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# Biochemical basis of Systemic acquired resistance induced by different SAR elicitors against downy mildew of muskmelon

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## ABSTRACT

***Pseudoperonospora cubensis*, an Oomycetous fungus causing downy mildew in muskmelon 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 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 muskmelon against downy mildew. Concentration of Salicylic acid, Jasmonic acid and Bion @ 500 µM, and β amino butyric acid @ 50 mM gave good control of disease. Protein content of treated muskmelon plant varied from 9.6 to 12.7 mg/g fresh weight compared to 3.9 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 muskmelon plants as compared to control. Electrophoretic protein profiling of treated muskmelon 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 2% to 91 % in response to elicitors. Salicylic acid gave best results with 93.8 % disease control followed by Jasmonic acid with 87.2%; whereas Bion and β amino butyric acid were almost at par with each other and gave 76 % disease control as compared to control plants. Thus integration of disease tolerance and salicylic acid spray resulted in effective and eco-friendly control of downy mildew in muskmelon.**

**Keywords:** Muskmelon, systemic acquired resistance, salicylic acid, Jasmonic acid, β amino butyric acids (BABA), Benzothiadiazole (BTH), downy mildews.

## INTRODUCTION

*Pseudoperonospora cubensis* (Bert. Et. Curt.) Rost. is the causal agent of muskmelon downy mildew. *P. cubensis* is an obligate oomycete pathogen, infecting crops within the family *Cucurbitaceae* and can be found worldwide, causing significant yield losses in USA, Europe, and Asia (Thomas, 1996). It is reported from over 70 countries, across diverse environments (semi-arid to tropical) (Cohen *et al*., 2003). Angular chlorotic lesions and a downy or feltlike appearance on the abaxial side of the leaf characterize the disease ( Lebeda and Urban, 2007). Continuous use has led to resistance to commonly used fungicides like mefenoxam, metalaxyl and the strobilurin (Reuveni *et al*., 1980). 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, for 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). After a pathogen invasion there is activation of variety of plant defense responses via signal transduction cascade activation, which involves oxidative burst, reinforcement of cell walls, hypersensitive response, production of phytoalexins, etc. Delayed active defenses include containment of the pathogen, wound repair, expression of pathogenesis-related proteins and the acquisition of systemic resistance (Jones and Dangl, 2006). These mechanisms restrict the spread of the pathogen after infection is established and contain the damage to host tissues. The success of defense responses can be increased, if activated in combination i.e. SAR elicitors are sprayed prior to infection to prime the plant system and plant's own defence mechanism can be exploited by means of systemic acquired resistance for control of diseases (Ryals *et al*., 1996). 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). BTH is a functional analogue of salicylic acid in the signal transduction pathway of SAR, inducing cell wall strengthening, phytoalexin accumulation and pathogenesis related (PR) protein synthesis (Gorlach *et al*., 1996). Therefore, the present study was conducted to test application of SAR elicitors *i.e.* Jasmonic acid (JA), Salicylic acid (SA) and Benzothiadiazole (BTH) and Amino butyric acid (BABA) to enhance plants own defense mechanism to control downy mildew disease and reduce fungicide load on muskmelon.

## MATERIAL AND METHODS

**Muskmelon cultivars/hybrids:** Three varieties of muskmelon i.e. Kajri, Hara madhu and Punjab hybrid 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 muskmelon were raised on 3 X 3m wide beds and replicated thrice using standard package of practices in the month of February. 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 (Kajri) and susceptible (Punjab Hybrid) cultivars muskmelon was done. Jasmonic acid and BABA was procured from Sigma Aldrich, USA and BTH was procured from Syngenta, India.

