# Study on Beta Amino Butyric acid induced defence response in *Solanum lycopersicum*L. against *Alternaria solani*

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**ABSTRACT**

The present investigation deals with to study the effect of Beta Amino Butyric Acid (BABA) induced defence responses in tomato against *Alternaria solani*. The BABA induced defence were observed significantly in the seedlings which were pre-treated with BABA as compared to the seedling which were not pre-treated with BABA, Moreover, the pathogen attack was more prevalent in the seedling without BABA pre-treatment. The defence response were studied by assessing the $H_2O_2$ localization, callose deposition, Peroxidise activity and expression of *sl*WRKY transcription factors, as per the various defence responses under study, it is found that the BABA was found to be a potent defence inducer in tomato against fungal pathogen *Alternaria solani*.

**Keywords:***Beta Amino Butyric Acid; Alternaria solani;* induced defence.

## Introduction:

Tomato (*Solanum lycopersicum* L.)is the second most important vegetable crop after potato in world for local consumption & exportation. Tomatoes has outstanding vitamin content, the most important being ascorbic acid (vitamin C), thiamine(vitamin B), riboflavin(vitamin $B_2$) also rich in lycopene, Carotenoids, mineral phenolic acids, flavonoids, organic acids, essential amino acids & dietary Fibres. Research suggests that lycopene may play role in the fight against cancer, especially Prostate cancer.(Boss and Som, 1986). Tomato is attacked by several diseases one of the most severe being Early Blight of Tomato, is one of the most common foliar disease of tomatoes, caused by the necrotrophic fungus *Alternaria solani*. The disease causes significant reduction in yield up to 35-78% (Datar & Mayee, 1972). Early Blight disease to the tomato plants can result in a complete loss of the crop yields which is due to destruction of foliage and the fruits directly by the pathogen (Rotem, 1994).

***Alternaria solani*:**

Alternaria is belongs to division Deuteromycotina with no known sexual stages. The key taxonomic features of the genus *Alternaria* are the large, multicellular, dark coloured (melanised) conidia with longitudinal as well as transverse septa. The two major features of *Alternaria* spores are the production of melanin and the production of host-specific toxins, for example Alternaric acid in pathogenic *A.solani.* The pathogen was first described by Ellis and Martin, 1882. Alternaria has the ability to grow over a wide range of temperature from 4 -36 $^0$C (Pound, 1951) and requires only a short wet period of at least four hours for successful infection (Vloutoglou, 1999). Under favourable conditions, Alternaria

spores germinate within hours and can produce more than one germ tube per spore as the spores consist of several cells.

**Early blight of tomato:**

Early blight is the major disease symptom caused by the fungus *Alternaria solani* (Ellis & Martin), this disease severe cause leads to complete defoliation, it is found to be most damaging to *Solanum lycopersicum* L. Peralta *et al*., 2005, in the regions with heavy rainfall, high humidity, and fairly high temperatures (24°–29°C). Epidemics can also occur in semiarid climates where frequent and prolonged nightly dews occur (Rotem 1994). Apart from the leaf symptoms it is known as early blight (EB), Moreover, *A. solani* can cause symptoms on whole plant body including collar rot (basal stem lesions at the seedling stage), stem lesions on the adult plant, and fruit rot (Walker 1952).

**β- aminobutryic acid (BABA):**

β-aminobutryic acid (BABA) is a non– protein amino acid. It is structurally related to γ-aminobutryic acid (GABA), a highly bioactive substance and neurotransmitter in animals. β-aminobutyric acid (BABA) is known to induce resistance against several microbial pathogens, nematodes and insects in several host plants.BABA was shown to induce resistance in grapevines against *P. viticola*, tomato plants against *P. infestans*, tobacco plants against *P. tabacina* and *Arabidopsis* against *B. cinerea* (Zimmerli *et al*., 2001).

