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Biochemical basis of systemic acquired resistance in potato induced by different SAR elicitors in response to challenge inoculation of late blight pathogen

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ABSTRACT

The *Omycetes*, also known as "water molds", are a group of several hundred organisms that include some of the most devastating plant pathogens. Among oomycete pathogen; *Phytophthora infestans* causing late blight of potato is most important foliar pathogen, causing significant yield losses. The present study was conducted to reduce fungicide load and work out alternate method for control of this disease. Different SAR compounds were tested and foliar sprays of different conc. of Salicylic acid, Jasmonic acid and Bion (Benzothiadiazole-BTH) and β- amino butyric acid were given for inducing resistance in potato against late blight pathogen. Concentration of Salicylic acid, Jasmonic acid and Bion @ 500 µM, and β- amino butyric acid @ 50 mM gave best control of disease among all tested concentrations. Protein content of treated potato plants varied from 6.4 to 7.7 mg/g fresh weight compared to 4.0 mg/g fresh weight in control. Induction of proteins and defense enzymes was systemic in nature in response to all the four elicitors. The SAR compounds tested also stimulated the activities of pathogenesis related proteins (Pr- proteins) i.e. β-1,3 glucanase, Peroxidase (POD), and defense related proteins i.e. Polyphenol oxidase (PPO), Phenylalanine ammonia lyase (PAL) from 26 to 99 % indicating induced resistance in treated potato plants as compared to control. Electrophoretic protein profiling of treated potato plants also confirmed the induction of pathogenesis-related proteins ranging from 15- 75 kDa along with some other proteins. Total chlorophyll and carotenoids also showed spike of 1% to 100 % in response to elicitors. Salicylic acid gave best results showing 77.7 % disease control followed by Jasmonic acid showing 75.1% while Bion and Beta amino butyric acid gave 69 % disease control as compared to control plants. Thus integration of disease tolerance and salicylic acid spray schedule resulted in effective, eco-friendly as well as economical control of late blight of potato.

KEYWORDS: Potato, systemic acquired resistance, salicylic acid, Jasmonic acid, β-amino butyric acids (BABA), Benzothiadiazole (BTH).

INTRODUCTION

Potato (*Solanum tuberosum* L.*)* is fourth most important food crop of the world after wheat, rice and corn. Late blight caused by the *Phytophthora infestans* (Mont.) de Bary, is the major decimator of potato cultivation costing over 12 billion USD losses worldwide (Haverkort *et al.,* 2008). Late blight has tremendous potential to cause up to 80% reduction in the yield in susceptible varieties of potato. All the commercial varieties of potato cultivated in Punjab state of India are moderate to highly susceptible to late blight. Moreover, host resistance to *P. infestans* is not generally stable due to development of new races of the pathogen. Therefore, fungicides play an important role in management of this disease. However, a strain of *P. infestans* resistant to fungicide metalaxyl was reported in Europe in 1981, in Canada and the US in the 1990s and thereafter worldwide; this resulted in the loss of effective late blight disease control, especially against tuber blight (Dowley and O'sullivan, 1991). Similarly, in India, Metalaxyl resistant strains of *P. infestans* were also reported (Kaur *et al.*, 2010). To date, Mancozeb and Ridomil gold fungicides are commonly used to control potato late blight in India.

Application of signaling molecules i.e. Jasmonic acid (JA), Salicylic acid (SA) and β-Amino butyric acid (BABA), etc. is a new promising way of disease management. These molecules are reported to induce systemic acquired resistance (SAR) against pathogens in many crops by activating genes encoding PR-proteins like β -1,3-glucanase (PR-2), chitinase (PR-3), peroxidase (PR-9) and several other stress related proteins (Enkerli *et al.*, 1993). According to Durrant and Dong (2004) Systemic Acquired Resistance is a mechanism of induced defense that vest in long lasting protection against broad spectrum of pathogens. Walters and Fountaine (2009) and Pieterse *et al* (2009) asserted that, at least three types of systemic acquired resistance is known, which is shown to be effective against biotrophic fungi and oomycetes: systemic acquired resistance (SAR), induced systemic resistance (ISR) and β-amino butyric acid induced resistance (BABA-IR). SAR can be induced by treatment with various agents, such as acibenzolar-S-methyl (ASM), a photostable functional analogue of salicylic acid (SA) that is associated with the accumulation of SA and pathogenesis related (PR) proteins, and is dependent on the regulatory protein NPR1 (non-expressor of PR-gene 1) (Durrant and Dong, 2004). Exogenous application of SA, or of its functional analogues 2, 6-dichloroisonicotinic acid (INA) and ASM, can activate PR gene expression and resistance in plants without pathogen inoculation (Van Loon *et al.*, 2006). Many biochemical, cytological and molecular changes are associated with SAR in plants that are systemically protected against pathogens. Among these, cell-wall strengthening, the production of phytoalexins and PR proteins, the hypersensitive reaction (HR), and callose deposition is associated with BABA-induced resistance to *Helminthosporium parasitica* in *Arabidopsis thaliana* (Zimmerli *et al.*, 2000). In view of these observations, the present study was conducted to test application of SAR elicitors Jasmonic acid (JA), Salicylic acid (SA), Benzothiadiazole (BTH) and β-Amino butyric acid (BABA) to enhance plant defense to control late blight disease and reduce fungicide load on potato crop. .

MATERIAL AND METHODS

Potato cultivars/hybrids: Three varieties of potato i.e. Kufri Badshah (moderately tolerant), Kufri Jyoti (tolerant) and Kufri Pukhraj (susceptible) were procured from department of vegetables, Punjab Agricultural University, Ludhiana and used for the study.