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:** Leaf tissue (0.5 g) was weighed and was 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 $4^0$C. Supernatant was used for protein estimation. To 0.1 mL of the protein, extract added 0.9 mL of distilled water. Then 5 mL of reagent (2% Sodium carbonate in 0.1N sodium hydroxide and 5% Copper sulphate in 1% sodium potassium tartarate, mixed in ratio of 50:1) was added and properly mixed. After 10 min, 0.5 mL of Folin Ciocalteau reagent (diluted in 1:1 with DDW) was added, mixed and kept for 30 min at room temperature. The intensity of blue color developed was then read at 520 nm against a reagent blank (Lowry *et al*., 1951). Bovine serum albumin (BSA) standards (20-100 g) 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 muskmelon 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 13,000 g 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 and Polyphenol oxidase. For estimation of -1, 3 glucanase, DNSA was used as reagent. Reddish brown colour developed was read 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, 0.2g 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. cubensis* sporangial solution at conc. of approx. 4.0 x $10^4$ sporangia per mL was prepared by dislodging sporangia from sporulating leaves in double distilled water and used for challenge inoculations in muskmelon experiments.

**Disease data on lesion development:** Data on lesion development was taken on three cultivars of muskmelon. Plants treated with elicitors were challenge inoculated with *P. cubensis* sporangial suspension and lesion size in mm was recorded after $5^{th}$ and $7^{th}$ day of challenge inoculation.

## 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 muskmelon namely; Punjab Hybrid, Kajri, Hara Madhu (**Table 1**). Mean maximum protein induction was observed at 500 µM of SA in Kajri i.e. 11.9 mg/gFW followed by 11.8 mg/gFW in Punjab Hybrid and 11.8 mg/gFW in Hara Madhu. JA at 500 µM proved to be second best treatment with mean maximum protein induction observed in Kajri i.e. 11.8 mg/gFW followed by 11.7 mg/gFW in Punjab Hybrid and by 11.2 mg/gFW in Hara Madhu. Amongst the different elicitors treatment in Punjab Hybrid; SA caused 154% increase in protein content in leaves, whereas JA resulted in 150%, BABA gave 126% and BTH gave 110% increase in protein concentration w.r.t. control. Similarly, in Kajri SA caused 158 % increase in protein

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

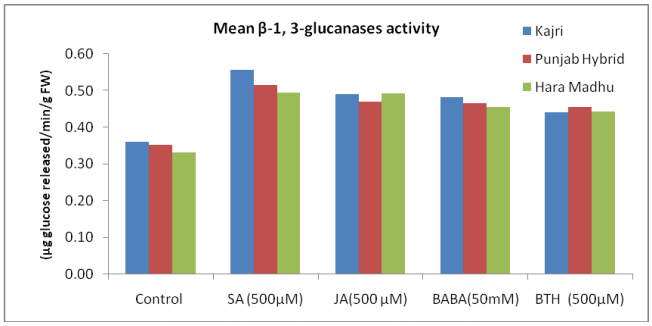
| Total protein content (mg /g FW) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Variety** | **Treatment** | **Days after spray** | | | | | | | **Mean** |
| | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | |
| Kajri | Control | 4.1 | 4.2 | 4.4 | 4.6 | 4.8 | 5.0 | 5.0 | 4.6 |
| | SA (500µM) | 11.9 | 12.0 | 12.7 | 12.4 | 11.8 | 11.5 | 11.1 | 11.9 |
| | JA(500 µM) | 11.4 | 11.8 | 12.5 | 12.5 | 11.9 | 11.5 | 11.2 | 11.8 |
| | BABA(50mM) | 10.7 | 11.1 | 11.5 | 11.2 | 10.6 | 10.3 | 9.9 | 10.7 |
| | BTH (500µM) | 10.0 | 10.2 | 10.8 | 10.4 | 9.9 | 9.7 | 9.5 | 10.1 |
| Punjab Hybrid | control | 4.1 | 4.3 | 4.4 | 4.7 | 4.9 | 5.0 | 5.0 | 4.6 |
| | SA (500µM) | 11.7 | 11.8 | 12.5 | 12.2 | 11.6 | 11.3 | 10.9 | 11.7 |
| | JA(500 µM) | 11.0 | 11.5 | 12.2 | 12.1 | 11.6 | 11.2 | 10.9 | 11.5 |
| | BABA(50mM) | 10.4 | 10.8 | 11.2 | 10.9 | 10.3 | 10.0 | 9.6 | 10.4 |
| | BTH (500µM) | 9.6 | 9.8 | 10.4 | 10.0 | 9.5 | 9.3 | 9.1 | 9.7 |
| Hara Madhu | Control | 3.9 | 4.1 | 4.2 | 4.5 | 4.7 | 4.8 | 4.8 | 4.4 |
| | SA (500µM) | 11.8 | 11.9 | 12.6 | 12.3 | 11.7 | 11.4 | 11.0 | 11.8 |
| | JA(500 µM) | 10.7 | 11.2 | 11.9 | 11.8 | 11.3 | 10.9 | 10.6 | 11.2 |
| | BABA(50mM) | 10.2 | 10.6 | 11.0 | 10.7 | 10.1 | 9.8 | 9.4 | 10.2 |
| | BTH (500µM) | 9.9 | 10.1 | 10.7 | 10.3 | 9.8 | 9.6 | 9.4 | 10.0 |
| CD (5%) | Variety (A) -0.025; Elicitor (B) - 0.033; Time interval (C) -0.041 ; AB - 0.057; AC -0.067; BC - 0.087; ABC - 0.15 | | | | | | | | |