## Materials and Methods:

**Plant material:**Seeds of *Solanum lycopersicum* L showing susceptibility to the pathogen *Alternaria solani* were obtained from Seming, MonsantoIndia. The seeds were sown in pots containing garden soil and seedlings were grown for 4 weeks under in the green house in botanical garden up to 4-leaf stage. The plants were then transplanted to a new soil bags for luxuriant growth.

**Fungal cultures of *A. solani* :**

Fungal cultures of *A. solani* were obtained from the MTCC (Microbial type culture collection) Chandigarh and subsequently grown on the Potato Dextrose Agar medium in a growth chamber at 20$^{o}$C in the dark. Diseased leaf samples were collected from field, surface sterilized and grows on PDA medium in growth chamber at 28$^{0}$ C in the dark. Sporulation occurred after 45-40 days, after which the cultures were stored in a refrigerator at 4$^{o}$C. Spores were collected in a small volume of water and the spore density was adjusted to approximately 5X10$^{3}$ spores /ml before application.

**Chemicals and treatments:**

BABA was obtained from Sigma-Aldrich Corporation (St.Louis, MO, USA). Aqueous solution of 4mM BABA was sprayed till run off point on the adaxial surface of the leaves, 24 h before inoculation. Water was sprayed on some plants which wereconsidered as control.

**Plant Inoculation**:

Inoculations with *A. solani* were performed on 4 week old plants of *Solanum lycopersicum*. *Alternaria solani* spores in the form of suspension were applied as 10ul of droplets (approximately 50 spores) by the droplet inoculation method on either side of the midrib lamina portion of the first & second leaf from

the shoot apex.The inoculum droplet was allowed to dry & then the plantlets were sprayed with water to run off and covered by transparent polythene.

**Spore germination and penetration on Tomato leaves:**

Infected leaves were detached from plants after 5 dpi. The leaves were cleared in 95% ethanol so that the chlorophyll bleaches out. To note spore germination, the pieces of cleared leaves were stained with cotton blue for few seconds then de-stained with lacto-phenol & mounted in 50% glycerol for microscopic observations.

**Disease scoring by five point scale: Pande *et al.*,2003.**

Four week old 32 plants leaves of tomato variety Naina were used for disease scoring. Disease scoring was done at 3dpi, 5dpi and 7dpi after water & BABA pre-treatment with pathogen inoculation

**In situ localization of H2O2: Thordal, 1997.**

1 ml DAB (3, 3-Diaminobenzinine) solution was taken in eppendorf tube. Slanty Cut were made to the petiole of leaves and kept in DAB solution (4 week old plants pre-treated 24h before with Water & BABA (4mM) were used).Spores droplets were applied on either side of midrib simultaneously. After drying the spore droplets the water spread on the leaves and eppendorf tube were kept in strand some water were spread for humidity.Tray coverd with polythene bag & kept at room temp for 48 hrs. After 48hrs leaves cleared in ethanol & Mounted in 50% glycerol for microscopic observation.

**Callose detection:**

Deposition of Callose was studied by observing the green yellow fluorescence after aniline blue staining (Yim & Bradford ,1998) by using Epifluorescence microscope (Olympus -B×40,Japan).Water & BABA 24h pretreated tomato plants of variety Naina were inoculated with A.solanispores, After 5dpi ,the inoculated leaves were cleared in boiling ethanol ,& stained with 0.05% aniline blue solution prepared in 0.1M phosphate buffer (pH 8.5) for 15 min and destined with the same buffer. Mounted in 50% glycerol & observed under Epifluorescence microscope.

