that negative feedback regulation resulted in lower endogenous BR levels, and, hence, an increase in basal defence.

In addition, role of other plant growth regulators during nematode pathogenesis have also been documented. Cooper et al. in 2005 determined the role of JA-dependent defences in tomato infected with root-knot nematodes. There was a significant interaction between tomato cultivars and chemical treatment. Nematode performance was dramatically reduced on the resistant cultivar Motelle (Mi+) as compared to the susceptible cultivar Moneymaker (Mi-). Also, in the susceptible cultivar, egg mass production was lower on plants treated with JA than on plants sprayed with control solution. Egg mass numbers were also low on Motelle plants treated with control solution, and no egg masses were observed on Motelle plants treated with JA. Similarly, Sirohi and Pankaj (2005) studied the effect of bare root dip treatment on SAR inducing chemicals (salicylic acid, rose bengal and gibberellic acid) in tomato with simultaneous or delayed *M. incognita* inoculation. All three chemicals had varied effect as resistance inducers and of all these chemicals; SA was the most effective molecule for inducing resistance. Later, Gawade and Sirohi, 2011 also reported SA induced resistance in eggplant cv. Pusa Purple Long against *M. incognita* where significant reduction in number of root galls, egg masses and overall population of RKN when compared to untreated inoculated plants. Thus, indicating significant increase in plant growth parameters of eggplant after SA application.

Positive impacts of BRs have also been observed in plants under abiotic stress. Chon et al. (2000) in rice observed that application of brassinolide strongly stimulated mesocotyl and coleoptile elongation of rice cultivars. Further, Anuradha and Rao, (2001; 2003) showed that EBL reduced inhibition of percentage seed germination,
seedling growth and also prevented the loss of photosynthetic pigment induced by salinity stress in rice. Vardhini and Rao (2003) demonstrated alleviation of osmotic stress in BRs application in three varieties of *Sorghum vulgare* (L.) Moench by enhancing length, fresh and dry biomass of seedlings. Almost similar observations have also been reported by other groups working on anti-stress properties of brassinosteroids on plants under heavy metal toxicity. Pre-soaking treatments of EBL improved the shoot length and biomass production in *B. juncea* seedlings and plants under Cu, Zn, Mn, Co, Ni, Cr and Cd metals stress. Besides this, EBL was also observed to reduce the heavy metal uptake and accumulation in *B. juncea* seedlings and plants (Sharma and Bhardwaj, 2007a; 2007b; Bhardwaj et al., 2008; Arora et al., 2010; Sharma et al., 2010).

### 5.1.3. Alterations in Antioxidative Enzymes during Nematode Pathogenesis

Antioxidative enzymes are those key enzymes that are actively involved in the plant’s defence against excessive ROS produced during stress. These include enzymes of “Asada-Foyer-Halliwell pathway such as SOD, CAT, GR, APOX, MDHAR and DHAR that act in a highly co-ordinated manner (Noctor and Foyer, 1998; Arora et al., 2002; Asada, 2006). In the present study, along with the morphological parameters, alterations in antioxidative enzymes (CAT, APOX, GPOX, GR, GPOD and SOD) were also examined. It was found that in susceptible tomato plants, nematode inoculation brought about changes (either activation or suppression) in the activities of enzymes while in resistant tomato plants, the overall activities of enzymes were slightly altered during nematode pathogenesis. Thereby, suggesting that in both the cultivars, host’s defence system was modulated during nematode infection. Studies conducted by Rajasekhar et al. (1997) revealed similar investigations where the activity of CAT increased in susceptible tomato cultivar but decreased in resistant cultivar post nematode inoculation. An increase in the POX activity was observed in roots and shoots of both cultivars with senescence and inoculation. While in case of SOD, it increased due to infection in susceptible cultivar but decreased in resistant cultivar. Similar reports were also given by Molinari and Miacola (1997) and Molinari (1998). Following the entry of nematode, an increased peroxidase level in infected plants has suggested a greater *de novo* synthesis of peroxidase. Further studies by Molinari and
Mi cola (1997) on galls produced by *M. incognita* during compatible response in susceptible and resistant tomato roots cultured in vitro were conducted. Galls were analyzed for their content of antioxidative enzymes: CAT, APOX and SOD with respect to non-galled tissue. The study reported an induction in CAT in all tested interactions in fully developed galls. SOD activity was higher in galls produced in susceptible roots and lower in resistant roots compared with non-galled tissue. Also, variations in APOX activity during the infestation period in galls were similar to that of CAT.

