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Research Article

Biochemical Basis of Systemic Acquired Resistance Induced by Different SAR Elicitors against Late Blight of Potato

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ABSTRACT

Phytophthora infestans is an oomycete that causes a serious potato disease known as late blight of potato. 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 exogenous foliar sprays of different conc. of Salicylic acid, Jasmonic acid and Bion (Benzothiadiazole-BTH) @ 50µM, 250µM, 500µM, 1000µM and of β- amino butyric acid of 20 mM, 30mM, 50 mM, 100mM were given for inducing resistance in potato against late blight disease. 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 plant varied from 4.3 to 8.1 mg/g fresh weight compared to 3.2 mg/g fresh weight in control. Induction of proteins and defense enzymes was systemic in nature in response to all the four elicitors. The inducers 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 137 % in response to elicitors. Salicylic acid gave best results with 82.6 % disease control followed by Jasmonic acid with 79.2%; whereas Bion and Beta amino butyric acid were almost at par with each other and gave 75 % disease control as compared to control plants. Thus integration of disease tolerance and salicylic acid spray resulted in effective and eco-friendly control of late blight of potato.

Keywords: *Potato, Systemic acquired resistance, Salicylic acid, Jasmonic acid, β-amino butyric acids (BABA), Benzothiadiazole (BTH), Late blight.*

INTRODUCTION

More than 10,000 species of oomycetes and fungi can cause diseases in plants, with the resultant severe reductions in the quantity and quality of the plant products (Agrios 2005). Late blight of potato caused by *Phytophthora infestans*, belonging to oomycete has become quite severe in the past recent years.

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All the commercial varieties of potato cultivated in Punjab state of India are moderately to highly susceptible to late blight. Moreover, host resistance to *P. infestans* is not generally stable due to development of new multigene races of the pathogen and so fungicides play important role in disease management. In India and abroad, fungicide (Metalaxyl) resistant strains of *P. infestans* were also reported (Dowley & O'sullivan 1991; Kaur et al., 2010). Heath (2000) reported that there are two types of resistance in plant species i.e. host and non- host specific resistance. Non-host resistance is shown by whole plant against particular pathogen and host specific in interaction between specific host and specific pathogen. Biochemical changes may be accompanied by production of phenolics and related enzymes, termed as hypersensitive response (Siqueira et al., 1991). Walters and Fountaine (2009) asserted that, at least three types of systemic induced resistance are known, which are 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-genes 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 even without pathogen inoculation (Van Loon et al., 2006). PR-1 inhibits *in-vitro* zoospore germination of *P. infestans*, as well as *in-vivo* differentiation of infection hyphae of *Uromyces fabae* in leaves of the broad bean (Rauscher et al., 1999). 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 are found to induce systemic acquired resistance (SAR) against various pathogens in many crops by activating various genes coding for PR-proteins e.g. β -1,3-glucanase (PR-2), chitinase (PR-3), peroxidase (PR-9) and a number of other proteins in stress conditions (Enkerli et al., 1993). Therefore, the present study was conducted to test application of SAR elicitors i.e. Jasmonic acid (JA), Salicylic acid (SA) and Benzothiadiazole (BTH) and β-Amino butyric acid (BABA) to enhance plants own defense mechanism to control late blight disease and reduce fungicide load on potato.

MATERIAL AND METHODS

Potato cultivars/hybrids

Three varieties of potato i.e. Kufri Badshah, Kufri Jyoti and Kufri Pukhraj were procured from department of vegetables, PAU, Ludhiana and were used for standardization of concentration of all the four elicitors.

Sowing of crop and testing of different doses of elicitors

The selected varieties of potato were raised in rows and replicated thrice using standard package of practices in month of October with plot size of 2 X 3m. Standardization of concentration of Jasmonic acid (JA) and Salicylic acid (SA), Benzothiadiazole (BTH) and β- amino butyric acids (BABA) for the induction and over expression of proteins in tolerant (Kufri Jyoti) and susceptible (Kufri Pukhraj) cultivars of potato was done. Different concentrations of elicitors tried as spray (Prepared in 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

These doses were sprayed on three-week-old sprouts using an atomizer. Water sprayed plants of corresponding genotypes were kept as control.

Collection of plant tissue samples

Periodical leaf sampling was done after 24, 48, 72, 96, 120, 144 hrs and at weekly intervals after elicitors spray. Samples were brought to laboratory under refrigerated conditions and were stored at -80°C in deep freezer to prevent denaturation of proteins.