**Activity of Peroxidase Isozymes by Native-PAGE:**

Preparation of Enzyme extract. 200mg of the 5dpi plant material (C,P, B,B+P) was used, Quantification of protein by Bradford's method (Bradford, 1976) using BSA as a standard, sample bufferwas preparedfrom mixture containing 25% sucrose (w/v) +0.1%bromophenol blue (w/v) + water, 60 µg of enzyme extract was loaded on gel and runned at 50 v till the bands migrates 1cm to the bottom of gel. Gel was stained by Guaicol and de-stained by using potassium phosphate buffer pH 7.0

**RNA isolation:**

Total RNA was isolated using RIBOZOL (AMERESCO, Solon, Ohio) according to the manufacturer's instructions.RNA concentration was determined by measuring the O.D of samples at 260 nm, assuming that an absorbance of 1 unit corresponds to a concentration of 40 µg RNA per ml. Purity of RNA was assessed from the ratio of the absorbance at 260 nm and 280 nm.

Formaldehyde agarose gel electrophoresis: RNA was separated by formaldehyde agarose gel electrophoresis; Gel was stained with ethidium bromide and observed on a UV transilluminator.

**RT-PCR:**

One µg of RNA was reverse transcribed using a RT-PCR kit (SuperScript™ III First-Strand Synthesis System,Invitrogen, USA) according to the manufacturer's directions. Slwrky3 primers were designed used for PCR amplification:F: 5'GTACTAGTGGAGCTGATCAGG3', R: 5' GCTGCTGATGTTGTTGTTG 3'

**PCR Amplification of Slwrky3 gene:**1 µl of cDNA synthesized was amplified by PCR using gene specific Slwrky3 primers, 18s rRNA amplification was also carried out

## Observation and result:

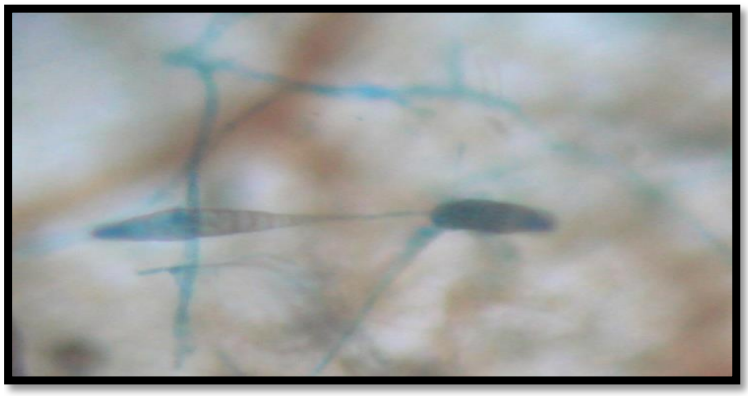
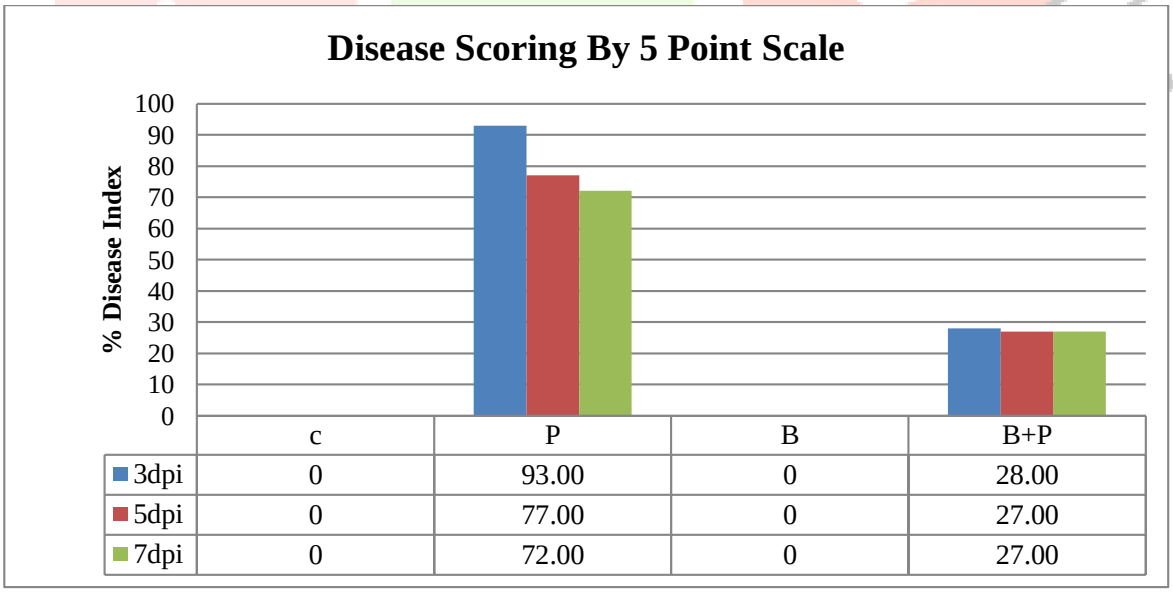