Changes in CAT activity in sugarcane due to *P. zeae* infection were also analysed by Kathiresan and Mehta (2003) in resistant (Co7717) and susceptible (CoC671) clones of sugarcane 3, 7, 14, 21, 28 and 35 days after infection. In resistant clone, maximum percent decrease in catalase activity over uninfected clone was at 21 and 14 days after infection in roots and leaves respectively. In susceptible clone, maximum percent increase of catalase activity over uninfected clone was at 7 days in roots and 35 days in leaves. Catalase activity increased in *P. zeae* infected susceptible clone suggesting that this was because of the nematode infected tissues of susceptible clones which maintained the lower levels of superoxides and lipid peroxidation. Thus, reflecting an efficient scavenger system to the site of superoxide production in susceptible plants. The investigations were further carried out on the sequential development of SOD in resistant (Co 7717) and susceptible (CoC 671) sugarcane clones infected with *P. zeae*. SOD activity was found to increase quantitatively in both roots and leaves from nematode-infected susceptible clones compared to non-infected clones. It was decreased in roots and leaves from nematode infected resistant clones, when compared to the non-infected-resistant clones (Kathiresan and Mehta, 2005).

Furthermore in another experimental study, effects of *M. incognita* infection on reactive oxygen metabolism and the activities of SOD, CAT and POD were investigated and compared between resistant (*S. torvum*) and susceptible (*S. intergriflium*) seedlings by Xiao-ming *et al.* (2008). The activities of SOD and CAT were rather higher in the resistant variety than susceptible one, while POD activity acted contrarily in both the varieties. Observations have also been recorded in sugar beet genotypes pertaining to physiological and biochemical changes infected with root-knot nematode by Korayem *et al.* (2012). Ten genotypes of sugar beet plant were screened under greenhouse
conditions for reaction to *M. incognita* for SOD and CAT activities. Nematode infection significantly increased the activities of these enzymes in most genotypes according to the degree of infection with *M. incognita*. Alterations in enzyme activities following nematode inoculation have also been reported in other studies by Chakrabarti and Mishra, 2002; Gopinatha *et al.*, 2002; Sundararaju and Pandi Suba, 2006; Chawla and Pankaj, 2007; Rani *et al.*, 2008; Sundararaju and Kurinji, 2008; Kalaiarasan, 2009 and Oliveira *et al.*, 2012.

