

β-amino Butyric Acid as Novel Defense Activator against *Xanthomonas campestris* pv *campestris* causing Black Rot of Cabbage

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Received 27 June 2016, Accepted 25 July 2016; Published online 619 August 2016

Abstract The non-protein amino acid, β-amino butyric acid (BABA) is known to induce systemic acquired resistance (SAR) which is generally effective against a broad range of pathogens, is associated with the production of pathogenesis related (PR) proteins and is mediated via a salicylic acid depended process. BABA is capable of inducing local and systemic resistance against numerous plant pathogens. Experiment was conducted to evaluate the effect of the BABA on the *X. campestris* pv *campestris* causing black rot disease in cabbage. In order to determine the effect of BABA, the *X. campestris* pv *campestris* was inoculated 4 days after spraying with BABA on to the cabbage cv golden Acre where in the control plants were treated with sterilized distilled water. The disease severity was recorded 15 days after inoculation on the cabbage plants. The higher disease severity index was observed in non-treated plants (33.33%) as compared to cabbage

plants treated with BABA (18.51%), indicating the efficiency in reducing the progress of the disease.

Keywords *Xanthomonas campestris* pv *campestris*, β-amino butyric acid, Systemic acquired resistance.

Introduction

Cruciferous vegetables are one of the dominant food crops worldwide. Cabbage is an important cole crop cultivated in India. Black rot disease in cabbage is incited by *Xanthomonas campestris* pv *campestris* (Pammel) Dowson. The bacterium cause losses upto 50% in favorable environmental conditions like high relative humidity and high temperature. Black rot disease has been reported from major crucifers growing parts of the world. In India, the black rot disease is an major production limiting factor in cabbage (*Brassica oleracea* var *capitata*), cauliflower (*B. oleracea* var *botrytis*), knol-khol (*B. oleracea* var *gongylodes*), broccoli (*B. oleracea* var *italica*), brussels sprout (*B. oleracea* var *gemmifera*), brown mustard (*B. juncea*), yellow sarson (*B. rapa* var *trilocularis*), brown sarson (*B. rapa* sub sp. *sarson*) and toria (*B. campestris* L. var *toria*). Growing regions of Rajasthan, Uttar Pradesh, Bihar, Orissa, Assam, West Bengal, Haryana, Punjab, Himachal Pradesh, North Eastern states, Karnataka, Andhra Pradesh, Maharashtra, Tamil Nadu and Kerala which are belonging to diverse agro climatic regions.

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Disease control is difficult because of a lack of commercially acceptable resistant cabbage cultivars. Chemical control of the disease relies upon the use of antibiotics (such as streptomycin) and copper compounds, which prevent bacterial multiplication and further infection. Unfortunately, the antibiotics have led to the selection of resistant bacterial populations. Use of antibiotics is banned or severely limited in many countries. There is currently an urgent need to consider alternative environmentally friendly management strategies for the control of black rot of cabbage. Chemical inducers of disease resistance play the key role for the integration of the SAR concept in modern plant protection strategies, as the immune-stimulated plants are able to respond more rapidly and adequately to various biotic stresses allowing them to efficiently combat an infection. During the priming phase, plants are stimulated in absence of a challenge and can accumulate and store conjugates or precursors of molecules as well as other compounds that play a role in defense. These molecules can be released during the defensive phase following stress [1].

Induced resistance can be attained by using chemical analogues of salicylic acid (such as 2, 6 dichloroisonicotinic acid (INA), β -aminobutyric acid (BABA) and benzothiadiazole-S-ester (Bion) for systemic acquired resistance (SAR) or jasmonic acid (Methyl jasmonate) for induced systemic resistance (ISR) [2]. The non-protein amino acid BABA is known to induce systemic acquired resistance (SAR) which is generally effective against a broad range of pathogens, is associated with the production of pathogenesis related (PR) proteins and is mediated via a salicylic acid depended process. The efficiency of BABA in inducing the disease resistance has been reported against several bacterial, fungal and oomycetes such as bacterial head rot disease of broccoli [3], *Pseudomonas syringae* pv *phaseolicola* infection in the common bean [4], bacterial speck disease caused by *Pseudomonas syringae* pv tomato [5], *Bremialactuae* infection in lettuce [6], downy mildew of grape [7] and rust infection in sunflower plants [8]. In spite of the intriguing capacity of BABA to confer protection against an extraordinarily wide range of biotic stresses [3–8]. No information is available on the

