SHORT COMMUNICATION

Effect of β -amino butyric acid in induction of resistance against *Xanthomonas campestris* pv. *campestris* in cabbage

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ABSTRACT: A defense activator chemical, β -aminobutyric acid (BABA) is capable of inducing local as well as systemic resistance against numerous plant pathogens. An experiment was conducted to evaluate induction of defense related enzyme peroxidase (POX) by BABA for imparting systemic resistance against *Xanthomonas campestris* pv. *campestris* (Xcc) inciting black rot disease of cabbage. Effect of BABA (1000 μ g/ml) on the induction of POX activity in the leaves of cabbage resistant cv. Pusa Mukta and susceptible cv. Golden Acre in response to BABA and *X. campestris* pv. *campestris* revealed that the POX was found increasing in BABA + Xcc treated plants and reached maximum level at 5 days after inoculation, in comparison with BABA or Xcc treated alone. The bacterial population of *X. campestris* pv. *campestris* was also reduced significantly in both the cultivars of cabbage treated with BABA (1000 μ g/ml) as compared to control (water) treated plants. The level POX activity was observed to be low in both uninoculated and inoculated susceptible cv. Golden Acre as compared to resistant seedlings. Thus, explaining the enhancing activity of POX with application of BABA in reducing population level of *X. campestris* pv. *campestris*.

Keywords: Peroxidase, systemic acquired resistance, *Xanthomonas campestris* pv. *campestris*, β -amino butyric acid

Cabbage is one of the major cruciferous vegetable which has been cultivated worldwide. Yield potential of cabbage is limited by many of the bacterial, fungal and viral diseases, among them black rot disease caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson is the most serious bacterial disease affecting the yield and marketable quality of the produce. *X. campestris* pv. *campestris* is also known to infect other economically important cruciferous vegetables of *Brassica oleracea* group such as cabbage, cauliflower, broccoli, brussels sprouts as well as cruciferous weeds and ornamentals plants such as stock (*Matthiola* sp.) and wallflower (*Cheiranthus cheiri*) (Singh *et al.*, 2016).

β -amino butyric acid (BABA) has shown inhibition of the disease development in canker of tomato caused by *Clavibacter michiganensis* (Baysal *et al.*, 2005), bacterial leaf spot of tomato caused by *Pseudomonas syringae* (Baysal *et al.*, 2007), fire blight of apple incited by *Erwinia amylovora* (Hassan and Buchenauer, 2007), rust of sunflower incited by *Puccinia helianthi* (Amzalek and Cohen, 2007) and citrus canker caused by *X. citri* subsp. *citri* (Sharifi *et al.*, 2011) which is not directly shown antifungal and antibacterial activities. However, it induces a wide variety of defense mechanisms in the plants like accumulation of PR proteins involved in the antimicrobial activity, depending on the pathogen and its host plant (Altamiranda *et al.*, 2008; Sasek *et al.*, 2011). Interestingly, it has been observed that BABA

induces resistance in cauliflower, cabbage, brown mustard, sunflower and lettuce against diseases, but it does not induce the accumulation of PR proteins (Silue *et al.*, 2002; Amzalek and Cohen 2007; Kamble and Bhargava 2007; Cohen *et al.*, 2010). Thus, it indicates that the accumulation of PR proteins is not the only mechanism of resistance in the plant. Hassan and Buchenauer (2007) reported the BABA reduced fire blight in apple seedlings and peroxidase activity, total and free salicylic acid (SA) in leaf tissues was found to be increased by treating with BABA. Raghavendra *et al.* (2017) demonstrated the potentiality of BABA in controlling black rot disease in cabbage. The present study was undertaken to explore the ability of BABA in induction of defense related enzyme such as peroxidase in imparting the systemic resistance against *X. campestris* pv. *campestris* inciting black rot of cabbage. Thus, enhance the understanding of mechanism of resistance induced by BABA in crop plants in general and cabbage in particular.

Plant material

The seed material of resistant cv. Pusa Mukta and susceptible cv. Golden Acre of cabbage supplied by the ICAR-IARI, regional station Katrain, India was used. The seeds were sown in plastic pots having peat/vermiculite mixture based media and are grown under controlled conditions with 18-22°C day and 16-18°C night temperature in the National Phytotron Facility at ICAR-IARI, New Delhi.