**5.1.3.1. Effect of HBl Treatment on Antioxidative Enzymes**

In addition to nematode inoculation, seed pre-soaking treatment of tomato cultivars with HBl brought about marked changes in the activities of defensive enzymes. In both the cultivars, specific activities of antioxidative enzymes were enhanced in brassinosteroid treated seedlings. Further these changes were more pronounced in the resistant cultivar when compared with the susceptible check. Regulation of enzyme activities with brassinosteroid treatment during abiotic stress has been well documented. Anuradha and Rao in 2007 studied the effect of 24-Epibrassinolide and 28-Homobrassinolide on seed germination and seedling growth of radish under cadmium toxicity. Also, the impact of BRs on the activity of antioxidant enzymes CAT, POX, SOD, APOX and GPOX in radish seedlings under Cd toxicity was evaluated. It was found that BRs supplementation alleviated the toxic effect of the heavy metal and increased the percentage of seed germination and seedling growth. Out of the two substances, HBl was found to be more effective than EBl in stress alleviation. Sharma *et al.* (2007) reported that pre-sowing treatments of 28-Homobrassinolide lowered the uptake of zinc and enhanced the activities of antioxidative enzymes and protein concentrations of seven days old *B. juncea* seedlings. Moreover, the activities of antioxidant enzymes CAT, SOD, APOX and GPOX were stimulated in the seedlings from treatments with Cd along with BRs. Regulation of enzyme activities with 24-Epibrassinolide treatment under abiotic stress have also been documented by Arora *et al.* (2010) where it was analyzed that seed pre-soaking treatments with 24-Epibrassinolide improved the growth of plants and increased the activities of antioxidative enzymes of *B. juncea* during chromium metal toxicity. Sharma *et al.* (2010) reported similar results in seven days old *R. sativus* seedlings.
under cadmium (Cd) stress. Here also, Cd toxicity resulted in reduced seedling growth in terms of root and shoot length and biomass but HBl treatment lowered the Cd toxicity and enhanced the length and biomass. Similarly, modifications in the antioxidative enzyme activities during exogenous application of BRs have also been reported in stressed plants like mustard, maize, wheat and groundnut (Hayat et al., 2007; Sharma et al., 2007; Bhardwaj et al., 2008; Sirhindi et al., 2009; Yusuf et al., 2010 and Verma et al., 2012).

Besides, the role of steroids in host plant defence, assays have also been carried out on the protective role played by other phytohormones and elicitors in reducing nematode stress. Molinari in 2001, studied near-isogenic lines of tomato, resistant and susceptible to virulent *M. incognita* juveniles and treated with SA. CAT activity determined was significantly inhibited in resistant roots by either nematode infestation or salicylic acid treatment. Such inhibition did not occur in susceptible roots and in resistant roots inoculated with a virulent isolate of *M. incognita*. The $\text{H}_2\text{O}_2$ degrading activities of CAT and APOX were both inhibited in the upper green tissues of the resistant seedlings 24hrs after nematode inoculation, although, they were restored or enhanced compared with those of controls 48 hrs after inoculation. Resistant seedlings incubated overnight in SA showed marked inhibition of CAT activity in green tissues whilst such inhibition was much less consistent with susceptible seedlings. In another study, Molinari and Loffredo (2006) investigated the possible involvement of SA in defence responses of tomato to *Meloidogyne* spp. SA was found not to be responsible for the inhibition of CAT detected in the early stages of *Meloidogyne*-tomato incompatible interactions. Inhibition of CAT extracted from leaves was observed only after treatment of seedlings with SA concentrations as high as 4mM and most of the free SA found in plants after SA treatment was detected in the leaves. The results reported by Nguyen et al. (2011) showed similar results where cucumber plants were infected with *M. incognita* and then treated with *C. cassia* crude extracts (CCE). The activities of antioxidative enzymes CAT, APOX and SOD were detected in the leaves. Twenty-eight days after treatment with CCE, SOD activities of leaves were greater than that of leaves treated with nematode alone. In addition, CAT and APOX activities increased as the concentrations of CCE increased. Also, relative activities of
antioxidative enzymes decreased as the galling formation in the roots increased in response to *M. incognita* infection.

In a study by Sahebani *et al.* (2011), effects of BABA on activities of GPOX and CAT in cucumber against *M. javanica* was evaluated. The treatment of cucumber roots by BABA and BABA+ nematode, significantly increased GPOX and CAT activities in root tissues one day after nematode inoculation, in comparison to control and reached to a maximum level at four and three days after nematode inoculation, respectively. Biochemical changes in grape rootstocks resulted from humic acid treatment in relation to *M. incognita* and *R. reniformis* infection were analyzed by Kesba and El-Beltagi (2012). As a result of humic acid application, antioxidative defense enzymes APOX, SOD and CAT showed significant increase in their specific activities in treated plants compared with nematode treated check. El-Beltagi *et al.* (2012) in another study on tomato against *M. incognita* using fertilizers (organic and inorganic) determined the activities of SOD, APOX and CAT in shoots and roots of healthy and infected tomato plants. The results showed that nematode infection significantly increased the activity levels of SOD, APOX and CAT and greater increasing rates were observed in all treatments. In addition, Pokhare *et al.* (2012) also assessed the biochemical changes induced by foliar applications of BABA, JA, SA and carbofuran in susceptible wheat roots during *H. avenae* infection 5 and 10 days after nematode inoculation. Results revealed a little variation of enzyme activity in the inoculated control and carbofuran treated plants. On the other hand, significant increase in enzyme activity was observed following the foliar spray of three elicitors and this increase in enzyme activity was found greater with increase in their concentrations.