Each value is mean of values of protein concentration at different time interval (1 -7 days) for each treatment of respective elicitors.

content in leaves, whereas JA resulted in 156 %, BABA gave 132% and BTH gave 119% increase in protein concentration w.r.t. control. Minimum protein varied from 4.1 to 5.0 mg/gFW for control treatment between all the respective time intervals with mean value of 4.6 mg/gFW. The total protein activity increased up to 3rd day and thereafter registered decline irrespective of variety and elicitor treatment. In general, 500 µM SA resulted in higher mean protein activity followed by JA, followed by BABA and then BTH. Kajri showed better protein induction followed by Punjab Hybrid and Hara Madhu.

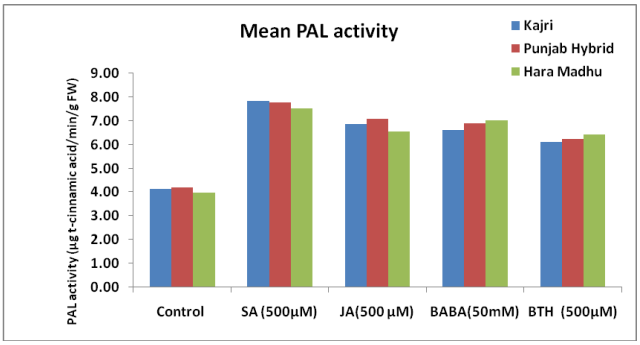
**β-1, 3 glucanase (Pr-protein) Activity:** Mean maximum β-1, 3 glucanase activity was observed at 500 µM of SA in Kajri i.e. 0.56 µg glucose released/min/gFW followed by 0.52 µg glucose released/min/gFW in Punjab Hybrid and 0.50 µg glucose released/min/gFW in Hara Madhu. JA at 500 µM proved to be second best treatment with mean maximum β-1, 3 glucanase activity observed in Kajri i.e. 0.49 µg glucose released/min/gFW followed by 0.47 and 0.49 µg glucose released/min/gFW in Punjab Hybrid and Hara Madhu, respectively. BTH at 500 µM gave mean maximum β-1, 3 glucanase activity of 0.44 µg glucose released/min/gFW in both, Hara Madhu and Kajri, whereas 0.46 µg glucose released/min/gFW in Punjab Hybrid. BABA gave mean maximum β-1, 3-glucanase activity of 0.48 and 0.47 µg glucose released/min/gFW at 50mM of concentration in Kajri and Punjab Hybrid and 0.46 µg glucose released/min/gFW in, Hara Madhu, respectively (**Fig 1.).** The total β-1, 3 glucanase activity increased upto 4th 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. In general, 500 µM SA resulted in higher mean β-1, 3-glucanase activity followed by JA followed by BABA and then BTH. Kajri showed better β-1, 3-glucanase activity followed by Punjab Hybrid and Hara Madhu. BABA and BTH treatments were at par w.r.t. β-1, 3-glucanase activity in all the three varieties.