Sowing of crop and testing of different doses of elicitors: The selected varieties were raised in rows and replicated thrice using standard package of practices in month of October with plot size of 2m X 3m. Standardization of concentration of Jasmonic acid (JA), Salicylic acid (SA), Benzothiadiazole (BTH) and β- amino butyric acids (BABA) for the induction and over expression of proteins in 30 plants (10 X 3 replication) of each variety i.e. tolerant (Kufri Jyoti) and susceptible (Kufri Pukhraj) cultivars of potato was done. Different concentrations of each elicitor tried as spray (prepared in 500 ml double distilled water) are as follows:

1. Jasmonic acid of 50µM, 250µM, 500µM, 1000 µM,
2. Salicylic acid of 50 µM, 250 µM, 500 µM,1000 µM,
3. Bion (Benzothiadiazole) of 50 µM, 250 µM, 500 µM,1000 µM, and
4. β-amino butyric acid of 20 mM, 30mM, 50 mM, 100mM

Three-week-old sprouts were sprayed using an atomizer. Water sprayed plants of corresponding varieties were kept as untreated control.

Collection of plant tissue samples: Leaf sampling was done after 24, 48, 72, 96, 120, 144 hrs and at weekly intervals after elicitors spray. Samples were brought to laboratory in ice packs and stored at -80°C to prevent denaturation of proteins.

Estimation of total soluble proteins: Leaf blade tissue excluding veins (0.5 g) was weighed and homogenized in 25 mM Tris HCl buffer (pH 8.0) in a precooled pestle and mortar on the ice bath and centrifuged at 10,000 rpm for 25 minutes at 4^0C. Supernatant was used for protein estimation (Lowry *et al.*, 1951). Bovine serum albumin (BSA) standards (20-100 g) were run along with the test samples and the concentration of protein was calculated from the standard curve of BSA and expressed as mg/g fresh weight of tissue. Tissue was sampled from at least three leaves.

Protein profiling: Protein profiling by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Walker, 1996) was done for leaves of all the three varieties sprayed with 500 µM of SA, JA, BTH and 50 mM of BABA. Standard protein ladder with molecular weights ranging from 6-180 kDa was also run. Gels were stained with Comassie brilliant blue for visualizing changes in bands in the range of 6-50 kDa and compared with their respective control.

Extraction and estimation of enzymes: One hundred mg of leaf tissue from each plant was crushed in a pre chilled pestle and mortar with 2 mL 0.1M sodium phosphate buffer (pH 7.5) containing 10 mM 2-mercaptoethanol in the presence of 1% polyvinyl-pyrrolidone (PVP). The homogenate was centrifuged at 13,000 g at 4^0C for 30 min (Eppendorf 5804R) and clear supernatant was used for estimating peroxidase, polyphenol oxidase and phenylalanine ammonia lyase activity. Standard procedures of Claiborne and Fridovich (1979), Burrell and Rees (1974), and Zauberman *et al* (1991) were employed for the estimation of Peroxidase, phenylalanine ammonia lyase, polyphenol oxidase, respectively. For estimation of -1, 3 glucanase, Dinitrosalicylic acid (DNSA) was used as reagent. Reddish brown colour developed was measured at 575 nm. The enzyme activity has been expressed as µg of glucose released/min/g FW (Kauffmann *et al.,* 1987)

Estimation of chlorophylls and carotenoids: For estimation of chlorophylls, 0.2 g of leaf sample was taken and to this added 1 mL of Dimethyl Sulfoxide (DMSO). This solution was kept overnight for colour development. The optical density was measured at 649 nm and 665 nm (Barnes *et al.,* 1992). The amount of carotenoids was estimated by the method of Kirk and Allen (1965).

Preparation of sporangial suspension for challenge inoculation: Fresh sporulations of *P. infestans* sporangial solution at conc. of approx. 4.0 x 10^4 sporangia per mL were prepared by dislodging sporangia from sporulating leaves in double distilled water and used for challenge inoculations in potato experiments.

Determination of disease severity: Elicitors were sprayed after 21 days of sowing. After 2 days of elicitor spray pathogen inoculations with *P. infestans* sporangial solution at conc. of 4.0 x 10^4 sporangia per mL was done using an atomizer to create disease. High relative humidity was maintained for next 72 hrs by spraying water. Observations on disease severity were recorded using the scale given by Thind and Mohan (1998). Per cent Disease Index was calculated using following expression:

$$\text{Per cent Disease Index (PDI)} = \frac{\text{Sum of numerical rating}}{\text{Total no of samples x Maximum of rating scale}} \times 100$$

Per cent disease incidence and disease severity was observed from 7 to 14 days post inoculation. The leaves were collected for seven consecutive days after inoculation and analyzed for various biochemical estimations. The statistically significant difference (CD 5%) in biochemical parameters in response to foliar spray of SA, JA, BABA and BTH on three varieties was calculated using CRD factorial analysis.

RESULTS AND DISCUSSION

Total Protein: The data pertaining to changes in protein concentration recorded at periodical interval of 24 hrs till a week in response to selected elicitors and pathogen inoculation revealed statistically significant difference. JA, SA and BTH i.e., at 500 µM and BABA at 50 mM was sprayed after 21days of planting followed by challenge inoculation of pathogen. These elicitors were applied on three different varieties of potato, namely Kufri Badshah, Kufri Jyoti, Kufri Pukhraj **(Table 1)**. Mean maximum protein induction was observed at 500 µM of SA in Kufri Jyoti i.e. 7.7 mg/g FW followed by 7.3 mg/g FW in Kufri Badshah and 6.9 mg/g FW in Kufri Pukhraj. JA at 500 µM proved to be second best treatment with mean maximum protein induction observed in Kufri Jyoti i.e. 7.1 mg/g FW followed by 7.0 mg/g FW in Kufri Badshah and by 7 mg/g FW in Kufri Pukhraj. BTH at 500 µM gave mean maximum protein induction of 6.5 and 6.7 mg/g FW in Kufri Badshah, and Kufri Jyoti, whereas 6.4 mg/g FW in Kufri Pukhraj. BABA gave mean maximum protein induction of 6.8 mg/g FW at 50mM of concentration in Kufri Jyoti and 6.7 and 6.5 mg/g FW in Kufri Badshah, Kufri Pukhraj, respectively.