### 5.1.4. Changes in Non-enzymatic Antioxidants during Nematode Pathogenesis

Non-enzymatic antioxidants play a key role in the host defence against invading pathogen. These help the plants to reduce accumulation of ROS to sub-lethal amounts thereby improving plant growth (Ahmad *et al.*, 2009). Various antioxidants that play leading role in reducing acclimatization of plants to stresses includes phenols, isoflavonoids, terpenoids, α-tocopherols, ascorbic acid, glutathione, alkaloids etc. In the present study, total phenolic content, total flavonoid content, ascorbic acid content
and total glutathione content were estimated during nematode pathogenesis and post HBl treatment. Data collected showed variations in the content of antioxidants post nematode inoculation and further with HBl treatment in both the cultivars. Moreover, considerable differences were observed in the content of antioxidants when the two cultivars were compared with each other highest being in the resistant one. Similar study by Rani et al. (2008) on biochemical basis of resistance to root-knot nematode in tomato showed that among the parents CLN 2026C and SL 120, SL 120 showed the highest level of phenol. Among the hybrids, CLN 2026C x SL 120 had highest levels of total phenols. Further, it was reported that the susceptible check (COTH-1) registered the lowest value of phenols. Later on, Rani et al., 2009 in another investigation studied biochemical components in tomato using twenty three hybrids highly resistant to M. incognita. In the study, total phenols, ortho dihydroxy phenol, IAA oxidase, chlorogenic acid and ascorbic acid were determined. It was found that total phenol content was highest in resistant plants, SL 120 and LE 812.

Further, the results obtained from the current investigation are also in concordance with the investigations conducted by El-Beltagi et al. in 2011 where effect of M. incognita and two species of A. tumefaciens (A; A1 or B1 = 2.5 x 10⁷ CFU/pot) and A. vitis (B; A2 or B2 = 5 x 10⁷ CFU/pot) on antioxidant activity of grape leaves was studied. The results indicated that M. incognita at two levels: 2000 J₂ (M1) and 4000 J₂ (M2) and two Agrobacterium species (M1A1 and M1B1), significantly enhanced the contents of GSH and ascorbate. While M1A2, M2A1, M1B2 and M2B1 showed an obvious increase in GSH and AsA as compared to the healthy plants. Modulations in ascorbic acid content in Japanese black pine (P. thunbergii) during pine wood nematode (B. xylophilus) infection were determined by Jia-jin et al. (2011). After nematode invasion, ascorbic acid in stems of pine seedlings decreased gradually compared to that in the control with time elapsed after inoculation. While during whole experiment, the content of ascorbic acid in control plants was almost constant. Kumar et al. (2012) also carried out investigations to screen the in vitro derived mutants of banana cv. Robusta (resistant) and Rasthali (susceptible) against P. coffeae and R. similis. Mutants belonging to Robusta registered the highest total phenol content than the susceptible check. Studies on the evaluation of total phenolic content have also been carried out in
ten sugar beet genotypes infected with *M. incognita* under glass house conditions. The data was procured sixty days after nematode inoculation and significant increase was demonstrated in total phenolic contents (both shoot and root) in most infected genotypes as compared to non-infected genotypes (Korayem *et al.*, 2012).