activity of BABA against black rot disease in cabbage. Therefore, our major objective was to explore the ability of BABA to protect cabbage against *Xanthomonas campestris* pv *campestris*, which might allow the development of a novel environmentally friendly method for disease protection in the cabbage crop plants, which also will improve quality and productivity of the crop. Although, exploitation of natural defense mechanism for management of this bacterial disease offers an alternative to traditional methods of disease management in cabbage.

Materials and Methods

Plant material

Seeds of cabbage cv golden acre was used in this study. The seed material have been supplied by the ICAR-IARI, regional station Katrain, India. These seeds were sown in plastic pots filled with peat/vermiculite mixture based media and are maintained in green house (National phytotron facility in ICAR-IARI, New Delhi) with 18–22°C day, 16–18°C night. Plants were fertilized with 0.5% N : P : K (20 : 20 : 20) once a week.

X. campestris pv *campestris* culture and inoculum preparation

The characterized strain of *X. campestris* pv *campestris* belonging to race 1 was used in this study for challenge inoculation on cabbage. This culture has been 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 are stored under 4°C for short term storage. It was also maintained for long term storage at -80°C in nutrient sucrose broths with 20% glycerol. For preparing inoculum suspension, culture from -80°C were grown on nutrient sucrose agar (NSA) media for 24 h at 28°C and single colonies were transferred to nutrient sucrose broth and incubated 24 h at 28 °C with shaking at 150 rpm. Bacteria was pelleted with centrifugation for 5 min at 6,000 rpm and resuspended in distilled water to give concentrations of 10⁸ CFU/ml.

Pathogenicity test

X. campestris pv *campestris* was tested for pathogenicity on 4 weeks-old plants of cabbage cv golden acre. Inoculation was made on young leaves by clipping the secondary vein margins as described by Vicente and co-workers [9]. Then the development of symptoms were observed.

Defense activating compound and its application

DL-3- β -aminobutyric acid (BABA) was purchased from sigma aldrich. BABA was used at 500 μ g/ml on the 30 days old cabbage seedlings. The chemical was applied by spraying whole plants. Spraying will be done with the aid of a fine atomizer onto the upper leaf surfaces. Control plants were treated similarly with sterilized distilled water. Later challenge inoculation was done 4 days after spraying and disease scoring was done 15 days after inoculation.

Method of inoculation and disease scoring

Cabbage leaves were inoculated by giving a small minute cut of about 1 mm, near the leaf margins, with scissor dipped in the inoculum suspension. The youngest leaves on each plant were inoculated. The

disease severity was recorded at 15 days after inoculation. The severity of symptoms was assessed [8] on a six-point scale of 0—9 based on the relative lesion size as 0 = no symptom, 1 = small necrosis or chlorosis surrounding the infection point, 3 = typical small V - shaped lesion with black veins, 5 = typical lesion half way to the middle vein, 7 = typical lesion progressing to the middle vein and 9 = lesion reaching the middle vein. The disease severity index (DSI) will be calculated using the formula,

$$DSI (\%) = \frac{\sum (\text{Rating no.} \times \text{no. of plants in rating}) \times 100}{\text{Total no. of plants} \times \text{highest rating}}$$

Results and Discussion

The characterized strain of *X. campestris* pv *campestris* belonging to race 1 was used in this study for challenge inoculation on cabbage. The culture was grown on nutrient sucrose agar (NSA) media for 24 h at 28°C and single colonies were transferred to nutrient sucrose broth and incubated 24 h at 28°C with shaking at 150 rpm. Bacteria was pelleted with centrifugation for 5 min at 6,000 rpm and re-suspended in distilled water to give concentrations of 10⁸ CFU/ml. The prepared inoculum was tested for pathogenicity on 4 weeks-old plants of cabbage cv golden acre. Inoculation was made on young leaves by clipping the secondary vein margins. The typical