B-1, 3 glucanase activity: The data pertaining to changes in β-

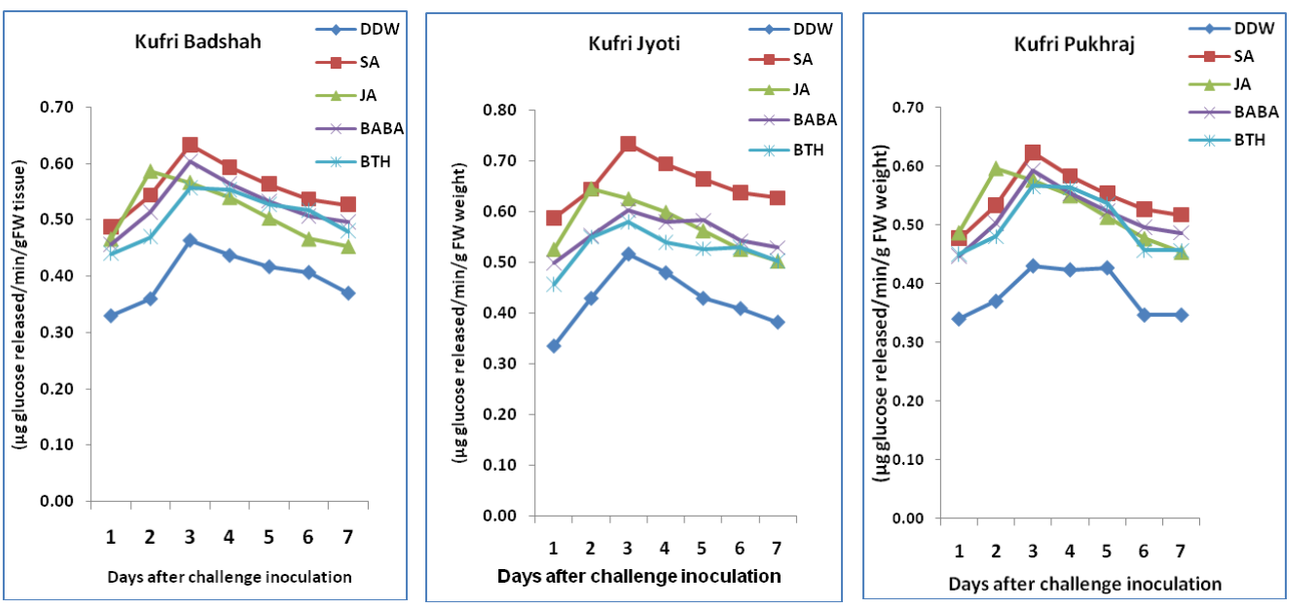
Table 1: Leaf protein (mg/gFW) induction studies in response to elicitors in potato varieties challenge inoculated with *P. infestans*.

Total protein content (mg /g FW)									
		Days after challenge inoculation							
Variety	Treatment	1	2	3	4	5	6	7	Mean
Kufri Badshah	Control	3.4	3.8	5.2	4.4	4.4	3.7	3.6	4.1
	SA (500µM)	6.4	6.8	8.4	8.2	7.8	7.2	6.6	7.3
	JA(500 µM)	6.4	6.8	7.8	7.4	7.2	6.8	6.5	7.0
	BABA(50mM)	6.0	6.5	7.5	7.1	6.8	6.5	6.3	6.7
	BTH (500µM)	6.0	6.4	7.5	6.9	6.6	6.3	6.1	6.5
Kufri Jyoti	Control	3.7	4.1	5.5	4.8	4.4	4.2	3.9	4.4
	SA (500µM)	6.6	7.3	8.7	8.5	8.1	7.8	6.8	7.7
	JA(500 µM)	6.4	6.8	8.1	7.6	7.2	6.8	6.5	7.1
	BABA(50mM)	6.2	6.7	7.7	7.3	7.0	6.6	6.5	6.8
	BTH (500µM)	6.1	6.5	7.6	7.1	6.8	6.5	6.3	6.7
Kufri Pukhraj	Control	3.5	3.9	4.8	4.3	4.1	3.9	3.8	4.0
	SA (500µM)	6.0	6.7	7.8	7.5	7.2	6.9	6.5	6.9
	JA(500 µM)	6.2	7.3	7.7	7.5	7.0	6.6	6.4	7.0
	BABA(50mM)	5.9	6.3	7.3	6.9	6.7	6.4	6.3	6.5
	BTH (500µM)	5.8	6.2	7.3	6.8	6.6	6.2	6.0	6.4
CD (5%)	Variety (A)-0.022; Elicitor (B)- 0.029; Time interval (C)-0.035 ; AB- 0.051; AC-0.060; BC-0.078; ABC- 0.135								

1, 3 glucanase activity recorded at periodical interval in combined response to elicitors and challenge inoculations of pathogen revealed statistically significant difference. SA caused 37 % increase in β-1, 3 glucanase activity in leaves, whereas JA and BTH resulted in 27%, BABA gave 30% increase in β-1, 3 glucanase activity w.r.t control in Kufri Badshah. Similarly, in Kufri Jyoti SA caused 53 % increase in β-1, 3 glucanase activity in leaves, whereas JA and BABA resulted in almost 32%, and BTH gave 23% increase in β-1, 3 glucanase activity w.r.t control. In Kufri Pukhraj SA caused 42% increase in β-1, 3 glucanase activity in leaves, whereas JA resulted in 37%, BABA gave 34% and BTH gave 31% increase in β-1, 3 glucanase activity w.r.t control indicating that SA is better inducer of β-1, 3 glucanase activity among all the four elicitors with maximum β-1, 3 glucanase activity in Kufri Jyoti (**Fig. 1**). The total β-1, 3 glucanase activity increased up to 3rd day in all three elicitors except JA where peak value was observed on second day of spray and thereafter registered decline irrespective of variety and elicitor treatment. In general, 500 µM SA resulted in higher mean β-1, 3 glucanase activity followed by JA followed by BABA and then BTH. Kufri Jyoti showed better β-1, 3-glucanase activity followed by Kufri Badshah and Kufri Pukhraj. BABA and BTH treatments were at par w.r.t. β-1, 3 glucanase activity in all the three varieties.