Fig.1 Cotton blue stained water pre-treated leaves with pathogen inoculation showing germinating spores (400 xs).




| | c | P | B | B+P |
|---|---|---|---|---|
| 3dpi | 0 | 93.00 | 0 | 28.00 |
| 5dpi | 0 | 77.00 | 0 | 27.00 |
| 7dpi | 0 | 72.00 | 0 | 27.00 |

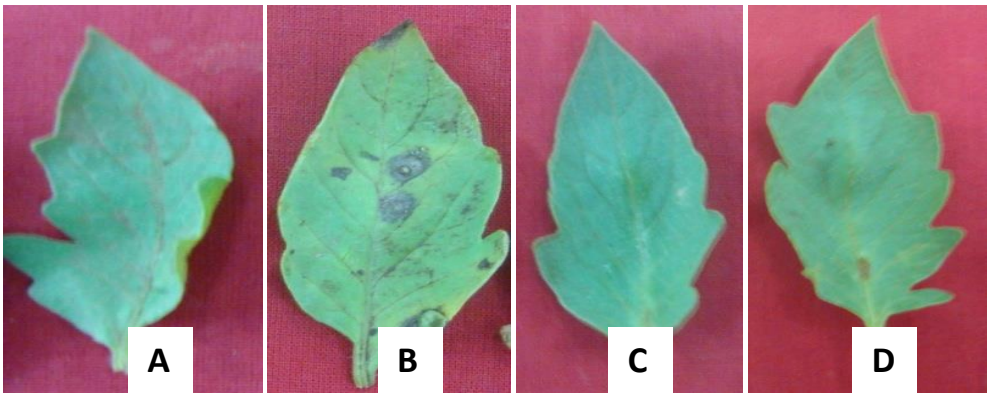



Fig.2: Effect of water &BABA pre-treatment on lesion development intomatoleaves, after 5dpi. (A) Control-Water pre-treated, (B) Water pre-treated with Pathogen inoculated, (C) BABA (4mM) pre-treated (D) BABA (4mM) - pre-treated with pathogen inoculated.