Estimation of total soluble proteins

Soluble proteins in the supernatant were estimated according to Lowry et al. (1951). Bovine serum albumin (BSA) standards (20-100 µg) were also 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 (Walker 1996) was done for leaves of three varieties of potato sprayed with 500 µM of SA, JA, BTH and at 50mM of BABA. SDS-PAGE was carried out to study the protein profile of these varieties. Standard protein marker 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 extracted 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 13,000g at 4^0 C for 30 min (Eppendorf 5804R) and clear supernatant was used for estimating enzymes viz. peroxidase, Polyphenol oxidase and phenylalanine ammonia lyase. Then the standard procedures by Clariborne and Fridovich (1979), Burrell and Rees (1974), and Zauberman et al. (1991) were employed for the estimation of Peroxidase, Phenylalanine ammonia lyase, Polyphenol oxidase. For estimation of β-1, 3 glucanase, DNSA was used as reagent. Reddish brown colour developed was read at 575nm. 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, 0.2g of leaf sample was taken and to this added 1mL of DMSO. This solution is kept for overnight for colour development. The optical density was read 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. 4.0×10^4 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

After one week of elicitor spray pathogen inoculations of *P. infestans* with sporangial solution at conc. of 4.0×10^4 sporangia per mL was sprayed using an atomizer to create disease, and high relative humidity was maintained for next 72 hrs by spraying water. Observations on disease severity were recorded. Disease rating system for late blight of potato was done using scale given by Thind and Mohan (1998).

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

RESULTS

Total proteins

The data pertaining to changes in protein concentration recorded at periodical interval of 24 hrs till a week in response to selected best doses of JA, SA and BTH i.e, at 500 µM and BABA at 50 mM, revealed statistically significant difference amongst the various elicitors 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. 7.3 mg/gFW followed by 6.8 mg/gFW in Kufri Badshah and 6.4

mg/gFW in Kufri Pukhraj. JA at 500 µM proved to be second best treatment with mean maximum protein induction observed in Kufri Jyoti i.e. 7.0 mg/gFW followed by 6.4 mg/gFW in Kufri Badshah and by 6.0 mg/gFW in Kufri Pukhraj. BTH at 500 µM gave mean maximum protein induction of 5.2 mg/gFW in both Kufri Badshah, and Kufri Jyoti, whereas 4.6 mg/gFW in Kufri Pukhraj. BABA gave mean maximum protein induction of 6.0 mg/gFW at 50mM of concentration in Kufri Jyoti and 5.8 and 4.9 mg/gFW in Kufri Badshah, Kufri Pukhraj respectively. Statistically significant difference for all four elicitors w.r.t. time interval was observed. SA was found to induce mean maximum protein 8.1mg/gFW at 3rd day interval respectively followed by JA showing maximum protein 7.7 mg/gFW at 3rd day interval. BABA and BTH showed maximum protein 6.7 and 5.9 mg/gFW at 3rd day interval in Kufri Jyoti. Whereas minimum protein varied from 3.2 to 4.2 mg/gFW for control treatment between all the respective time intervals with mean value of 3.7 mg/gFW. Similar pattern was also observed in other two varieties.

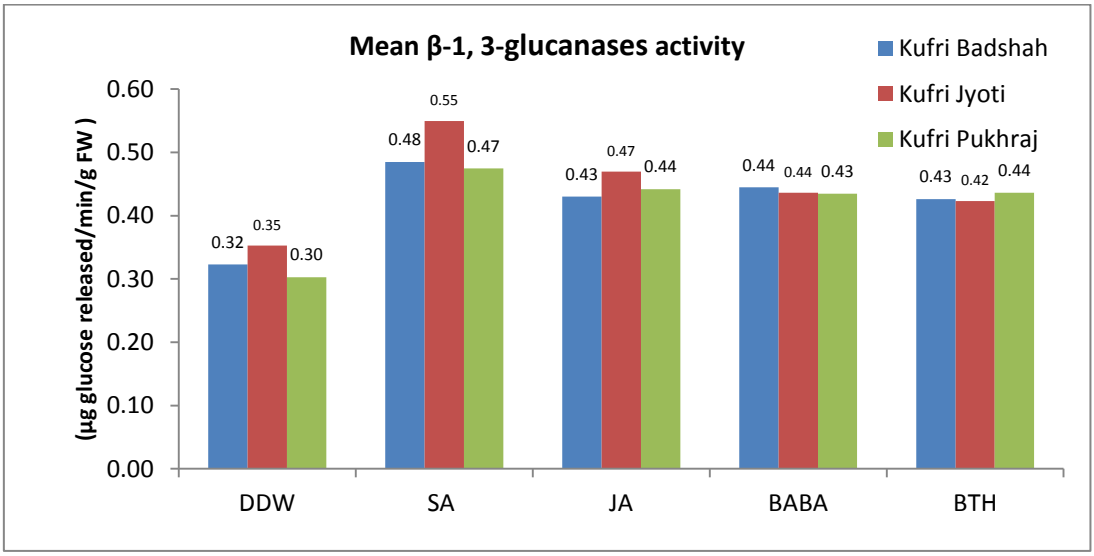
Table 1. Effect of different doses of JA, SA, BABA and BTH on leaf protein concentration (mg/gFW) potato leaves after 21 days of sowing