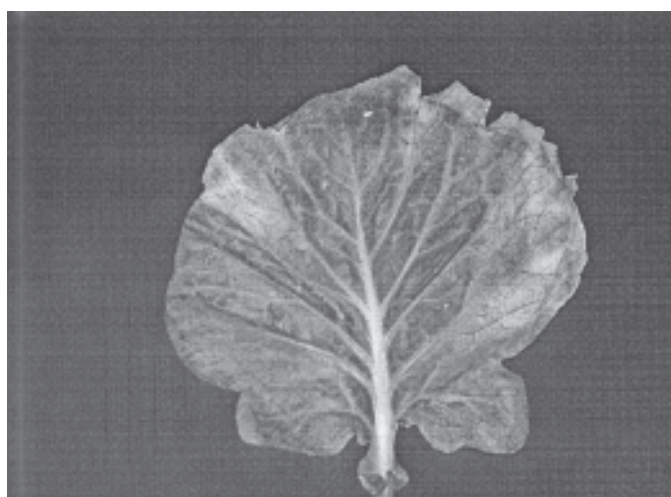


Fig.1. Typical black rot symptom on cabbage leaf inoculated with *X. campestris* pv *campestris*.

Table 1. Disease severity recorded on the control and BABA treated cabbage plants 15 days after inoculation with *X. campestris* pv *campestris*. *DSI = Disease severity index.

Disease score (0-9 scale) recorded after 15 days of inoculation	
Control (water sprayed) plants	BABA sprayed plants
3	1
1	3
3	3
5	1
3	1
3	3
3	3
3	1
3	1
3	1
1	1
3	1
3	3
3	1
5	1
*DSI (%) 33.33	*DSI (%) 18.51

black rot symptoms were observed on the cabbage leaf confirming the pathogenicity of the *X. campestris* pv *campestris* culture (Fig. 1).

Later to determine the effect of the DL- β – aminobutyric acid against the black rot disease causing pathogen an concentration of 500 μ g/ml was used on cabbage seedlings. Seeds of cabbage cv golden acre supplied by the ICAR-IARI, regional station Katrain, was sown in plastic pots filled with peat/vermiculite mixture based media and are maintained in green house (National phytotron facility in ICAR-IARI, New Delhi) with 18-22°C day, 16-18°C night. Plants were fertilized with 0.5% N : P : K (20 : 20 : 20) once a week. Later, the chemical was applied by spraying whole plants. Spraying will be done with the aid of a fine atomizer onto the upper leaf surfaces. Control plants were treated similarly with sterilized distilled water. Later challenge inoculation was done 4 days after spraying and disease scoring was done 15 days after inoculation. The disease development was more pronounced on the control plants as compared to the BABA treated plants (Table 1). The disease severity index was higher in non-treated plants (33.33%) whereas reduced disease severity index was recorded on the cabbage plants sprayed with BABA (18.51%). Similar kinds of reduced disease progress with the application of BABA has been

observed in case of bacterial head of broccoli [3], halo blight of common bean [4], bacterial speck of tomato [5]. Recent studies also suggested that, BABA-treatment leads to a more rapid induction of the priming phase, through a boost of the primary metabolism through the tricarboxylic acids (TCA) such as citrate, fumarate, (S)-malate and 2-oxoglutarate and the potentiation of phenylpropanoidbiosynthesis and the octadecanoic pathway [2]. Thus, BABA makes an efficient alternative for the traditional chemical method of managing the disease. So the use of BABA against *Xanthomonas campestris* pv *campestris* in cabbage might result in a novel environmentally friendly method for disease protection in the cabbage crop plants, which also will improve quality and productivity of the crop.

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