5.1.4.1. Effect of HBl Treatment on Non-enzymatic Antioxidants

Besides nematode inoculation, when seeds of both the cultivars were treated with different concentrations of HBl, modulations in the content of antioxidants were observed. With HBl application, overall content of antioxidants was improved, thus, suggesting the increase in resistance capacity of plants during nematode stress. Moreover, when the two cultivars were compared, higher values of antioxidants were seen in the resistant cultivar. Increase in the antioxidant level in plants treated with brassinosteroids has been reported by Ding *et al.* (2009). In the study, root and foliar applications of EBL significantly reduced symptoms of fusarium wilt and the pathogen induced flavonoids and phenolic compounds. Thereby, revealing enhanced resistance by EBL to fusarium wilt by increase in antioxidant system. Recently, Serna *et al.* (2013) reported observations pertaining to effects of brassinosteroid analogues on total antioxidant activity and phenols in endive. All treatments with DI-100 and DI-31 (brassinosteroid analogues) sprayed by dissolving in Tomex Amin resulted in significant increase in total antioxidant activity and total phenols in the endives. Treatment with other plant growth regulators and growth elicitors has also demonstrated observable alterations in antioxidant content. Studies by Sun *et al.* (2011) regarding enhanced levels of total phenolics, and flavonoids reported that elevated CO₂ influenced nematode (*M. incognita*) induced defence response of tomato genotypes [wild-type (Wt), jasmonate deficient *spr2* mutants (*spr2*) and 35S::Prosystemin transgenic tomato plants (35S)] differing in the JA pathway. In other experiment, the effects of β-amino-butyric acid (BABA) on *M. javanica* infection on cucumber and accumulation of total phenolic compounds were investigated. It was observed that inoculation of cucumber plants by BABA significantly increased the total phenols in comparison to control with maximum level observed seven days after nematode inoculation. Thus, suggesting inhibitory effect of BABA on the root-knot nematode and relating its ability to enhance defence responses in the cucumber roots (Sahebani *et al.*, 2011). Further, investigations
were carried by Campos et al. (2012) to evaluate the metabolic responses of plants susceptible or resistant to *M. incognita* during first 96h after inoculation in seeds of common beans, tomato and soybean resistant and susceptible to *M. incognita*. Soybean cultivars resistant to nematode produced more soluble phenols (107% higher than susceptible plants 48h past inoculation). In tomato also, resistance to *M. incognita* was correlated with the production of soluble phenols with higher concentrations in the resistant cultivar.

Studies regarding salicylic acid-induced glutathione status in tomato crop and resistance to *M. incognita* have been evaluated by Meher et al. (2011). Salicylic acid was sprayed on tomato foliage (Pusa Ruby) at 5.0-10.0 μg mL⁻¹. It was found that SA reduced the root infection by *M. incognita*, nematode reproduction and improved the resistance of tomato but reduced crop growth and redox status. Hence, suggesting that SA can be applied for metabolic engineering of tomato at transplanting to combine host-plant resistance and health benefits in formulating a strategic nematode management decision. Also, Kesba and El-Beltagi (2012) analysed biochemical changes in grape rootstocks due to humic acid treatments in relation to *M. incognita* and *R. reniformis*. It was observed that as a result of humic acid applications, the contents of ascorbic acid and total phenols were increased significantly when compared with the control. In another experiment, El-Beltagi et al. (2012) speculated the response of antioxidant substances as a defence mechanism against root-knot nematode infection in tomato treated with fertilizers (organic and inorganic). The results when analysed, demonstrated that the levels of glutathione (GSH) and ascorbic acid in the healthy plants were low in shoots and roots. Also, nematode infection resulted in a slight but significant increase in both glutathione and ascorbic acid in shoots and roots. Thus, concluding that all treatments significantly increased the antioxidant substances comparing to healthy and infected untreated tomato plants.