Total protein content (mg /g FW)								
Variety	**Treatment**	**Days after spray**						**Mean**
		1	**2**	**3**	**4**	**5**	**6**	**7**	
Kufri Badshah	Control	2.8	3.2	3.5	3.7	3.9	3.9	3.7	3.5
	SA (500µM)	6.3	6.6	7.4	7.2	6.9	6.7	6.4	6.8
	JA(500 µM)	6.3	6.4	6.8	6.7	6.4	6.2	5.8	6.4
	BABA(50mM)	5.6	5.8	6.5	6.3	5.7	5.4	5.2	5.8
	BTH (500µM)	4.6	5.0	5.8	5.7	5.4	5.1	5.0	5.2
Kufri Jyoti	Control	3.2	3.3	3.6	3.7	4.0	4.2	4.2	3.7
	SA (500µM)	7.3	7.4	8.1	7.8	7.2	6.9	6.5	7.3
	JA(500 µM)	6.6	7.0	7.7	7.7	7.1	6.7	6.4	7.0
	BABA(50mM)	6.1	6.3	6.7	6.5	5.9	5.6	5.2	6.0
	BTH (500µM)	5.0	5.4	5.9	5.5	5.1	4.9	4.6	5.2
Kufri Pukhraj	Control	2.7	3.1	3.4	3.5	3.8	3.8	3.6	3.4
	SA (500µM)	5.6	6.1	7.2	6.9	6.4	6.2	6.0	6.4
	JA(500 µM)	6.0	6.1	6.5	6.3	5.8	5.6	5.4	6.0
	BABA(50mM)	4.5	4.8	5.5	5.2	4.9	4.7	4.6	4.9
	BTH (500µM)	4.5	4.6	5.1	4.9	4.5	4.4	4.3	4.6
CD (5%)	Variety (A)-0.028; Elicitor (B)- 0.037; Time interval (C)-0.043 ; AB- 0.064;AC-0.075; BC-0.097; ABC- 0.16								

β-1, 3 glucanase

Amongst the different elicitors treatment in Kufri Badshah; SA caused 50 % increase in β-1, 3-glucanase activity in leaves, whereas JA resulted in 34%, BABA gave 37% and BTH gave 43% increase in β-1, 3-glucanase activity concentration w.r.t control. Similarly, in Kufri Jyoti SA caused 57 % increase in β-1, 3 glucanase activity in leaves, whereas JA resulted in 34%, BABA gave 26% and BTH gave 20% increase in β-1, 3 glucanase activity w.r.t control. In Kufri Pukhraj SA caused 57% increase in β-1, 3 glucanase activity in leaves, whereas JA resulted in 47%, BABA gave 43% and BTH gave 47% 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 (Fig1).

The total β-1, 3 glucanase activity increased upto 3rd day in all three elicitors except JA were peak value observed on second day of spray and thereafter registered decline irrespective of variety and elicitor treatment.

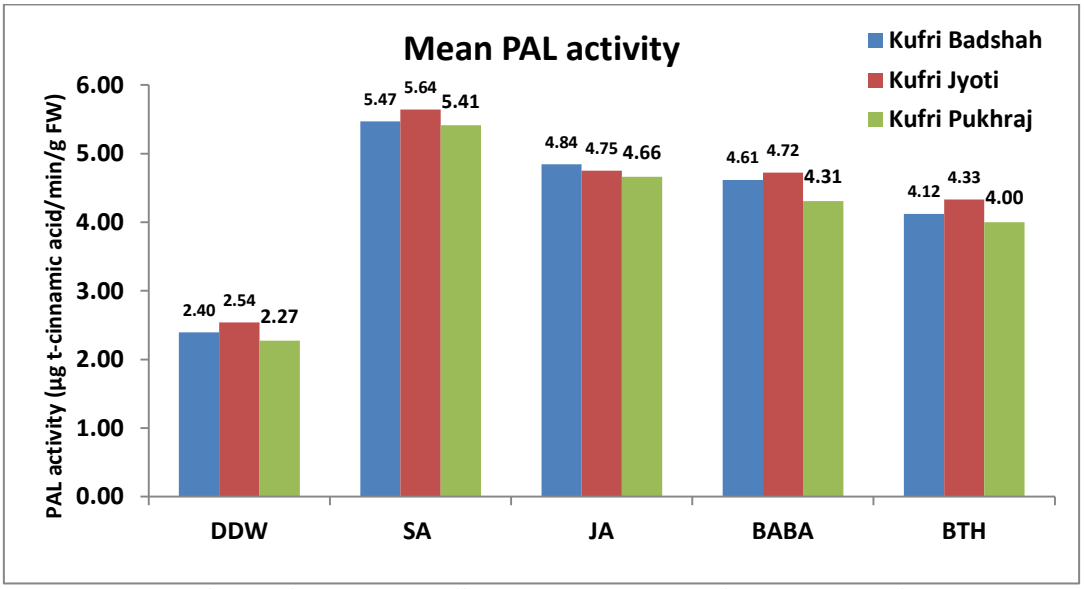


Each value is mean of values of β-1, 3-glucanases activity at different time interval (1-7 days) for each treatment of respective elicitor

Fig. 1: Effect of foliar spray with different elicitors i.e. SA, JA, BABA and BTH on β-1, 3-glucanases activity (µg glucose released/min/gFW) in leaves of potato varieties