**Phenylalanine ammonia lyase (PAL) Activity:** Mean maximum PAL activity was observed at 500 µM of SA in Kajri i.e. 7.84 µg t-cinnamic acid/min/g FW followed by 7.77 µg t-cinnamic acid/min/g FW in Punjab Hybrid and 7.52 µg t-cinnamic acid/min/g FW in Hara Madhu. JA at 500 µM




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 muskmelon varieties.




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

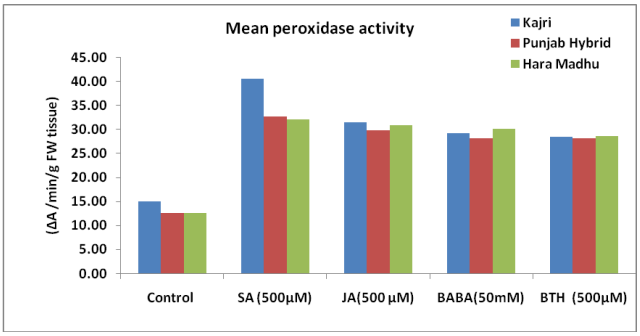
**Fig. 2**: Effect of foliar spray with different elicitors i.e. SA, JA, BABA and BTH on PAL activity (µg t-cinnamic acid/min/g FW) in muskmelon leaves.

proved to be second best treatment with mean maximum PAL activity observed in Kajri i.e. 6.85 µg t-cinnamic acid/min/g FW, and 7.08 and 6.56 µg t-cinnamic acid/min/g FW in Punjab Hybrid and Hara Madhu. Similar was the pattern with BTH and BABA (**Fig 2.**). Minimum PAL activity varied from 3.87 to 4.47µg t-cinnamic acid/min/g FW for control treatment between all the respective time intervals with mean value of 4.14 µg t-cinnamic acid/min/g FW. In general, 500 µM SA resulted in higher mean PAL activity followed by JA followed by BABA and then BTH. Kajri showed better PAL activity followed by Punjab Hybrid and Hara Madhu. BABA gave good results than JA and BTH in Hara Madhu and it proved to be second best elicitor for PAL activity particularly in this variety. Our findings of field trials showed that elicitors help in eliciting the activity of PAL as compared to control.

**Peroxidase (PR-protein) Activity:** Amongst the different elicitors treatment in Punjab Hybrid; SA caused 159% increase in Peroxidase activity in leaves, whereas JA resulted in 137%, BABA and BTH gave 123% increase in peroxidase activity w.r.t. control. Similarly, in Kajri SA caused 144% increase in Peroxidase activity in leaves, whereas JA resulted in 110%, BABA gave 95% and BTH gave 89% increase in peroxidase activity w.r.t. control. In Hara Madhu SA caused 154% increase in peroxidase activity in leaves, whereas JA resulted in 143%, BABA gave 139% and BTH gave 127% increased 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 Kajri at 36.56 ΔA/min/g FW (**Fig. 3**).

Minimum peroxidase activity varied from 11.37 to 18.37 A /min/g FW for control treatment between all the respective time intervals with mean value of 14.96 ΔA /min/g FW. The total Peroxidase activity increased up to 3rd day in all the four elicitors and thereafter registered decline irrespective of variety and elicitor treatment.

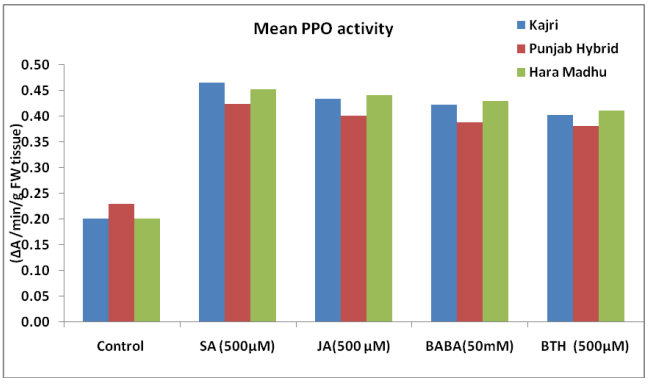
**Polyphenol oxidase (PPO):** Mean maximum PPO activity was observed at 500 µM of SA in Kajri i.e. 0.47 ΔA /min/g FW followed by 0.42 ΔA /min/g FW in Punjab Hybrid and 0.45 ΔA /min/g FW in Hara Madhu. JA at 500 µM proved to be second best treatment with mean maximum PPO activity