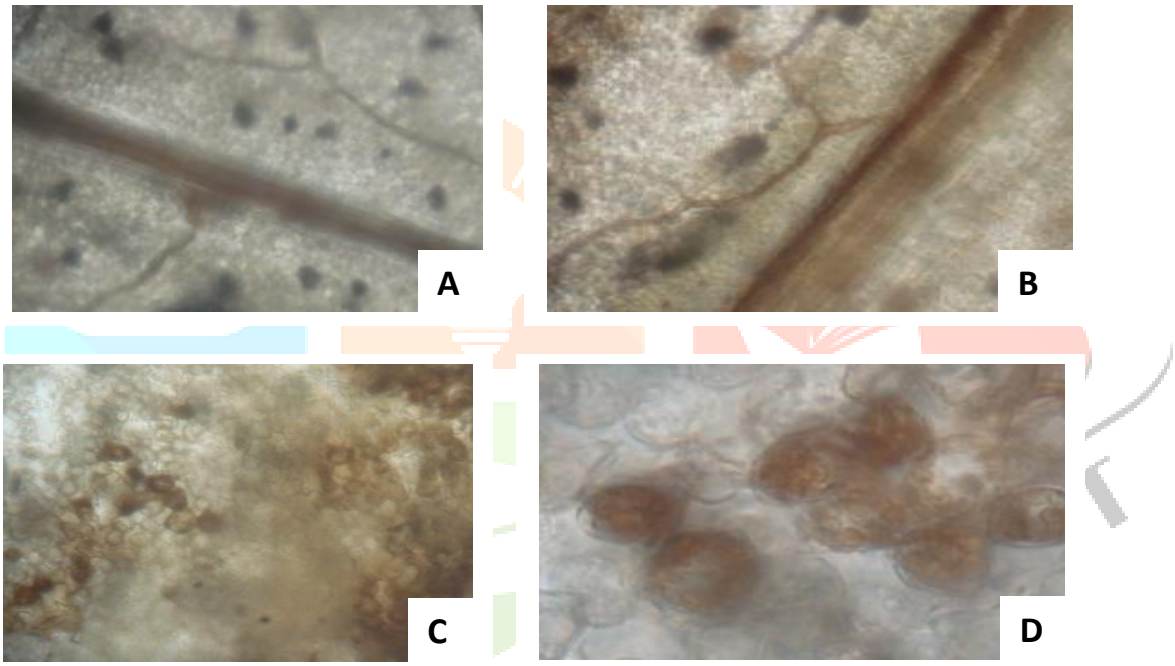



Fig. 3:*In situ* localization of $H_2O_2$ in*Solanum lycopersicum* L leavesin response to inoculation of *A. Solani*,whole mount of leaves cleared in ethanol after DAB uptake for 24h were observed under a light microscope. Reddish brown coloration in the region of inoculation indicated sites of $H_2O_2$ accumulation were observed. (A) Water pre-treated Leaves (100x). (B) BABA (4mM) with no inoculation (100X). (C) Water pre-treated Leaves with pathogen inoculation. (D) BABA pre-treated leaves with pathogen inoculation.

**In situ localization of $H_2O_2$**:

Reddish brown patches formed due to peroxidase catalyse DAB oxidation was observed at the site of spore's application on leaves; appearance of these brown patches indicates that the generation of $H_2O_2$ in the region of infection. It is observed that the BABA pre-treated and pathogen inoculated as well as water pre-treated pathogen inoculated plants shows high intensification of reddish brown coloration at the site of infection, whereas, control- water and BABA pre-treated non inoculated plants shows no accumulation of reddish brown patches. Moreover, the BABA pre-treated pathogen inoculated plants

showing high intensity of accumulation of reddish brown patches at the site of infection and found no spread throughout the leaf area.In case of water pre-treated pathogen inoculated plants shows high accumulation of reddish brown patches and it slightly spread throughout leaf area.