Phenylalanine ammonia lyase (PAL)

Amongst the different elicitors treatment in Kufri Badshah; SA caused 128 % increase in PAL activity in leaves, whereas JA resulted in 100%, BABA gave 92% and BTH gave 72% increase in PAL activity w.r.t control. Similarly, in Kufri Jyoti SA caused 122% increase PAL activity in leaves, whereas JA resulted in 87%, BABA gave 86% and BTH gave 70% increase PAL activity w.r.t control. In Kufri Pukhraj SA caused 138% increase in PAL activity in leaves, whereas JA resulted in 105%, BABA gave 89% and BTH gave 76% increase in 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. Statistically significant difference for all four elicitors w.r.t. time interval was observed. SA was found to induce mean maximum PAL activity 6.23 µg t-cinnamic acid/min/g FW at 4th day interval respectively followed by JA showing maximum PAL activity 5.23 µg t-cinnamic acid/min/g FW at 4th day interval (Fig 2).

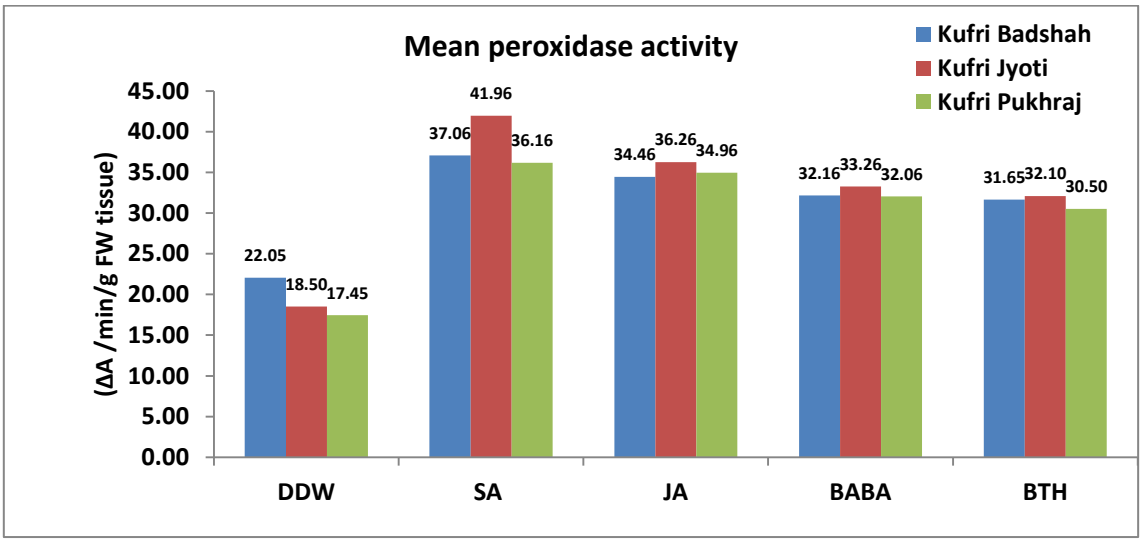


Each value is mean of values of PAL activity at different time interval (1-7 days) for each treatment of respective elicitor

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

Peroxidase (POD)

Mean maximum peroxidase activity was observed at 500 µM of SA in Kufri Jyoti i.e. 41.96 ΔA /min/g FW followed by 37.06 ΔA /min/g FW in Kufri Badshah and 36.16 ΔA /min/g FW in Kufri Pukhraj. JA at 500 µM proved to be second best treatment with mean maximum peroxidase activity observed in Kufri Jyoti i.e.36.26 ΔA /min/g FW, and 34.46 and 33.96 ΔA /min/g FW in Kufri Badshah and Kufri Pukhraj. BTH at 500 µM gave mean maximum peroxidase activity of 31.65 and 32.10 ΔA /min/g FW in Kufri Badshah, and Kufri Jyoti, whereas 30.5 ΔA /min/g FW in Kufri Pukhraj. BABA gave mean maximum peroxidase activity of 33.26 and 32.16 ΔA /min/g FW at 50mM of concentration in Kufri Jyoti and Kufri Badshah and 32.06 ΔA /min/g FW in, Kufri Pukhraj respectively (Fig 3). The total peroxidase activity increased upto 3rd day in all four elicitors and thereafter registered decline irrespective of variety and elicitor treatment. In general, 500 µM SA resulted in higher mean peroxidase activity followed by JA followed by BABA and then BTH. Tolerant variety, Kufri Jyoti showed better peroxidase activity followed by Kufri Badshah and Kufri Pukhraj. BABA and BTH treatments were at par w.r.t. peroxidase activity in all the three varieties.