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β -aminobutyric acid and its application

The chemical DL-3- β -aminobutyric acid (BABA) was purchased from Sigma Aldrich. BABA was used at 1000 μ g/ml on the 30 days old cabbage plants. The chemical was applied by spraying whole plants with the aid of a fine atomizer onto the upper leaf surfaces. A set of plants were treated with sterilized distilled water and maintained as control. pathogen

Inoculation of *X. campestris* pv. *campestris* on plants

The characterized strain of *X. campestris* pv. *campestris* belonging to race 1 was taken from Plant Bacteriology Laboratory, Division of Plant pathology, ICAR-IARI, New Delhi. The culture was maintained on yeast glucose calcium carbonate agar (YGCA) slants and is stored under 4°C for a short period. For preparing inoculum suspension, culture of *X. campestris* pv. *campestris* was transferred into nutrient sucrose broth and incubated for 24 h at 28°C with shaking at 150 rpm. Bacteria were pelleted with centrifugation for 5 min at 6,000 rpm and resuspended in distilled water to give concentrations of 10⁸cfu/ml. *X. campestris* pv. *campestris* race 1 was inoculated on young leaves of cabbage by clipping the secondary vein margins to the cabbage plants after 4 days of spraying of chemical. The disease scoring was done at 15 days after inoculation of pathogen as described by Vicente *et al.* (2001).

Assessment of Peroxidase (POX) (EC 1.11.1.7) enzyme activity

From the inoculated leaves, leaf disks of 1.0 cm in diameter was taken at 0, 2, 3, 5 and 7 days post-inoculation and examined for peroxidase activity. Assessment of these defense related enzymes was performed according to the following standard procedure. Crude enzyme extract was prepared by grinding about 0.5 g of leaf tissue which was homogenised in potassium phosphate (KH₂PO₄) buffer (1:5 w/v) (pH 7.0) containing 1 mM EDTA and 1% polyvinyl pyrrolidone (PVP). The homogenate was centrifuged at 15,000 X g for 30min at 4°C. The supernatant was collected and it was used for enzyme assays. Total soluble protein was estimated by using BSA as a standard for the enzyme activity (Bradford, 1976). Peroxidase activity was assayed by using standard method described by Castillo *et al.* (1994) with slight modifications. Three ml reaction mixture contained one ml of 100 mM phosphate buffer (pH 7.0), 0.5 ml each of 96 mM guaiacol and 12 mM H₂O₂, 50 μ l of enzyme extract and 950 μ l of distilled water. At 470 nm changes in absorbance due to the formation of tetra-guaiacol was recorded after every 30 seconds for 3 min. The enzyme activity was calculated as per the extinction coefficient of its oxidation product, tetra-guaiacol E= 26.6 nM/cm and expressed as μ mol/mg protein/min.

Enumeration of bacteria *in-planta*

BABA and water treated leaves were sampled. Excised leaf discs were homogenized in 1 ml of sterile 0.06%

NaCl solution and serially diluted up to 10⁻⁷ and 0.1 ml aliquots of dilution were plated on NSA medium. The Petri plates were incubated at 28°C for 48 h, and emerging colonies of *X. campestris* pv. *campestris* were counted. The population of *X. campestris* pv. *campestris* were enumerated only pathogen treated plants at 1, 2, 5, 7 and 11 days after inoculation.

Experimental design and statistical analyses

All the experiments were maintained in a CRD (completely randomized design) with three replicates having 20 plants in each treatment and these were repeated twice. For peroxidase activity, variance (anova) analysis was carried out. The significant variations among the treatments was calculated using Duncan's Multiple Range Test at $P < 0.05$.

Effect of β -amino butyric acid treatment on the induction of peroxidase (POX) activity in the leaves of resistant cv. Pusa Mukta, the POX activity was found increasing in BABA + Xcc treated plants and reached maximum level of 4.197 (mM mg protein⁻¹min⁻¹) at 5 days after inoculation and shown decreasing tendency at 7 days after inoculation (Fig. 1). BABA treated plants which were not inoculated with *X. campestris* pv. *campestris* shown higher POX activity (1.054mM mg protein⁻¹min⁻¹) 2 days after spraying and continued up to 5 days after spraying and was reached maximum level (3.071 mM mg protein⁻¹min⁻¹) significantly higher than that of control plants (inoculated or not). In susceptible cv. Golden Acre also, the level POX activities was reached higher (3.697 mM mg protein⁻¹min⁻¹) at 5 days after inoculation in BABA + Xcc treated plants but showed decreased tendency at 7 days after inoculation (Fig. 2). Also, the POX activity in BABA treated plants which were not inoculated with *X. campestris* pv. *campestris* increased with time and reached highest level (2.571 mM mg protein⁻¹min⁻¹) 5 days after spraying.

The growth of *X. campestris* pv. *campestris* was reduced in BABA treated plants of both resistant cv. Pusa Mukta Acre (Fig. 3) and susceptible cv. Golden Acre (Fig. 4) compared with the control plants. The inhibitory effect was first observed 2 days after inoculation and monitored until 11 days after inoculation with *X. campestris* pv.