5.1.5. **Qualitative Analysis of Phytohormones**

Plant hormones are the signalling molecules that are produced in extremely low concentrations in plants. They regulate cellular processes both locally and at far. Any alterations in the indigenous levels of these hormones, thereby, activate plant’s defence
responses in addition to the physico-chemical modulations. Currently, investigations are being carried out on the interaction of different hormones. Cross-talk between phytohormones such as IAA, SA, ABA, JA, BRs, polyamines etc. revealed their role in stress management. Therefore, in the present study, qualitative analyses of phytohormones were also carried out using LC/MS. When the responses of both the cultivars were compared with each other at all time intervals, in susceptible cultivar, $10^{-9}$ M was the most potent concentration with 120 hrs after nematode inoculation as the effective day. In contrast, in resistant cultivar, $10^{-7}$ M was the most effectual HBl concentration with 72 hrs post-nematode inoculation as the effective time interval. The analysis showed the presence of three phytohormones: Put, JA and SA in both the cultivars after nematode inoculation and HBl treatment.

Phytohormone signalling in response to herbivory mediates changes in the plants (Pieterse et al., 2009). Number of investigations asserted the role of JA and SA related gene expression levels during induced responses. Moran and Thompson, (2001) investigated the feeding of green peach aphid (Myzus persicae Sulzer) on Arabidopsis. The study showed induced transcription of two genes associated with SA-dependent responses to pathogens (PR-1 and BGL2) 10 and 23 fold respectively. 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 by de Vos et al. (2005). 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 carried out by Heidel and Baldwin, 2004; Kuśnierzcyk et al., 2007, 2008, 2011; Wubben et al., 2008; Kutyniok and Müller, 2012 and Beneventi et al., 2013.

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 (van Breda de Haan) Luc and Goodey were carried out by Nahar et al. (2012). The study demonstrated prerequisite requirement of intact ET, JA and SA biosynthesis pathway while negative role of ABA was revealed. De
Vleesschauwer et al. (2012) demonstrated the involvement of BR pathway in rice root susceptibility to root pathogen, *Pythium graminicola* Subraman in suppressing SA and GA mediated signal transduction pathways during disease resistance. Also, Nahar et al. in 2013 demonstrated the balance between BR and JA pathway which is an effective regulator of the outcome of the rice-*M. graminicola* interaction. Results showed a negative cross-talk between BR and JA pathway. JA-related gene transcripts were strongly down-regulated in low brassinolide concentrations 24 hrs but when brassinolide concentrations were high, JA genes were up-regulated.

The primary effect of BRs is to promote cell elongation and division. They are highly effective in stimulating growth in young vegetative tissues. They have been reported in promoting elongation of mung bean, soybean, pea epicotyls, bean, sunflower and cucumber hypocotyls, *Arabidopsis* peduncles and *Hordeum vulgare* L. seedlings (Gregory, 1982; Clouse et al., 1992; Yu et al., 2004; Clouse, 2008; Kartal et al., 2009). In addition, the ability of BRs in incrementing plant resistance against environmental stresses (abiotic/biotic) has also been carried out both under laboratory and field conditions. In the present study, application of HBl improved the overall plant growth and regulated the activities of enzymes of Asada-Halliwell pathway i.e. SOD, APOX, GR and water-water cycle enzyme activities i.e. CAT and POD under nematode stress. This increase in the level of these enzymes may be attributed to the fact that HBl treatment helped the plants to overcome the stress generated by *M. incognita* by enhancing the resistance capacity of plants.

The outcomes of both past and the current study have indicated that BRs enhances the capacity of plants to fight against an array of biotic and abiotic stresses. But, a key question how plants uses BRs under stressed conditions still needs an explanation. Further, the mechanism by which it suppresses the stress is also in its budding stage. However, a combination of genetic, proteomic and genomic approaches would be required in solving these mechanisms. Investigations pertaining to these questions will help in advancing our current understanding of plant growth regulation, which is a necessity for food, bio-energy production as well for safer environmental conservation.