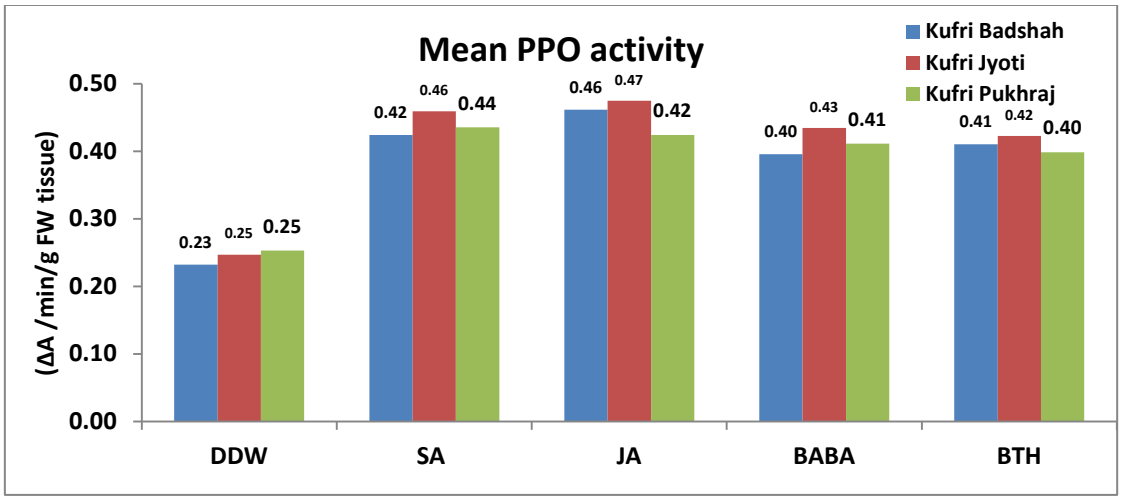
Each value is mean of values of Peroxidase (POD) activity at different time interval (1-7 days) for each treatment of respective elicitor

Fig. 3: Effect of foliar spray with different elicitors i.e. SA, JA, BABA and BTH on peroxidase activity (ΔA /min/g FW) in leaves of potato varieties

Polyphenol oxidase (PPO)

Statistically significant difference for all four elicitors w.r.t. time interval was observed. JA was found to induce mean maximum PPO activity 0.55 ΔA /min/g FW at 4TH day interval respectively followed by SA showing maximum PPO activity 0.54 ΔA /min/g FW at 4TH day interval. BABA and BTH showed maximum PPO activity 0.51 and 0.50 ΔA /min/g FW at 4TH day interval in Kufri Jyoti. Whereas minimum PPO activity varied from 0.22 to 0.27 ΔA /min/g FW for control treatment between all the respective time intervals with mean value of 0.25 ΔA /min/g FW. Similar pattern were also observed in other two varieties. The total PPO activity increased upto 4TH day in all four elicitors and thereafter registered decline irrespective of variety and elicitor treatment. In general, 500 µM JA resulted in higher mean PPO activity followed by SA followed by BABA and then BTH. Kufri Jyoti showed better PPO activity followed by Kufri Badshah and Kufri Pukhraj (Fig 4).

Spike in protein content in control samples was also observed because proteins (enzymes, *Pr* proteins etc) increase in plants on basis of age upto 30-60 days depending upon crop and variety. This daily change especially on micro molar level (biochemically) is also controlled by environmental conditions but control plants do not show peaks as in case of primed (elicitor treated) plants.



Each value is mean of values of Polyphenol oxidase (PPO) activity at different time interval (1-7 days) for each treatment of respective elicitor

