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# Gene encoding bacterial wilt related protein as a target for stress resistant transgenic plants

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**Abstract:** To explore molecular mechanism of defense against bacterial wilt; medium resistant and resistant variety of tomato (Roma & Riogrande) were selected. Salicylic acid (SA) was deliberated for expression analysis studies of iron ATP-binding cassette (ABC) transporters, ATP synthase and chaperonin along with activities of antioxidants peroxidase and catalase. RT-PCR showed expression of Iron ABC transporters in control and within 1st day of SA treatment in both varieties, while chaperonin was expressed after two days in Roma and after three days in Riogrande of SA treatment. ATP synthase was expressed after three days in Riogrande while started from 1st day in Roma after SA treatment. Peroxidase and catalase activity increased in both varieties after one day; with little decrease after two days of SA treatment. Expression of iron ABC transporters, ATP synthase and chaperonin in response to SA application signifies importance of these genes in response to bacterial wilt in tomato.
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**Keywords:** Salicylic acid, Tomato, ATP synthase, Iron ABC transporter, Chaperonin.

**Abbreviations:** Salicylic acid (SA), Systemic acquired resistance (SAR), Abscisic acid (ABA), Gibberellic acid (GA), Ethylene (ET), Jasmonic acid (JA), Pathogenesis related (PR), Catalase (CAT), Peroxidase (POD), ATP-binding cassette transporters (ABC transporters).

## Introduction

Tomato (*Solanum lycopersicum)* is second world most significant vegetable crop, and for last 25 years total world production and consumption of this important crop has raised moderately. Small genome, short life cycle similar to many commercially vital plants (potato, eggplant, peppers and tobacco), made it important as studies conducted on tomatoes are equally valid to these plants (Peterson et al., 1988; Kimura and Sinha 2008). It is challenged by various biotic stresses and abiotic stresses; became chief reason for tomato loss worldwide (Kissoudis et al., 2015). Tomato respond to biotic/abiotic stresses by multiple defense mechanisms including cellular reactions generated by the pathogens leads to signaling pathways (Pangesti et al., 2013; Schenk et al., 2000). Plant immune systems dependent on resistant/susceptible variety and their defense mechanisms (Da Cunha et al., 2006).

Main objective of studying plant pathogen interactions is to reveal the signaling molecules that leads to defense related pathways (Abu Qamar et al., 2009). Signaling molecules/hormones including auxins, cytokinins, salicylic acid (SA), abscisic acid (ABA), gibberellic acid (GA), ethylene (ET), jasmonic acid (JA), secreted by plants in response to environmental stresses have important functions in production of protein related to defense, growth and development (Clarke et al., 2000; Kunkel and

Brooks 2002). SA has an important role in plant defense against biotic and abiotic stresses, along with regulation of various physiological and biochemical processes (Plant growth, photosynthesis, respiration, thermotolerance, stomatal responses, nodulation, nitrate metabolism, flowering), and ET biosynthesis (Wu et al., 2015a; An and Mou, 2011; Zandalinas et al., 2016). Increased SA in plant cells deliberately/naturally is also coupled with augmentation of pathogenesis related proteins (PR) proteins along with defense and metabolism related proteins resulted in systemic acquired resistance (SAR) (Qi et al., 2012; Dong et al., 2011; Afroz et al., 2010). It had been reported for its critical role in defense aided with organization of SAR after biotrophic and hemi-biotrophic pathogens infections specifically in model plants (Yasuda et al., 2008; Chen et al., 1995). SAR is a potent form of innate immunity which is efficient and a long-term resistance effect against a wide variety of pathogens (Vlot et al., 2009). SA had been also reported to produce oxidative stress in plants at high concentration but at low concentrations enhanced antioxidative capability help to cope with stresses (Horvath et al., 2007). Antioxidant enzymes such as polyphenol oxidase, peroxidase (POD), catalase (CAT) were reported to increase 4 days after SA treatment (War et al., 2011). Antioxidant activity after SA treatment can be a measure of resistance or susceptibility of the varieties.

Taking into account the mechanisms which plants employ to defend themselves against pathogens may enable the researchers to design novel strategies to improve disease resistance in crop plants. ATP synthase, iron ATP-binding cassette (ABC) transporters and chaperonin related to protein destination, storage synthesis and photosynthesis expression were some of the important proteins expressed in plants resistant to biotic stress in response to SA treatment (Kohzuma et al., 2013; Eichhorn et al., 2006; Eichhorn et al., 2006). Previously SA induces proteins were explored in susceptible and resistant varieties of tomato (Roma and Pant Bahr) (Afroz et al., 2010). In current study ATP synthase, iron ABC transporters and chaperonin were confirmed using RTPCR analysis in response to SA in Riogrande (resistant) and Roma (medium resistant) to bacterial wilt. Along with these protein encoding genes, antioxidant enzymatic assays (POD, CAT) were carried out after one, two and three days of SA treatment.

## MATERIALS AND METHODS

### Plant Material

Tomato (*Solanum lycopersicum*) Riogrande (resistant) and Roma (medium resistant) cultivars were used. The seeds of Riogrande and Roma were obtained from National Agriculture Research Centre (NARC), Islamabad. Seeds were surface sterilized in 0.8 % (v/v) "Clorox" bleach (Sodium hypochlorite) as reported (Afroz et al., 2010). Seedlings were planted in pots containing sand and soil, grown in growth chamber under white fluorescent light (600 mol $m^{-2}$ $s^{-1}$; 16 h light/8 h dark) at 25ºC and 70% relative humidity.