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 (: A /min/g FW) in leaves of muskmelon varieties.

observed in Kajri i.e. 0.43 ΔA /min/g FW, and 0.40 and 0.44 ΔA /min/g FW in Punjab Hybrid and Hara Madhu. BTH at 500 µM gave mean maximum PPO activity of 0.38 and 0.40 ΔA /min/g FW in Punjab Hybrid, and Kajri, whereas 0.41 ΔA /min/g FW in Hara Madhu. BABA gave mean maximum PPO activity of 0.42 and 0.39 ΔA /min/g FW at 50mM of concentration in Kajri and Punjab Hybrid and 0.43 ΔA /min/g FW in Hara Madhu, respectively (**Fig. 4**). Statistically significant difference for all four elicitors w.r.t. time interval was observed. SA was found to induce mean maximum PPO activity 0.65 ΔA /min/g FW at 4$^{th}$ day interval, respectively followed by JA showing maximum PPO activity 0.61 ΔA /min/g FW at 4$^{th}$ day interval. BABA and BTH showed maximum PPO activity 0.60 and 0.58 ΔA /min/g FW at 4$^{th}$ day interval in Kajri. Whereas minimum PPO activity varied from 0.18 to 0.23 ΔA /min/g FW for control treatment between all the respective time intervals with mean value of 0.20 ΔA /min/g FW . Similar pattern in result was observed in other two varieties as well. The total PPO activity increased upto 4$^{th}$ day in all four elicitors and thereafter registered decline irrespective of variety and elicitor treatment. In general, 500 µM SA resulted in higher mean PPO activity followed by JA followed by BABA and then BTH. Kajri showed better PPO activity followed by Hara Madhu and Punjab Hybrid.




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

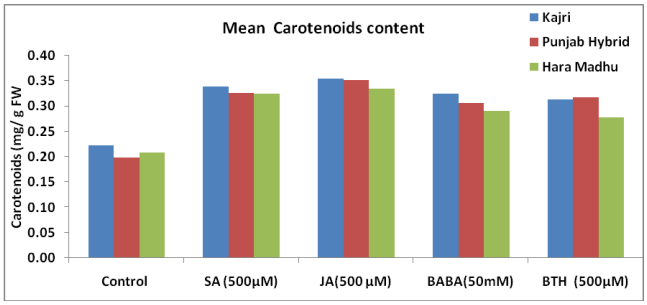
**Fig. 4**: Effect of foliar spray with different elicitors i.e. SA, JA, BABA and BTH on PPO activity (: A/min/g FW) in leaves of different muskmelon varieties

**Total chlorophyll:** Amongst the different elicitors treatment in Kajri; SA caused 13% increase in total chlorophyll (mg/ g FW) in leaves, whereas JA showed no increase in chlorophyll , BABA gave 11% and BTH gave 8% increase in total chlorophyll (mg/ g FW) w.r.t. control. Similarly, in Punjab Hybrid; SA caused 5% increase in total chlorophyll (mg/ g FW) in leaves, whereas JA resulted in 3% decrease, BABA gave 4% and BTH gave 3% increase in total chlorophyll (mg/ g FW) w.r.t. control. In Hara Madhu; SA treatment resulted in 11% increase in total chlorophyll (mg/ g FW) in leaves, whereas JA resulted in decrease of 1%, BABA gave 10 % and BTH gave 9% 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 Kajri. 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. Peak value of total chlorophylls was recorded on 5$^{th}$ day of elicitors treatment (**Table 2**).