Fig. 4: 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

Total chlorophyll

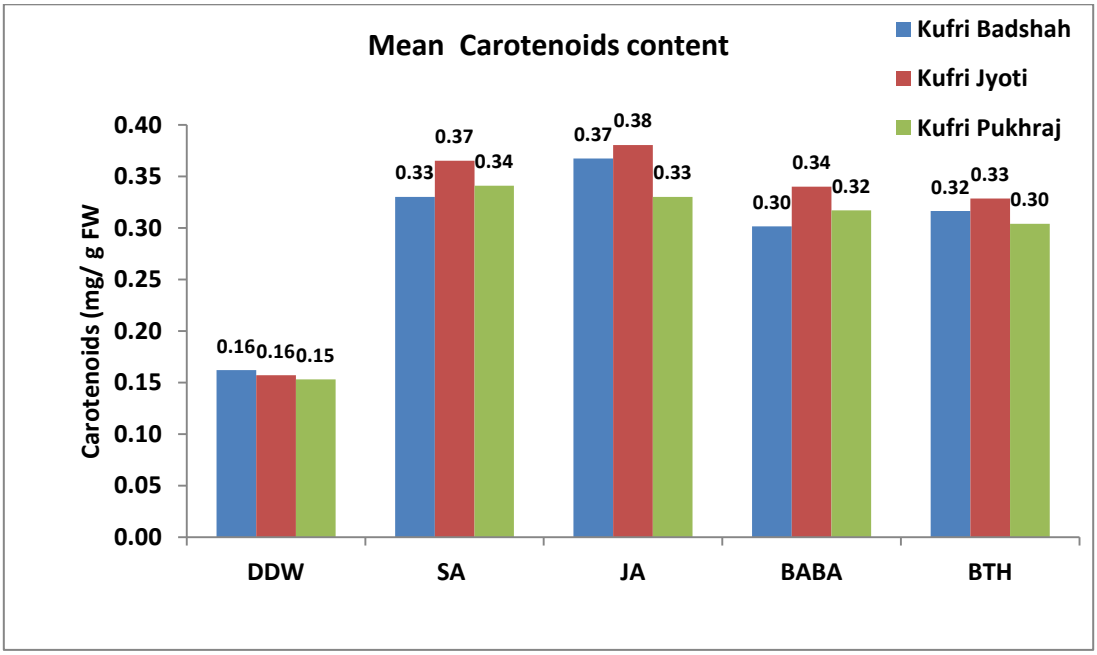
Amongst the different elicitors treatment in Kufri Badshah; SA caused 6% increase in total chlorophyll (mg/ g FW) in leaves, whereas JA resulted in 3% decrease, BABA gave 4% and BTH gave 2% increase in total chlorophyll (mg/ g FW) w.r.t control. Similarly, in Kufri Jyoti SA caused 13% increase in total chlorophyll (mg/ g FW) in leaves, whereas JA resulted in 1% decrease, BABA gave 12% and BTH gave 8% increase in total chlorophyll (mg/ g FW) w.r.t control. In Kufri Pukhraj SA caused 13% increase in total chlorophyll (mg/ g FW) in leaves, whereas JA resulted in decrease of 1%, BABA gave 11% and BTH gave 10% 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 maximum content in Kufri Jyoti. It was observed that the chlorophyll content decrease on treatment with JA in foliar application, even though control values were at par with 500µM JA treated samples (Table 2).

Table 2: Effect of foliar spray with different elicitors i.e. SA, JA, BABA and BTH on total chlorophyll (mg/gFW) in leaves of different potato varieties

Total chlorophyll (mg/gFW)									
Variety	**Treatment**	**Days After Spray**							**Mean**
		1	**2**	**3**	**4**	**5**	**6**	**7**	
Kufri Badshah	Control	1.33	1.35	1.36	1.37	1.39	1.40	1.42	1.38
	SA (500µM)	1.42	1.44	1.45	1.46	1.48	1.49	1.51	1.47
	JA(500 µM)	1.28	1.30	1.31	1.32	1.34	1.35	1.37	1.33
	BABA(50mM)	1.40	1.42	1.43	1.44	1.46	1.47	1.48	1.44
	BTH (500µM)	1.38	1.40	1.41	1.42	1.44	1.45	1.46	1.42
Kufri Jyoti	control	1.35	1.37	1.37	1.39	1.39	1.41	1.43	1.39
	SA (500µM)	1.53	1.55	1.58	1.66	1.63	1.57	1.57	1.58
	JA(500 µM)	1.31	1.33	1.39	1.41	1.41	1.40	1.39	1.38
	BABA(50mM)	1.50	1.52	1.56	1.64	1.60	1.55	1.55	1.56
	BTH (500µM)	1.46	1.48	1.51	1.59	1.56	1.50	1.50	1.51
Kufri Pukhraj	Control	1.35	1.37	1.37	1.37	1.38	1.39	1.40	1.38
	SA (500µM)	1.50	1.52	1.56	1.64	1.60	1.55	1.55	1.56
	JA(500 µM)	1.32	1.31	1.42	1.39	1.38	1.36	1.35	1.36
	BABA(50mM)	1.48	1.50	1.54	1.61	1.58	1.53	1.53	1.54
	BTH (500µM)	1.47	1.49	1.52	1.60	1.57	1.51	1.51	1.52
CD (5%)	Variety (A)-0.0021; Elicitor (B)- 0.0028; Time interval (C)-0.0033 ; AB- 0.0049; AC-0.0058; BC-0.0075; ABC- 0.013								

Carotenoids

The data pertaining to changes in leaf carotenoids (mg/ g FW) in response to various doses of JA, SA and BTH i.e., at 500 µM and BABA at 50 mM, revealed statistically significant difference amongst the various elicitors applied on three different varieties of potato namely; Kufri Badshah, Kufri Jyoti, Kufri Pukhraj (Fig 5).Amongst the different elicitors treatment in Kufri Badshah; SA caused 106% increase in carotenoids (mg/ g FW) in leaves, whereas JA resulted in 131% increase, BABA gave 87% and BTH gave 100% increase in carotenoids (mg/ g FW) w.r.t control. Similarly, in Kufri Jyoti SA treatment resulted in 131% increase in carotenoids (mg/ g FW) in leaves, whereas JA resulted in 137%, BABA gave 112% and BTH gave 106% increase in carotenoids (mg/ g FW) w.r.t control. In Kufri Pukhraj SA treatment resulted in 126% increase in carotenoids (mg/ g FW) in leaves, whereas JA resulted in increase of 120%, BABA in 113% and BTH in 100% 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.