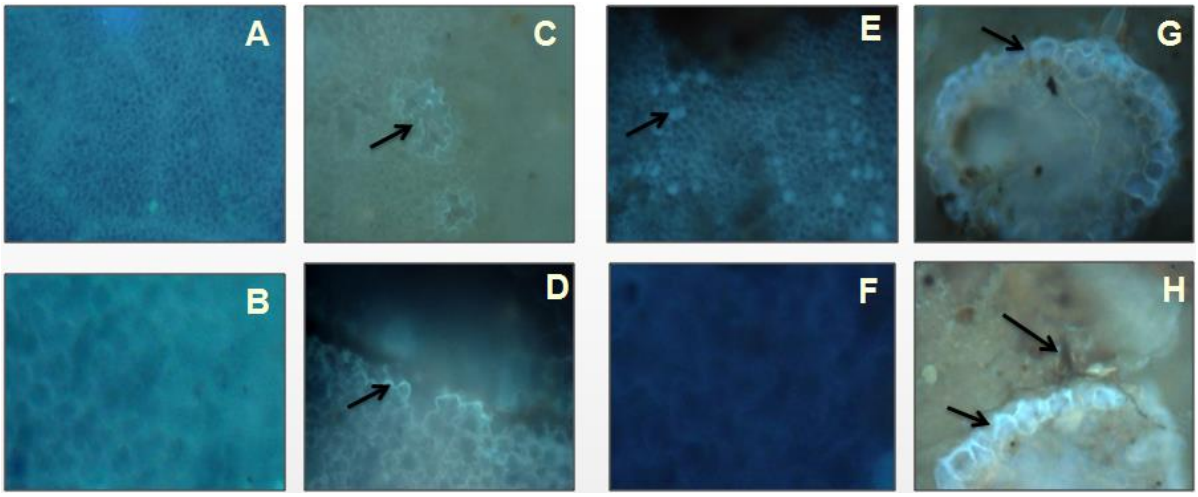



Fig 3: Detection of callose:thewhole mounts of leaves cleared in ethanol and stained with aniline blue were observed with an epifluorescence microscope (BP 330-385, LP 425 nm) in order to detect the green- yellow fluorescence due to callose deposition. All observations were made on five days post-inoculation. A).Leaves pretreated with water (200x) and B). (400x).C). Leaves pretreated with BABA (200x). D). (400x) E).Water pretreated leaves with pathogen inoculated (200x) and (F) (400x).G). BABA pretreated leaves with pathogen inoculation (200x) and H). (400x) pathogen with germination at the site of callose deposion.

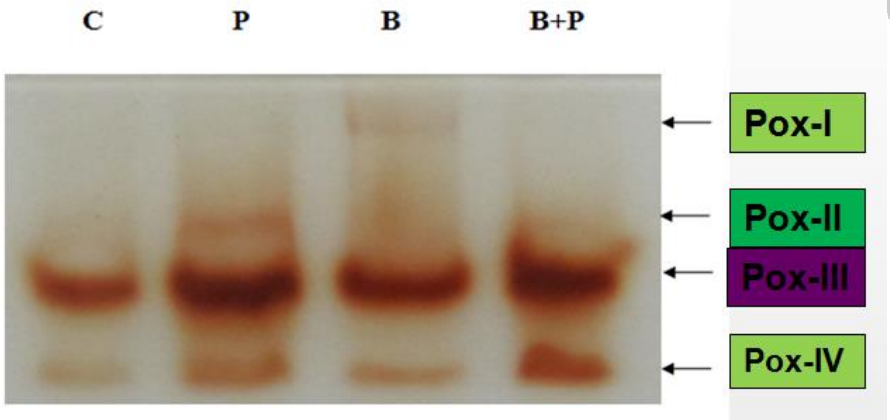



Fig.4 Activity of Peroxidase isozymes (Pox I-IV) by Native-PAGE after 5dpi.

A) Control-water pre-treated, B) Water Pre-treated with Pathogen inoculated. C) BABA (4mM) pre-treated D) BABA (4mM) Pre-treated with Pathogen inoculated