**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 muskmelon namely; Punjab Hybrid, Kajri, Hara Madhu (**Fig. 5**). The elicitors were sprayed after 21 days of planting. Amongst the different elicitors treatment in Kajri; SA caused 54% increase

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

| Total chlorophyll (mg/gFW) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Variety | Treatment | Days After Spray | | | | | | | Mean |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Kajri | Control | 1.62 | 1.64 | 1.65 | 1.65 | 1.65 | 1.66 | 1.66 | 1.65 |
| | SA (500µM) | 1.80 | 1.82 | 1.86 | 1.94 | 1.90 | 1.84 | 1.84 | 1.86 |
| | JA(500 µM) | 1.59 | 1.61 | 1.67 | 1.69 | 1.69 | 1.67 | 1.66 | 1.65 |
| | BABA(50mM) | 1.78 | 1.80 | 1.84 | 1.92 | 1.88 | 1.82 | 1.82 | 1.83 |
| | BTH (500µM) | 1.73 | 1.75 | 1.79 | 1.87 | 1.83 | 1.77 | 1.77 | 1.79 |
| Punjab Hybrid | control | 1.61 | 1.63 | 1.64 | 1.65 | 1.67 | 1.67 | 1.69 | 1.65 |
| | SA (500µM) | 1.70 | 1.72 | 1.73 | 1.74 | 1.76 | 1.76 | 1.78 | 1.74 |
| | JA(500 µM) | 1.56 | 1.58 | 1.59 | 1.60 | 1.62 | 1.62 | 1.64 | 1.60 |
| | BABA(50mM) | 1.68 | 1.70 | 1.71 | 1.72 | 1.74 | 1.74 | 1.75 | 1.72 |
| | BTH (500µM) | 1.66 | 1.68 | 1.69 | 1.70 | 1.72 | 1.72 | 1.73 | 1.70 |
| Hara Madhu | Control | 1.63 | 1.65 | 1.65 | 1.65 | 1.66 | 1.66 | 1.67 | 1.65 |
| | SA (500µM) | 1.78 | 1.80 | 1.84 | 1.92 | 1.88 | 1.82 | 1.82 | 1.83 |
| | JA(500 µM) | 1.59 | 1.58 | 1.70 | 1.67 | 1.66 | 1.63 | 1.61 | 1.63 |
| | BABA(50mM) | 1.75 | 1.77 | 1.82 | 1.89 | 1.86 | 1.80 | 1.79 | 1.81 |
| | BTH (500µM) | 1.74 | 1.76 | 1.80 | 1.88 | 1.84 | 1.78 | 1.78 | 1.80 |
| CD (5%) | Variety (A) -0.0031; Elicitor (B) - 0.0040; Time interval (C) -0.0048 ; AB- 0.0070; AC-0.0083; BC-0.010; ABC- 0.018 | | | | | | | | |




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/ g FW) in leaves of different muskmelon varieties

in carotenoids (mg/ g FW) in leaves, whereas JA resulted in 59% increase, BABA gave 45% and BTH gave 40% increase in carotenoids (mg/ g FW) w.r.t. control. Similarly, in Punjab Hybrid; SA treatment resulted in 65% increase in carotenoids (mg/ g FW) in leaves, whereas JA resulted in 75% increase, BABA gave 55% and BTH gave 60% increase in carotenoids (mg/ g FW) w.r.t. control. In Hara Madhu; SA and JA both resulted in 57% increase in carotenoids (mg/ g FW) in leaves, whereas in BABA treated plants 38% and in BTH application 33% increase in carotenoids (mg/ g FW) w.r.t. control was recorded indicating that JA is better inducer of carotenoids among all the four elicitors with maximum content in Punjab Hybrid followed by Kajri and Hara Madhu. It was observed that the carotenoids content showed peak on 4$^{th}$ day of treatment in all the tested elicitors.