Each value is mean of values of carotenoids at different time interval (1-7 days) for each treatment of respective elicitor

Fig. 5: Effect of foliar spray with different elicitors i.e. SA, JA, BABA and BTH on carotenoids (mg/gFW) in leaves of different potato varieties

Electrophoretic study (SDS- PAGE) protein extract of different potato

The potato leaf proteins extracts were subjected to SDS-PAGE electrophoresis. Total soluble leaf protein was resolved with standard protein marker ladder with molecular weights ranging from 6-180 kDa for reference. Specific bands lying in the range of 6-50 KDa were observed in treated samples as compared to their respective control (Plate 1). In gel figure bands in range of PR-2 family i.e. Beta-1,3-glucanases having hydrolytic enzyme activity on fungal cell wall lying in range of molecular weight of 25-35kDa, Antifungal PR-1 family having molecular weight of 14-17kd, and peroxidises (PR-9 family) lying in range of 35-45 kDa band can be seen. Thus indicating induction of PR-protein which induced defense against late blight of potato against *P. infestans*.

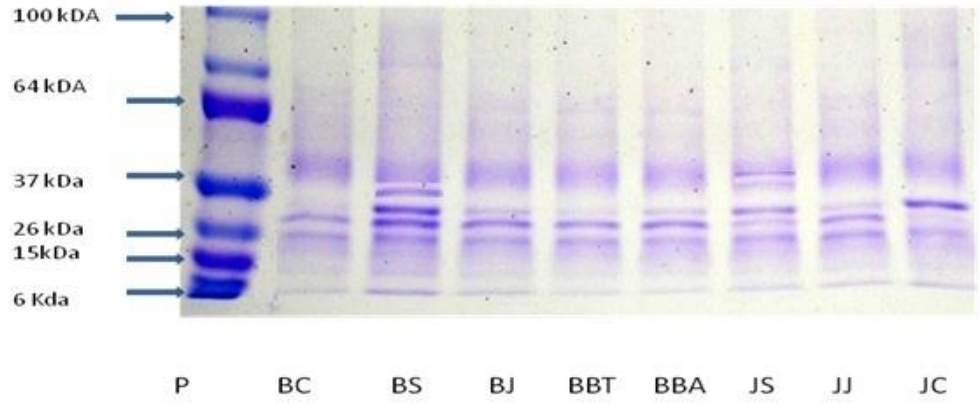


Plate 1: SDS- PAGE of leaf proteins of different potato varieties at 500 µM of SA, 500 µM of JA, 500 µM of BTH and 50mM for BABA

P- Protein Ladder, BC: Kufri Badshah control, BS: Kufri Badshah treated with SA, BJ: Kufri Badshah treated with JA, BBA: Kufri Badshah treated with BABA, BBT: Kufri Badshah treated with BION, JS: Kufri Jyoti treated with SA, JJ: Kufri Jyoti treated with JA, JC: Kufri Jyoti –control

Disease data

Testing of SAR inducers in various combination treatments was done to have better disease control. As replacement of contact fungicide i.e only selected doses of SA, JA, BTH and BABA were used for disease control then in combination of half recommended dose contact fungicide, i.e. & Mancozeb 75 % WP (Indofil M-45) @ 350 g per acre was also done. Selected doses of SA, JA, BTH and BABA were also tested in combination with systemic fungicide i.e. Ridomil @ 350 g per acre (Half dose than recommended). First spray of treatment was done on 21 days after the sowing of crop and followed by five sprays, at intervals of seven days. After one week of elicitor treatment; plants were challenge inoculated with disease (Table 3). The data on disease severity was calculated on weekly interval. All the treatments gave 100% disease control upto seven days of challenge inoculation. Per cent disease control varied from 75 % in BTH treated plots to 94 % in standard recommended fungicide treatment. Second best treatment comes out to be three sprays BABA followed by two sprays of contact fungicide Mancozeb with 92.8 % disease control. SA singly gave 82.6% disease control whereas in combination with Mancozeb and Ridomil gave 87.7 % and 92.8% respectively, which was at par with recommended fungicide control. Therefore it can be concluded that SA treatment can be combined with half dose of systemic fungicide Ridomil for late blight control in potato even for economical reasons.

Table 3: Testing efficacy of SAR elicitors along with Ridomil gold and Mancozeb against disease severity of late blight of potato

Variety	Treatment	No. of sprays	% Disease severity				% Disease control
			Days after challenge inoculation				
			7	14	21	28	
Kufri Pukhraj	SA	5	0	3.80	4.56	10.23	82.62
	JA	5	0	4.57	6.25	12.25	79.19
	BABA	5	0	5.20	7.45	14.36	75.61
	BTH	5	0	8.27	9.56	14.68	75.06
	SA & & Mancozeb	3+2	0	4.21	5.32	7.21	87.75
	JA & & Mancozeb	3+2	0	4.51	5.33	8.53	85.51
	BABA & Mancozeb	3+2	0	3.54	4.11	4.24	92.80
	BTH & Mancozeb	3+2	0	7.50	8.54	10.25	82.59
	SA & Ridomil Gold	3+2	0	3.50	3.53	4.56	92.25
	JA & Ridomil Gold	3+2	0	3.60	3.5	5.23	91.12
	BABA & Ridomil Gold	3+2	0	5.10	5.12	5.42	90.79
	BTH & Ridomil Gold	3+2	0	6.50	6.66	7.35	87.51
	Mancozeb & Ridomil Gold (Recommended spray schedule)	3+2	0	3.10	3.24	3.54	93.99
	Control	DDW	9.5	34.23	43.15	58.87	0.00
CD (5%)	Treatment (A)-1.03; Time interval (B)-0.55; Interaction (AB)- 2.07						