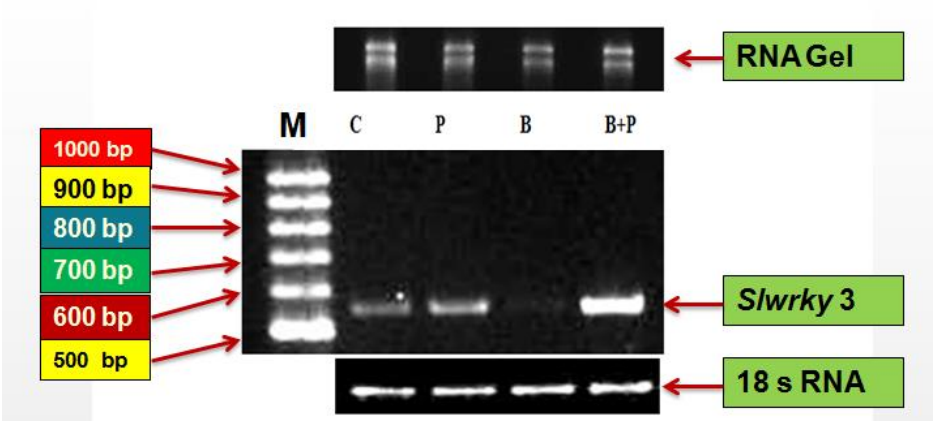



Fig. 5: Expression of *Slwrky 3 gene* in*Solanum lycopersicum* L.

Total RNA was extracted at 5 days inoculation of *Alternaria solani* spores. The different treatments include: Lane 1- water pre-treated plants; Lane 2- water pre-treated withpathogen inoculation; Lane-3 BABA pre-treated plants; Lane-4 BABA pre-treated with pathogen inoculation

## Discussion:

Inducing systemic resistance responses in crop plants is a promising alternative way of disease management.In the context of an increasing requirement for food security, the importance of tomato as one of the five major crop plants worldwide and the devastating crop losses due to *Alternaria solani* infection have prompted research into alternative ways to manage disease. In potato, resistance against *P. infestans* can be induced by infection with pathogens (Kombrink *et al*., 1996) and by exogenous application of compounds such as JA, arachidonic acid, or BABA (Cohen 2002; Cohen *et al*., 1993; Coquoz et al. 1995).BABA protected *Arabidopsis* independently of these defense signaling cascades, suggesting an additional mechanism of protection. Interestingly, the expression of BABA-IR against *H. parasitica* coincided with a rapid and enhanced deposition of massive callose-containing papillae (Fig. 1C; Zimmerli *et al*., 2000).Present study investigates the efficacy of BABA in induction of resistance against *Alternaria solani* L.During interactions of *S .lycopersicum* with *A solani*,the pathogen colonization resulted in the formation of the lesions at the site of inoculation , which ultimately resulted in the host cell death.The diseased scoring data on 3dpi, 5dpi & 7dpi shows that the percentage of diseased index is high (i. e 93%) in case of water pretreated pathogen inoculated plants, whereas, the plants pretreated with BABA & pathogen inoculated shows very less percentage of diseased index (i.e 28%) at 5dpi. The high intensified callose deposition was observed in BABA pretreated pathogen inoculated plants as compared to other treatments (i.e. C,P,BABA) on the site of pathogen inoculation. Transient production of ROS associated with increased activity of oxidative metabolism was frequently found in localized infection of foliar tissues by nacrotrophic pathogens. Moreover, it is observed that the level of ROS production is more in case of BABA pretreated Pathogen inoculated plants as well as water pretreated Pathogen inoculated plants, the enhancement ROS production during the oxidative burst is one of the earliest defence reactions elicited in response to infection with pathogenic micro organisms, in addition, it is also observed that the ROS were also produced together with the increased levels of ROS detoxifying enzymes, the cell death is only observed due to the $H_2O_2$ localization which causes a massive prolonged oxidative burst, for avoidance of unnecessarily cell death the antioxidative

enzymes protect the plants from oxidative stress. The peroxidase isoforms (POX) activity were assessed on Native PAGE,it was observed that the additional POX isoform were observed in BABA pretreated plants. The induction of defence gene expression was observed in plants by environmental stresses and by application of various chemicals which act as priming agents in plants. After pathogen attack or by chemical treatment, the defence related genes are expressed through accumulation of downstream signaling molecules which in turn regulate expression of defence through cell wall strengthening, lignification, phytoalexin synthesis and expression of localized cell death. It was observed that the expression of *Slwrky3* gene in tomato plants were pretreated with BABA & pathogen inoculated, shows high band intensity on the gel, whereas,in BABA pretreated non inoculated plants not shows *Slwrky3* gene expression.