**SDS-PAGE protein extract of different muskmelon varieties:** The muskmelon 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. β-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 with some other proteins. Thus indicating induction of PR-protein which induced defense

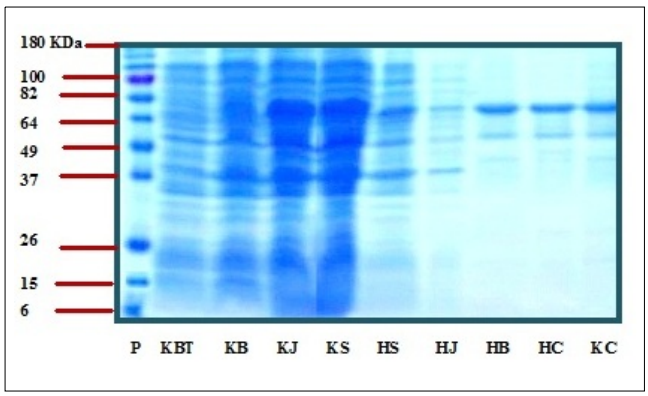



**Plate 1:** SDS- PAGE of leaf proteins of different muskmelon varieties at 500 µM of SA, 500 µM of JA, 500 µM of BTH and 50mM for BABA

against downy mildew of muskmelon caused by *P. cubensis.*

P- Protein Ladder, KS: Kajri treated with SA, KJ: Kajri treated with JA, KB: Kajri treated with BABA, KBT: Kajri treated with BION, HS: Hara Madhu treated with SA, HJ: Hara Madhu treated with JA, HB:Hara Madhu treated with BABA, HC: Hara Madhu control, KC- Kajri control

**Data on disease development:** SAR elicitors treated muskmelon plants show significantly reduced lesion size as compared to control (**Table 3** ). Five days after inoculation control plants produced lesions with average diameter of 15-16 mm whereas treated plants produced 0-4.5 mm-sized

**Table 3.** Effect of different elicitors on lesion development of *P. cubensis* on first Leaf position from stem base of different muskmelon varieties

| Variety | Treatment | 5$^{th}$ day after inoculation | | 7$^{th}$ day from inoculation | |
|---|---|---|---|---|---|
| | | Mean lesion diameter (mm) | Per cent protection | Mean lesion diameter (mm) | Per cent protection |
| Kajri | **Control** | 15.0 | 0.0 | 25.1 | 0.0 |
| | **SA** | 0.0 | 100.0 | 2.1 | 91.6 |
| | **JA** | 1.4 | 93.3 | 3.2 | 87.2 |
| | **BABA** | 2.3 | 86.6 | 5.4 | 78.4 |
| | **BTH** | 2.2 | 85.3 | 5.6 | 77.6 |
| | **Fungicide** | 0.0 | 100 | 3.2 | 87.2 |
| Punjab Hybrid | **Control** | 16.0 | 0.0 | 26.2 | 0.0 |
| | **SA** | 0.0 | 100.0 | 1.6 | 93.8 |
| | **JA** | 2.3 | 85.6 | 5.1 | 80.3 |
| | **BABA** | 3.5 | 78.1 | 5.6 | 78.4 |
| | **BTH** | 3.5 | 78.1 | 6.2 | 76.1 |
| | **Fungicide** | 0.0 | 100 | 1.8 | 93.1 |
| Hara Madhu | **Control** | 16.0 | 0.0 | 28.2 | 0.0 |
| | **SA** | 0.5 | 96.8 | 2.6 | 90.5 |
| | **JA** | 2.3 | 85.6 | 5.2 | 81.4 |
| | **BABA** | 3.3 | 79.3 | 6.2 | 77.8 |
| | **BTH** | 4.5 | 71.8 | 7.4 | 73.5 |
| | **Fungicide** | 0.0 | 100 | 2.8 | 90.1 |
| CD (5%) | Variety (A) -0.27; Elicitor (B) - 0.35; Time interval (C) -0.22 ; AB- 0.61; AC-0.38; BC-0.49; ABC- 0.865 | | | | |

lesions. After 7 days of challenge inoculations, control plants produced lesions with average diameter of 25.1-28.2 mm whereas treated plants produced 2.1-7.4 mm sized lesions. Per cent protection was maximum in case of SA treated plants, which comes out to be 91.6, 90.5 and 93.8 per cent for Kajri, Punjab Hybrid and Hara Madhu, respectively. Data given is the mean of three replications.