DISCUSSION

Elicitor treated plants have been shown to induce defense related PR-proteins in response to late blight pathogen. The present study depicted that elicitation could be a promising strategy to control late blight of potato by priming plant own defense system with SAR inducers. The results obtained in present study are corroborated with findings of Gao and Zhang (2013) who, demonstrated that SA treatment showed the best effect on disease control in pear fruit against rot as it significantly induced activities of the enzymes β-1, 3-glucanase, PAL, PPO, and POD, which are type of PR proteins. An important common feature of most PR proteins (chitinase and glucanase) is their antifungal effect, with some also exhibiting antibacterial, insecticidal, nematicidal, and antiviral action (Van Loon et al., 2006). Thaler et al. (2012) studied the influence of SA and JA against late blight of potato and studied the evolution of jasmonate and salicylate signal crosstalk. Complete control of late blight in tomato was reported with BABA, even when applied post- infection as reported by Cohen (2002). Induction of PR proteins i.e. P14a and β-1, 3-glucanase was higher in BABA-treated tomato plants as compared with control plots. Kone et al. (2009) studied in greenhouse that SA applied as soil drench or foliar spray at 25 or 50µg ml^{-1} significantly reduced severity of disease caused by *Phytothphora capsici*, compared with control. Tian et al. (2005) demonstrated that pear fruits treated with various elicitors like SA, oxalic acid, calcium chloride etc significantly enhanced defence-related proteins activities such as β,1-3 glucanase and reduced the disease incidence of *Alternaria alternata*. Olivieri et al. (2009) studied biochemical mechanisms of by which BABA increase resistance against *P. infestans*, as well the effect of BABA on the activity of a potential pathogenic factor of *Fusarium solani.* Mostafa and Gado (2007) reported that the application of ESA and JA, reduces the disease severity compared to check against late blight of potato. The study of Aldesuquy (2015) supports our study by demonstrating that in *Vicia faba*, SA application increases the total soluble protein content against *Botryis spp*. Similar, results were observed in case of, tomato plant treated with JA and SA showed higher total soluble protein and free amino acid content compared to infected control plants with *Fusarium* wilt (El-Khallal 2007).

Table 4: Correlation between various enzymes activity, chlorophyll, carotenoids, protein content and disease severity in different varieties of potato

Disease Severity on variety	Total protein	β-1, 3 glucanase	Pal	Peroxidase	PPO	Chlorophyll	Carotenoids
Kufri Badshah	-0.984	-0.970	-0.932	-0.911	-0.914	-0.759	-0.942
Kufri Jyoti	-0.975	-0.861	-0.919	-0.898	-0.907	-0.705	-0.976
Kufri pukhraj	-0.992	-0.994	-0.950	-0.991	-0.990	-0.647	-0.988

Critical Value of r at 5% = 0.878

There are many reports available on the accumulation of chitinases and β-1, 3-glucanases and other enzymes in many plant species in response to infection by pathogens, elicitor or chemical treatment. 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.* Higher activity of peroxidases and polyphenol oxidases have been reported in early maturing germplasm of maize when challenged by banded leaf and sheath blight pathogen, *R. solani* (Dahima et al., 2014). Exogenous application of SA was found to enhance the net photosynthetic rate, internal CO_2 concentration, water use efficiency, stomatal conductance and transpiration rate in *B. juncea* (Fariduddin et al., 2003). The maximum chlorophyll content and dry weight was recorded with 0.5 mM SA and 0.25 mM MJ application; so SA treatment increased the chlorophyll and carotenoid

contents in maize plants (Khodary, 2004). Our study revealed that carotenoids were further enhanced by all the elicitors treatments with maximum increase in case of JA application. This increase may be attributed to protection of plant cells from Reactive oxygen species and other oxidations factors. However, increase in PPO and PAL was higher in resistant variety than in susceptible cultivars after treatment with the SAR elicitor, indicating that defense related proteins reach an inhibitory level to the fungus in the potato plants. Increase in ß-1,3-glucanase and peroxidase activity, after treatment, was higher in SA treated plants as compared to other elicitors. There seems to be a definite role of total phenols, peroxidase and ß-1,3-glucanase in defense against *P. infestans* in potato. Therefore, it can be concluded that SA treatment can be combined in spray schedule with contact fungicide or with half dose of systemic fungicide for control of, late blight in potato, which will be economical and as well as environmental friendly.

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