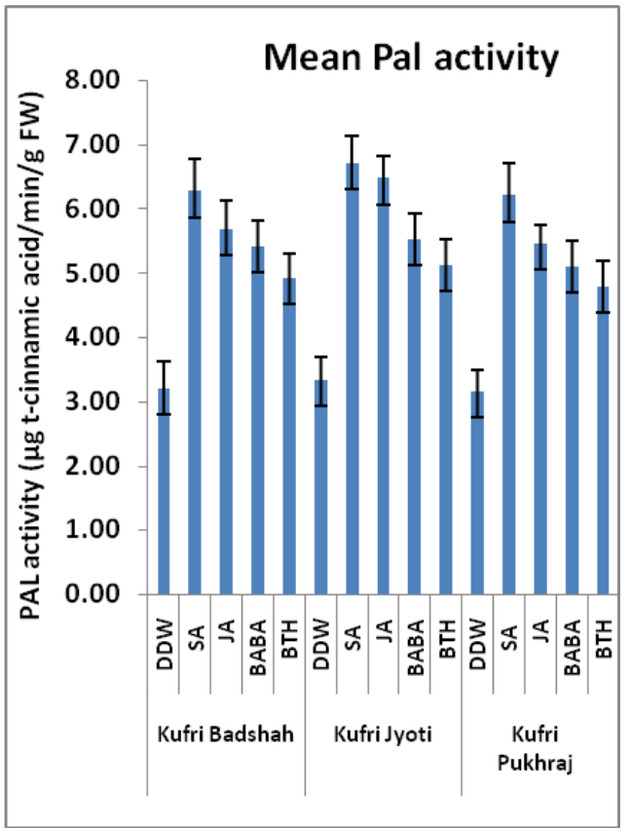
Phenylalanine ammonia lyase (PAL): The data pertaining to changes in Phenylalanine ammonia lyase (PAL) activity recorded at periodical interval of 24 hrs till a week in response to selected best doses revealed statistically significant difference. SA showed the spike of 96 % in PAL activity in leaves, whereas JA resulted in 78%, BABA gave 69% and BTH gave 54% increase in PAL activity w.r.t control in Kufri Badshah. Similarly, in Kufri Jyoti SA caused 102% increase in PAL activity in leaves, whereas JA resulted in 94%, BABA gave 65% and BTH gave 54% increase in PAL activity w.r.t control. In Kufri Pukhraj SA caused 102% increase in PAL activity in leaves, whereas JA resulted in 78 %, BABA gave 67% and BTH gave 56% increase PAL activity w.r.t control indicating that SA is better inducer of PAL activity among all the four elicitors with maximum PAL activity in Kufri Jyoti. The total PAL activity increased up to 4th day in all four elicitors and thereafter registered decline irrespective of variety and elicitor treatment (**Fig. 2**).

Peroxidase (POD): The data pertaining to changes in peroxidase (POD) activity revealed that in Kufri Badshah; SA caused 57 % increase in peroxidase activity in leaves, whereas JA resulted in 42%, BABA gave 34% and BTH gave 32% increase in peroxidase activity w.r.t control. Similarly, in Kufri Jyoti SA caused 107% increase in peroxidase activity in



Each value is mean of three replications, SA: Salicylic acid, JA: Jasmonic acid, BABA; Beta amino butyric acid; BTH, Benzothiadiazole, DDW: Double distilled water

Fig. 1 Effect of foliar spray with different elicitors i.e. SA, JA, BABA and BTH on -1, 3-glucanases activity (g glucose released/min/ gFW weight) in leaves of different potato varieties challenge inoculated with *P. infestans*.



Each value is mean of three replications, SA: Salicylic acid, JA: Jasmonic acid, BABA; Beta amino butyric acid; BTH, Benzothiadiazole, DDW: Double distilled water

Fig. 2 : Effect of foliar spray with different elicitors i.e. SA, JA, BABA and BTH on PAL activity (µg t-cinnamic acid/min/g FW) in leaves of different potato varieties challenge inoculated with *P. infestans*

leaves, whereas JA resulted in 81%, BABA gave 65% and BTH gave 53% increase in peroxidase activity w.r.t control. In Kufri Pukhraj SA caused 75% increase in peroxidase activity in leaves, whereas JA resulted in 69%, BABA gave 59% and BTH gave 52% increase in peroxidase activity w.r.t control indicating that SA is better inducer of peroxidase activity among all the four elicitors with maximum peroxidase activity in Kufri Jyoti (**Table 2**).