Fig. 1. Effect of β -amino butyric acid treatment on the induction of peroxidase activity in the leaves of resistant cabbage cv. Pusa Mukta. Bars with the same letters represent values that are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$)

Fig. 2. Effect of β -amino butyric acid treatment on the induction of peroxidase activity in the leaves of susceptible cabbage cv. Golden Acre. Bars with the same letters represent values that are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$)

Fig. 3. Effect of β -amino butyric acid treatment on the bacterial growth of *X. campestris* pv. *campestris* in the leaves of resistant cabbage cv. Pusa Mukta. Data are the means of two independent experiments, and bars represent standard error

Fig. 4. Effect of β -amino butyric acid treatment on the bacterial growth of *X. campestris* pv. *campestris* in the leaves of susceptible cabbage cv. Golden Acre. Data are the means of two independent experiments, and bars represent standard error

campestris. The Bacterial population was reduced in BABA treated resistant plants, the population level was varied between 3.42 log cfu/ml to 3.72 log cfu/ml at 2 and 7 days after inoculation respectively, and it again shown decreased tendency (3.64 log cfu/ml) at 11 days after inoculation. Whereas, in case of water treated control plants *X. campestris* pv. *campestris* population level increased from 4.73 log cfu/ml at 2 days after inoculation and showed increased tendency and reached maximum (6.23 cfu/ml) at 7 days after inoculation.

In case of susceptible cv. Golden acre, the *X. campestris* pv. *campestris* population recovered from BABA treated leaves showed reduced population level and it increased from 2.43 log cfu/ml at 2 days after inoculation and reached maximum level (4.32 log cfu/ml) at 7 days after inoculation and after that the population started decreasing at 11 days after inoculation. In control, susceptible cv. Golden acre, *X. campestris* pv. *campestris* population level increased from 4.93 log cfu/ml at 2 days after inoculation and showed increased tendency and reached maximum (7.63 cfu/ml) at 7 days after inoculation.

The BABA induces different types of defense mechanisms in plants against pathogen infection, which depends on the pathogen and its host. Biochemical mechanisms in response to the stress are initiated such as biosynthesis of phenols, anthocyanin, phytoalexins and induction of enzymes activities associated with active oxygene species. Interestingly, BABA induces resistance in cauliflower, cabbage, brown mustard, sunflower and lettuce to disease, but it does not induce the accumulation of PR proteins (Silue *et al.*, 2002; Kamble and Bhargava 2007; Amzalek and Cohen 2007; Cohen *et al.*, 2010).

In the present study, the effect of BABA treatment on the induction of defense related enzyme peroxidase (POX) activity in the leaves of cabbage resistant cv. Pusa Mukta and susceptible cv. Golden Acre in response to BABA and *X. campestris* pv. *campestris* revealed that the POX activity was found increasing in BABA + Xcc treated plants and reached maximum level at 5 days after inoculation in comparison with BABA or Xcc treated alone. This increased POX activity was in turn supported by the fact that less Xcc population recovered from BABA + Xcc treated leaf samples, whereas higher Xcc population was recovered from the water + Xcc treated (control) plants. The level of POX activity observed to be low in both uninoculated and inoculated susceptible cv. Golden Acre as compared to resistant seedlings during time course. It was observed that the increased level of POX activity is associated with the lower *X. campestris* pv. *campestris* population recovered from the respective tissue which has been tested for POX activity in both resistant and susceptible cabbage cultivars. Similarly, decreased level of POX activity is associated with the higher *X. campestris* pv. *campestris* population recovered from the respective tissue which has been tested for POX activity. Thus explaining the importance of POX activities in reducing *X. campestris* pv. *campestris* population level in turn its efficiency in determining the level of resistance induced by the BABA irrespective of resistant and susceptible cultivars of cabbage. This emphasizes the fact that as soon as the pathogen invades the plants, the activation of immune system of the plant by BABA diverts its defense responses to curb the pathogen colonization.

Thus explaining the importance of POX activity in inducing the systemic resistance against *X. campestris* pv. *campestris* immediately after infection in to cabbage plants pretreated with BABA. Similar effect of potentiality of BABA in reducing the black rot disease severity has

been demonstrated by Raghavendra *et al.* (2017). Hassan and Buchenauer (2007) reported that peroxidase and total free salicylic acid in leaf tissues were found to be increased on inoculation with BABA in apple seedlings against fire blight. The citrus canker was also effectively reduced in lime plants treated with BABA at 250 ppm (Sharifi *et al.*, 2011).

Present study support to the fact that candidature of BABA in inducing the systemic resistance in crop plants against bacterial diseases in general and *X. campestris* pv. *campestris* - cabbage pathosystem in particular was found effective. We can use BABA @ 1000 ppm as an alternative to traditional copper fungicide or antibiotics to control black rot of cabbage. It also offers a novel method of management of black rot disease for improving the quality and sustainable production of cabbage in India.

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