The results clearly depicted the effectiveness of the elicitor in controlling the disease. The minimum disease incidence was observed when crop was treated with salicylic acid (500µM conc) followed by jasmonic acid of 500µM conc, BABA 50 mM and then BTH @ 500µM concentration as foliar spray against downy mildew of muskmelon. The disease incidence on elicitor treated plants is significantly less compared to control. The results obtained in the present study are much in agreement with findings of, Kone *et al.* (2009) observed that SA applied via soil drench and foliar spray reduce the disease severity of *Phytophthora* blight in squash caused by *Phytophthora capsici*. Farouk *et al.* (2008) observed induction and expression of systemic resistance to downy mildew diseases in cucumber by salicylic acid. Kumar *et al* (2010) reported that minimum disease severity and incidence was observed in salicylic acid treated followed by Benzothiadiazole when compared to check in potato against Late blight.

## DISCUSSION

The study of Aldesuquy (2015) fully supports our study by demonstrating that in *vicia faba*, SA application increases the total soluble protein content against *Botrytis*. Similar, results were observed in case of SA as reported by Guleria *et al.* (2001) in peas (*Pisum sativum*) in which accumulation of PR proteins was reported by the application of SA. Ding *et al.* (2002) reported that pre-treatment of tomato fruit with MeJA induces the synthesis of some PR proteins such as Pr-2b, PR-2a, Pr-3b etc, which lead to increase in chilling tolerance and resistance to pathogens, therefore decreasing the incidence of decay. 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 treatments . In tomato seedlings treated with *Trichoderma asperellum* high activity of different defence related enzymes like Peroxidase (POD), Polyphenol

oxidase (PPO), phenylalanine ammonium lyase (PAL), and β-1, 3-glucanase have been reported resulting in more resistance against *Ralstonia solanacearum* as compared to the tomato seedlings in which low activity of these defense related enzymes was observed (Murthy *et al.*, 2013). Kim and Hwang (2014) also observed high activity of PAL enzyme in pepper plants showing resistance to the infection of *Xanthomonas campestris* pv. *vesicatoria.* These results are in conformity with the observations of Nawar and Kuti (2003) who indicated a positive relation between resistance and peroxidase activity. Peroxidase also produces free radicals and hydrogen peroxidase which are toxic to many microorganisms. 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).

When elicitors were tested, significant increase in the total chlorophyll pigments was observed except in JA. The present study revealed that the amount of carotenoids was further enhanced when treated with elicitors with maximum increase in case of JA application. This increase may be attributed to protection of plant cells from Reactive oxygen species and other oxidation factors. Cag *et al.* (2009) documented increase in chlorophyll, carotenoids, protein contents and PO activity in excised cotyledons of sunflower seedlings when SA was applied exogenously. Hayat *et al*. (2005) reported significant enhancement in pigment content of wheat seedlings, raised from the grains pre-treated with lower concentration (10−5 M) of salicylic acid, whereas, higher concentrations did not prove to be beneficial. Besides seed-soaking treatment, the foliar application of SA was also found to be equally fruitful in increasing the pigment contents in *Brassica napus* (Ghai *et al.*, 2002). Similar results were obtained when the plants of *B. juncea* were sprayed with lower concentrations (10−5 M) of SA. In this treatment the chlorophyll content was significantly enhanced, whereas, higher concentrations proved inhibitory. 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). However, increase in PPO and PAL was higher in resistant variety than in susceptible cultivars after treatment with the SAR elicitor, indicating that defense realted proteins reach an inhibitory level to the fungus in the muskmelon 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. cubensis* in muskmelon.

## CONCLUSION

In the present experiment after fourth week of post disease inoculations, although some tiny lesions could be seen on the leaves, their frequency and severity never reached the levels similar to those observed in control plants in case of all the treatments. The present results indicate that elicitors, especially SA stimulated the accumulation of PR proteins and enzymes in muskmelon leaves. This effect might be due to the impact of these substances on enzymatic activity and translocation of the metabolites to muskmelon plant. In conclusion, it may be possible, in certain cases, to replace conventional chemical fungicides with any of the four elicitors especially SA due to its safety for human and environment and thus providing both economical and ecological efficacy.

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