Table 2: Effect of foliar spray of SA, JA, BABA and BTH on peroxidase activity (ΔA /min/g FW) in leaves of potato varieties challenge inoculated with *P. infestans*

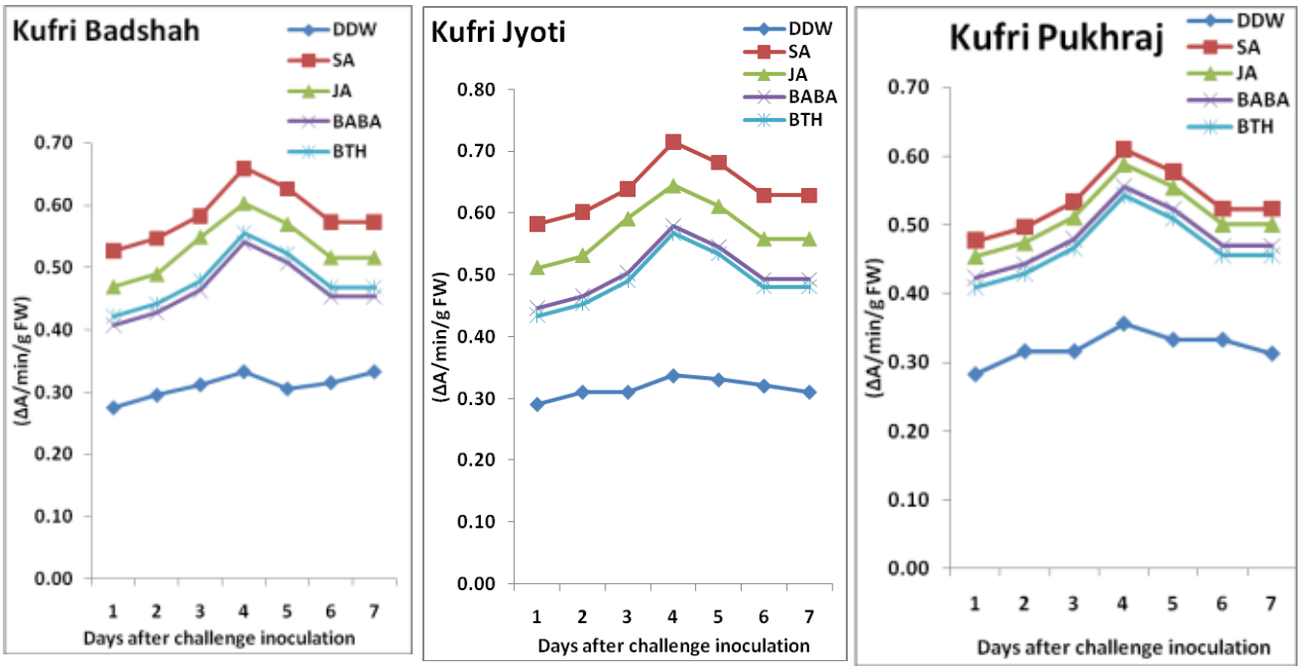
Peroxidase activity (ΔA /min/g FW)									
Variety	Treatment	Days after challenge inoculation							Mean
		1	2	3	4	5	6	7	
Kufri Badshah	Control	21.40	24.93	37.76	34.27	31.90	29.16	28.27	29.67
	SA(500µM)	37.70	42.74	54.94	52.74	49.20	45.54	44.97	46.83
	JA(500µM)	34.20	38.27	49.10	46.30	44.14	42.47	41.14	42.23
	BABA(50m)	32.50	36.37	47.90	43.77	40.87	40.14	37.94	39.93
	BTH (500µM)	32.67	35.97	46.77	43.77	40.94	37.94	37.87	39.42
Kufri Jyoti	Control	21.67	22.37	31.80	29.54	27.40	27.01	24.97	26.39
	SA (500µM)	45.49	50.53	62.73	60.53	56.99	53.33	52.76	54.62
	JA(500 µM)	39.00	43.07	53.90	51.10	48.94	47.27	45.94	47.03
	BABA(50m)	35.39	39.26	50.79	46.66	43.76	43.03	40.83	42.82
	BTH (500µM)	33.27	36.64	47.44	44.44	41.61	38.61	38.54	40.08
Kufri Pukhraj	Control	22.54	23.40	23.90	25.37	26.27	27.10	27.97	25.22
	SA (500µM)	34.80	39.84	52.88	50.48	46.71	42.64	42.07	44.20
	JA(500 µM)	34.70	38.77	49.60	46.80	44.64	42.97	41.64	42.73
	BABA(50m)	32.40	36.-27	47.80	43.67	40.77	40.71	38.35	40.00
	BTH (500µM)	31.52	34.82	45.62	42.62	39.79	37.01	37.31	38.38
CD (5%)	Variety (A)-0.16; Elicitor (B)- 0.21; Time interval (C)-0.25 ; AB- 0.37; AC-0.44; BC-0.57; ABC- 1.01								

Polyphenol oxidase (PPO): The data pertaining to changes in Polyphenol oxidase (PPO) activity revealed that amongst the different elicitors treatment in Kufri Badshah; SA caused 74 % increase in PPO activity in leaves, whereas JA resulted in 77%, BABA gave 53% and BTH gave 60% increase in PPO activity w.r.t control. Similarly, in Kufri Jyoti SA caused 100% increase in PPO activity in leaves, whereas JA resulted in 78%, BABA gave 56% and BTH gave 53% increase in PPO activity w.r.t control (**Fig 3**). Similar pattern was observed in Kufri Pukhraj as well. Statistically significant difference for all four elicitors w.r.t. time interval was observed. SA was found to induce maximum PPO activity 0.72 ΔA /min/g FW at 4TH day interval, respectively followed by JA showing maximum PPO activity 0.65 ΔA /min/g FW at 4TH day interval. BABA and BTH showed maximum PPO activity 0.58 and 0.57 ΔA /min/g FW at 4TH day interval in Kufri Jyoti. Whereas minimum PPO activity varied from 0.29 to 0.34 ΔA /min/g FW for control treatment between all the respective time intervals with mean value of 0.32 ΔA /min/g FW.

Raut and Borkar (2014) from their studies also reported that application of SA via seed dip treatment and seedling dip treatment elicited increase in chitinase and β-1, 3-glucanase activity in susceptible tomato cultivars and induced resistance to *Alternaria* leaf blight. Rajab *et al* (2009) showed prominent increase in the PAL activity when calli of *Sesamum prostratum* were challenged with *Fusarium oxysporum* f. *sesame* crude toxin metabolite of varying concentrations. The activated PAL activity leads to production of phenolics, which are further converted into more reactive species by phenol oxidases and peroxidases (Heath, 2000). ASM significantly induced β-1,3-glucanase activity which increased with time in inoculated seedlings, as confirmed by the presence of PR-2. Analysis of three other acidic (PR-1C, PR-5S, PR-8) and one basic (PR-6) PR proteins in the ASM-treated seedlings showed that only PR-1 and PR-5 were slightly and slowly induced (4-5 days after treatment), but this induction was more pronounced after inoculation with *P. parasitica* (Ziadi *et al.,* 2001).