**Conclusion:**from above discussion it has been concluded that the β- aminobutryic acid (BABA) act as a priming agent to activate the defence system in tomato against *Alternaria solani*.

## Conflict of interest:

The author declared no conflict of interest

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## References:


- Berri,S.,Abbruscato,P.,FaivreRampant,O.,Brasileiro,A.C.,Fumasoni,I.,Satoh,K.,Kikuch, S., Mizzi, L., Morandini, P., Pe, M. E. and Piffanelli, P. (2009) Characterization of WRKY co-regulatory networks in rice and Arabidopsis. BMC Plant Biology, 9,120.
- Bose, T.K. and Som, G.M. (1986) Vegetable Crops in India. Naya Prokash, Calcatta, India, 567-569.
- Cohen A, Perzov N, Nelson H, Nelson N (1999). A novel family of yeast chaperons involved in the distribution of V-ATPase and other membrane proteins. *J Biol Chem* 274(38):26885-93
- Conrath, U., Corné, M., Pieterse J. and Mauch-Mani B. (2002) Priming in plant–pathogen interactions. Trends in plant science, 7, 210-216.
- Datar, V.V. and Mayee, C. D. (1982). Conidial dispersal of Alternaria solani in tomato. Indian Phytopathology 35:68-70.
- Eulgem, T. And Somssich I. E. (2007) Networks of WRKY transcription factors in defence signaling. Current opinion in plant biology, 10, 366-371.
- Jalali B.L. and Bhargava S. (2002) Gene expression during host plant and fungal pathogen interactions. Proc. Nat. Acad. Sci. India., 72 B. (3&4), 236.
- Jalali B.L., Bhargava S. S., and Kamble A. C. (2006) Signal transduction and transcriptional regulation of plant defence responces. J. Phytopathology, 154,65-75.
- Karam, B. S., Rhonda, C. F., and Luis, O. S. (2002) Transcription factors in plant defence and stress responces. Current opinion in plant biology, 5, 430-436.

- Mabrouk, Y, Badr, E., Rakha, F. and Ghazy, A. H. (2008) Differential Expression of Potato *WRKY1* Gene Upon *Erwinia carotovora* Infection and Bioinformatics Analysis for a Novel Tomato WRKY-like Gene. Australian Journal of Basic and Applied Sciences, 2(1), 30-36.
- Pandey KK, Pandey PK, Kalloo G, Banerjee MK.(2003). Resistance to early blight of tomato with respect to various parameters of disease epidemics. Journal of Genetic and Plant Pathology 69, 364-371.
- Richard M. B. (2005) Signal crosstalk and induced resistance: Straddling the Line between Cost and Benefit. *Annu. Rev*. Phytopathology, 43, 545-80.
- Rotem, J. (1994) The Genus Alternaria. Biology, Epidemiology and Pathogenicity. APS Press, Saint Paul.
- Thordal-Christensen, H., Zhang, Z., Wei, Y., Collinge, D. B. (1997). Subcellular localization of H2O2 in plants. H2O2 accumulation in papillae and hypersensitive response during the barley—powdery mildew interaction. Plant J 11(6): 1187-94.
- Vloutoglou I, (1999). Evaluation of tomato cultivars and hybrids for resistance to *Alternaria solani* infection. *Annals of Applied Biology* 134 (Suppl. 20), 48 9.
- Walker, J. C. (1952). Diseases of Vegetable Crops. McGraw-Hill Book Company, Inc.: 190 – 191.
- Zimmerli L, Metraux J, Mauch-Mani B, (2001). *β*-aminobutyric acid-induced protection of *Arabidopsis* against the necrotrophic fungus *Botrytis cinerea*. *Plant Physiology* 126, 517– 23.