The maximum increases in chitinase, β-1, 3-glucanase, PO and PPO activity occurred in root and shoot tissue from Bion® seed treated material inoculated with fungus (Whan *et al.,* 2008).

Kim and Hwang (2014) also observed that pepper plants showing high activity of PAL enzyme were resistant to the infection of *Xanthomonas campestris* pv. *vesicatoria.* The expression of genes encoding hydrolytic enzymes such as chitinases and β-1,3-glucanases that degrade the cell wall of microbes and may



Each value is mean of three replications, SA: Salicylic acid, JA: Jasmonic acid, BABA; Beta amino butyric acid; BTH, Benzothiadiazole, DDW: Double distilled water

Fig. 3 Effect of foliar spray with different elicitors i.e. SA, JA, BABA and BTH on PPO activity (ΔA/min/g FW) in leaves of different potato varieties challenge inoculated with *P. infestans*

be involved in the release of elicitor molecules and the synthesis of pathogenesis related proteins (PR proteins). These responses might trigger in the whole plant a long-lasting systemic acquired resistance (SAR) which is effective against a large spectrum of pathogens (Ricci, 1997).

Present study reports that potato plants showed increase in the activities of defense related enzymes after treatment with different elicitors as compared to control. Additional enhancement in the activities was observed upon inoculation with pathogen. The biochemical defence response was better in response to combined exposure of elicitors followed by pathogen, as plant gets primed with elicitor spray. SAR plant defense does not always get directly activated upon first exposure to stimulus instead may need priming and is often associated with a faster and stronger induction of the plant defence on subsequent exposure to abiotic and/or biotic stress (Conrath, 2011). Elicitors alone without challenge inoculation of *P. infestans* primed the plants but biochemical activity showed higher response with combined effect of elicitor plus pathogen (**Table 3**). Of the various elicitors used, SA followed by JA, BABA and BTH were found to be more effective in eliciting the level of enzymes which play an important role in defense; in potato against late blight pathogen.

Total chlorophyll: The data pertaining to changes in leaf total chlorophyll in response to combined effect of elicitor followed by challenge inoculation with pathogen revealed statistically significant difference amongst the various elicitors applied on three different varieties of potato (**Table 4**). Amongst the different elicitors treatment in Kufri Badshah; SA caused 13% increase in total chlorophyll (mg/ g FW) in leaves, whereas JA resulted in 3.5% increase, BABA gave 11% and BTH gave 10% increase in total chlorophyll (mg/ g FW) w.r.t control. Similarly, in Kufri Jyoti SA caused 17% increase in total chlorophyll (mg/ g FW) in leaves, whereas JA resulted in 3% increase, BABA gave 16% and BTH gave 12% increase in total chlorophyll (mg/ g FW) w.r.t control. In Kufri Pukhraj SA caused 16% increase in total chlorophyll (mg/ g FW) in leaves, whereas JA resulted in increase of only 2%, BABA gave 14% and BTH gave 13% increase in total chlorophyll (mg/ g FW) w.r.t control indicating that SA is better inducer of total chlorophyll among all the four elicitors with

Table 3. Comparison of Biochemical activities in response to SA, JA, BABA and BTH in leaves of potato varieties; with and without challenge inoculation of pathogen.

Potato cultivar	Elicitor	Total proteins (mg /g FW)		β-1, 3 glucanase (µg glucose released/min/g FW)		PAL (µg t-cinnamic acid/min/g FW)		Peroxidase activity (ΔA /min/gFW)		Polyphenol oxidase (ΔA/min/g FW)	
		Data is mean of seven readings taken at 24 hours interval upto a week post challenge inoculation									
		Mean (DDW)	Mean (PI)	Mean (DDW)	Mean (PI)	Mean (DDW)	Mean (PI)	Mean (DDW)	Mean (PI)	Mean (DDW)	Mean (PI)
Kufri jyoti (tolerant)	Control	3.7	4.4	0.35	0.43	2.54	3.34	18.50	26.39	0.25	0.32
	SA	7.3	7.7	0.55	0.66	5.64	6.70	41.96	54.62	0.46	0.64
	JA	7.0	7.1	0.47	0.57	4.75	6.47	36.26	47.03	0.47	0.57
	BABA	6.0	6.8	0.44	0.56	4.72	5.51	33.26	42.82	0.43	0.50
	BTH	5.2	6.7	0.42	0.53	4.33	5.12	32.10	40.08	0.42	0.49
Kufri Pukhraj (susceptible)	Control	3.4	4.0	0.30	0.38	2.27	3.16	17.45	25.22	0.25	0.32
	SA	6.4	6.9	0.47	0.54	5.41	6.20	36.16	44.20	0.44	0.53
	JA	6.0	7.0	0.44	0.52	4.66	5.45	34.96	42.73	0.42	0.51
	BABA	4.9	6.5	0.43	0.51	4.31	5.10	32.06	40.00	0.41	0.48
	BTH	4.6	6.4	0.44	0.50	4.00	4.79	30.50	38.38	0.40	0.47

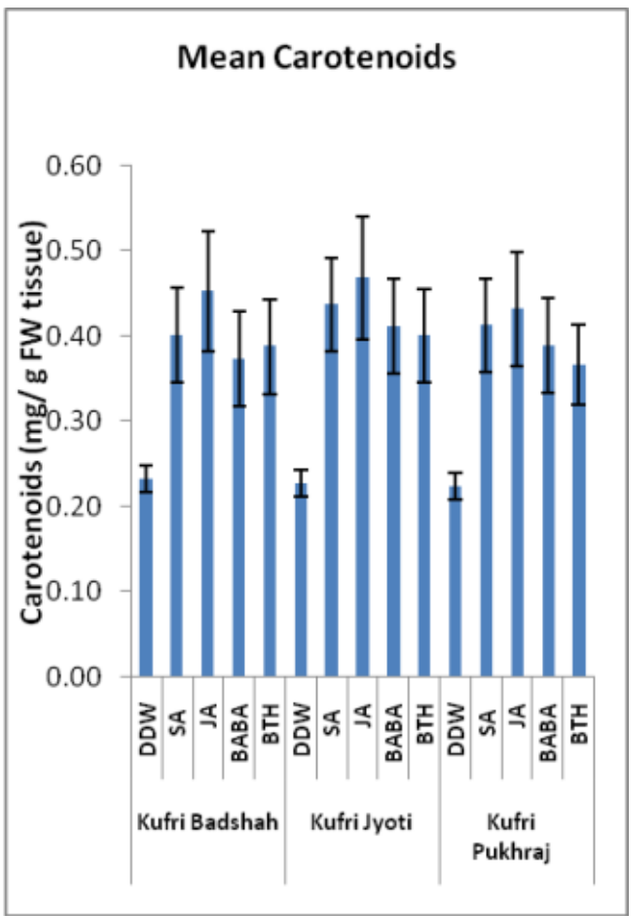
DDW- Double distilled water after 2 days of elicitor spray;
PI- *P. infestans* with sporangial solution at conc. of 4.0 x 10^4 sporangia per mL after 2 days of elicitor spray

Table 4: Effect of foliar spray of SA, JA, BABA and BTH on total chlorophyll (mg/gFW) in leaves of different potato varieties challenge inoculated with *P. infestans*

Total chlorophyll (mg/gFW)									
Variety	Treatment	Days after challenge inoculation							Mean
		1	2	3	4	5	6	7	
Kufri Badshah	Control	1.46	1.46	1.42	1.40	1.38	1.37	1.36	1.41
	SA (500µM)	1.55	1.57	1.58	1.59	1.61	1.62	1.64	1.60
	JA(500 µM)	1.41	1.43	1.44	1.45	1.47	1.48	1.50	1.46
	BABA(50mM)	1.53	1.55	1.56	1.57	1.59	1.60	1.61	1.57
	BTH (500µM)	1.51	1.53	1.54	1.55	1.57	1.58	1.59	1.55
Kufri Jyoti	control	1.48	1.50	1.48	1.46	1.44	1.42	1.41	1.46
	SA (500µM)	1.66	1.68	1.71	1.79	1.76	1.70	1.70	1.71
	JA(500 µM)	1.44	1.46	1.52	1.54	1.54	1.53	1.52	1.51
	BABA(50mM)	1.63	1.65	1.69	1.77	1.73	1.68	1.68	1.69
	BTH (500µM)	1.59	1.61	1.64	1.72	1.69	1.63	1.63	1.64
Kufri Pukhraj	Control	1.48	1.50	1.49	1.46	1.43	1.42	1.40	1.46
	SA (500µM)	1.63	1.65	1.69	1.77	1.73	1.68	1.68	1.69
	JA(500 µM)	1.45	1.44	1.55	1.52	1.51	1.49	1.48	1.49
	BABA(50mM)	1.61	1.63	1.67	1.74	1.71	1.66	1.66	1.67
	BTH (500µM)	1.60	1.62	1.65	1.73	1.70	1.64	1.64	1.65
CD (5%)	Variety (A)-0.0022; Elicitor (B)- 0.0028; Time interval (C)-0.0033 ; AB- 0.0049; AC-0.0058; BC-0.0076; ABC- 0.0131								

Each value is mean of three replications, SA: Salicylic acid, JA: Jasmonic acid, BABA; Beta amino butyric acid; BTH, Benzothiadiazole, DDW: Double distilled water

maximum content in Kufri Jyoti. It was observed that the chlorophyll content increased with JA treatment in foliar application, even though control values were at par with 500 µM JA treated plants.



Each value is mean of three replications, SA: Salicylic acid, JA: Jasmonic acid, BABA; Beta amino butyric acid; BTH, Benzothiadiazole, DDW: Double distilled water

Fig. 4: Effect of foliar spray with different elicitors i.e. SA, JA, BABA and BTH on carotenoids (mg/ g FW) in leaves of different potato

Carotenoids: The data pertaining to combined effect on leaf carotenoids (mg/ g FW) in response to various elicitors followed by challenge inoculation; revealed statistically significant difference amongst the various elicitors applied on potato **(Fig. 4).** After 21 days of planting, the plants were sprayed with elicitors, and were further, challenge inoculated with pathogen after 2 days. Amongst the different elicitors treatment in Kufri Badshah; SA caused 74% increase in carotenoids (mg/ g FW) in leaves, whereas JA resulted in 94% increase, BABA gave 61% and BTH gave 69% increase in carotenoids (mg/ g FW) w.r.t control. Similarly, in Kufri Jyoti SA treatment resulted in 91% increase in carotenoids (mg/ g FW) in leaves, whereas JA resulted in 100% increase, BABA gave 78% and BTH gave 74% increase in carotenoids (mg/ g FW) w.r.t control. In Kufri Pukhraj SA treatment resulted in 86% increase in carotenoids (mg/ g FW) in leaves, whereas JA resulted in increase of 91%, BABA in 77% and BTH in 73% increase in carotenoids (mg/ g FW) w.r.t control indicating that JA is better inducer of carotenoids among all the four elicitors with maximum content in Kufri Jyoti followed by Kufri Badshah and Kufri Pukhraj. It was observed that the carotenoids content showed peak on 4th day of treatment in all the tested elicitors.

Electrophoretic study (SDS-PAGE) of protein extract of different potato cultivars: Acidic extracellular forms of these PR proteins built up at the commencement of plant resistance, indicating that they have a value as molecular markers for the expression of SAR. PR proteins have a low molecular weight (5-75 kDa), and they are thermostable, highly resistant to proteases, extractable, and stable at low pH (<3). These PR proteins are known to provide resistance against various pathogens. PR proteins are categorised into structurally homologous families. Some of these PR-protein families have direct antimicrobial activities. Electrophoretic study of protein pattern for potato varieties in response to selected best doses of JA, SA and BTH i.e, at 500 µM and BABA at 50 mM, resolved in the molecular weights ranging from 6-180 kDa with respect to standard protein markers. Specific bands falling in the range of 6-75 kDa were reported in treated samples as compared to their respective control (**Plate 1**). It is known that PR proteins fall under the range of 15.5 KDa to 75 KDa (Van Loon *et al.,* 2006), Which, signified that application of SA, JA, BABA and BTH in potato resulted in PR protein induction along with some other proteins.

DISEASE DATA

Disease severity was observed from 14 days post challenge inoculation. SA treatment gave per cent disease control of 75.43, 77.07, and 77.29 in Kufri Badshah, Kufri Jyoti and Kufri Pukhraj. Minimum per cent disease control of 61.22 was observed in BTH treated Kufri Jyoti cultivar. Therefore, single spray of elicitors gave 61 to 77 % disease control of late blight disease **(Table 5)**.

Katoch (2007) showed that treatment with SA or

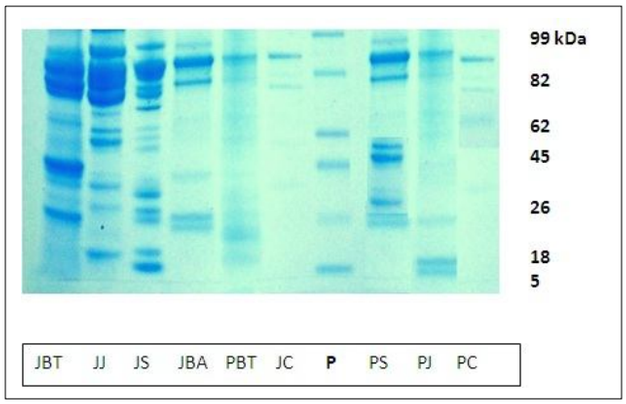


Plate 1: SDSPAGE of leaf proteins of different potato varieties at 500 M of SA, 500 M of JA, 500 M of BTH and 50 mM for BABA. JBT Kufri Jyoti treated with BTH, *JJ* Kufri Jyoti treated with JA, *JS* Kufri Jyoti treated with SA, JBA Kufri Jyoti treated with BABA , PBT Kufri Pukhraj treated with BTH, *JC* Kufri Jyoti-control, ***P***-protein ladder; PS Kufri Pukhraj treated with SA, *PJ* Kufri Pukhraj treated with JA; PC - Kufri Pukhraj control.

Inoculation with *Erysiphe polygoni* resulted in synthesis of new protein, whereas treatment with ABA did not show any variation in the protein profile as compared to the control. It may be that 5mM SA and inoculation with *E. polygoni* shares a mechanism for induction of synthesis of a new protein. Ziadi *et al* (2001) reported that foliar application of BTH induces systemic resistance in many crops and higher activity of β-1, 3-glucanases. Andreu *et al (*2006) showed that when BABA was applied to the foliage at early stages of crop development, a protective effect against late blight of potato was observed, and increase was observed in level of β-1, 3-glucanase and aspartyl protease (StAP1) in post harvest tuber samples. Sorokan *et al* (2014) reported that application of salicylic acid in potato against *P. infestans* showed increased peroxidase activity compared to control plants. Moharekar *et al* (2003) stated that salicylic acid increased the synthesis of many pigments like carotenoid, xanthophylls but it also decreases the amount of chlorophyll pigments in wheat and mung and chlorophyll *a/b* ratio in wheat plantlets. This stimulatory response of ascorbic acid, salicylic acid and other antioxidants might be due to the phenomenon of antioxidant scavenging to provide protection to chloroplast and chlorophyll against degradation caused by reactive oxygen species. Foliar application of SA (1.4×10^{-4} M) to *Brassica napus* was found to enhance chlorophyll concentration (Hayat *et al* 2005).

Table 5: Efficacy of elicitors in controlling late blight of potato 14 days after challenge inoculation with *P. Infestans*

% Disease severity after 14 days of challenge inoculation										
Sr. No.	**Variety**	**DDW/ control**	**SA**	**% Disease control**	**JA**	**% Disease control**	**BABA**	**% Disease control**	**BTH**	**% Disease control**
1	K Pukhraj	55.04	12.50	77.29	13.73	75.06	16.00	70.93	16.53	69.97
2	K Badshah	31.86	7.83	75.43	8.48	73.39	9.25	70.97	10.15	68.13
3	Kufri Jyoti	22.33	5.12	77.07	8.78	60.69	8.13	63.58	8.66	61.22
CD (5%)	Varieties (A)- 0.87 ; Elicitors (B)- 0.45; AB: 1.3									
DDW- Double distilled water										

CONCLUSION

In conclusion, it may be possible to, in certain cases, replace conventional chemical fungicides with any of the four elicitors especially SA, provided, spray is done before the onset of disease to prime the plants. It could also be concluded that primed plants with salicylic acid act better for suppressing the severity of late blight disease of potato, by stimulating their own inbuilt resistance under field conditions. Moreover, it is considered as safe, cost-effective and easily applied for such diseases. Blanket spray of salicylic acid can be done on vegetable crops before onset of diseases to enhance plant defense against oomycete pathogens. Only 8-10 grams of SA is required (Dissolved in 100 litres of water) for spraying crop and it approximately cost only Rs 15/ Acre per spray, making it very economical and safe.

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