### SA Treatment, RNA Isolation and cDNA Synthesis

SA (Wako) was used at 1 mM concentration prepared in water. Three-week-old seedlings of the Roma and Riogrande were treated with SA for 1, 2 and 3 days and RNA extraction was done for 3 consecutive days. Total RNA was isolated from leaf cells of controlled and treated tomato plants using the TRI Reagent (Cat. No. TRI. 118; Lot No. 4221) (Simms et al., 1993). RNA was precipitated by mixing it with isopropanol, centrifuged (12000 g) for 8 min at 4-25º C. RNA pellet was washed with 75% ethanol, centrifuged, dissolved in nano-pure water and stored. cDNA was prepared from total RNA samples using cDNA synthesis kit (Invitrogen, USA).

### RT-PCR Analysis

Accession number of five proteins (given in Table 1) were analyzed using bioinformatics tools, and relatively suitable length of aligned region of PCR primers were designed using Primer3 online software. Forward and reverse primers were designed for Iron ABC transporter, ATP synthase and 60-KDa chaperonin. cDNA was amplified with gene-specific primers using PCR. Confirmation of the desirable sequences in extracted cDNA from all species will be done by running PCR reaction with five set of primers under specific conditions. Primers used for ATP synthase F (5'TGACCTTAAATCTTAGTGTACTGACC3') and R (5'TTATGAAATCGGATTGATAGCC3'), Chaperonin were F (5'GAGATTGTGTTCGACCAGGAG3') and R (5'ACACGCCATCGTTGATAACC3'), iron ABC transporter were (F-5' ATGAAAAAAATTTTAATCATTATGAGTTTA 3') and R (5'TTAAAAATTTTGATTTTCTAATCTTGTTT T3').

20 µl PCR reaction mixtures contains 1.5 of the template cDNA, with 2 µl Taq buffer, 1 µl $MgCl_2$, 0.5 µl dNTP mixture, 0.5 µl of each forward and reverse primer, 1 µl Taq polymerase and 13 µl of nanopure water. Initial denaturation step for PCR is restricted to (95ºC) for 4 minutes, followed by 40

cycles of denaturation step at (95°C) for 30 seconds, annealing temperature (55°C for ATP synthase, 53°C 60-kDa chaperonin and iron ABC transporter) for 1.5 minutes and extension (72°C) for 30 sec, and a final extension cycle of 7 min (72°C). The amplified PCR products were analyzed by 1 % (w/v) agarose gel electrophoresis.

**Enzymatic Assays**

**POD activity**

POD activity was determined following the dehydrogenation of guaiacol as a substrate (Malik and Singh 1980). 2g of plant material is grinded in pre-chilled mortar with 3 ml of 0.1 M phosphate buffer (pH 7). Homogenate is centrifuged at 18000 g for 15 min at 5°C. Supernatant is used as enzyme source within 2-4 h. 3 ml of the buffer solution is pipetted along with 0.05 ml guaiacol solution (20 mM), 0.1 ml enzyme extract and 0.03 ml hydrogen peroxide solution (12.3 mM) in cuvette. Mixture absorbance was taken on spectrophotometer at 750 nm.

**CAT Assay**

CAT was assayed by monitoring the decrease of absorbance as described by Aebi (1984). The reaction mixture consisted of 50 µL of enzyme, 2.5 mL potassium phosphate buffer (50 mM; pH 7.6), 200 uL hydrogen peroxide (12.5 mM) and homogenate sample (50 µl), along with 250 µL of distilled water were taken in a cuvette. Absorbance was measured at 240 nm three times for each sample with a time interval of 30 seconds. One unit activity of CAT is described as an absorbance change of 0.01 as unit per minute.

## RESULTS

Bacterial wilt is one of the important bacterial stresses faced by tomato, restraining its production (Hayward, 1991). For the confirmation of genes expressed in resistant and susceptible varieties of tomato RTPCR is used (Park et al., 2015). Primarily RNA extraction was done using 3-W-Old seedlings leaves, followed by cDNA synthesis. It was followed by RTPCR for Iron ABC transporter, ATP synthase and chaperonin 60 in 2 varieties of tomato.

**Morphological variation**

Morphological variation was observed in Roma and Riogrande leaves 1-3 days after SA application. Leaves became light green after 1, 2 and 3 days of SA treatment in comparison to control in Roma (Figure 1A, 1B, 1C, 1D). Riogrande leaves almost remain same as control (Figure 1E, 1F, 1G, 1H).

**Enzymatic Assays**

POD and CAT activity was increased one day; decreased after 2 days and again increased after 3 days of SA treatment in Riogrande and Roma (Figure 2A; 2B). Enzymatic activity of both PD and CAT was more prominent in Riogrande than Roma (Figure 2A; 2B).

**Iron ABC transporter**

SA enhanced Iron ABC transporter expression in Roma and in Riogrande after 1, 2 and 3 days of SA treatment (Figure 3A). Iron ABC transporter, was also upregulated in the proteomics analysis of tomato cvs and soybean in response to application of either JA or SA (Afroz et al., 2010; Eichhorn et al., 2006). RTPCR also confirmed expression of Iron ABC transporter in Riogrande and Roma (resistant and medium resistant) cultivars of tomato after application of SA.

**ATP Synthase**

ATP synthase was found to be expressed within one day of SA treatment in Roma along with control, while in Riogrande it is expressed in control and 3 days after SA treatment (Figure 3B). Proteomic approach show reduced expression of ATP synthase with 1, 2 and 3 days of SA treatment in Roma (Afroz et al., 2010). While RT PCR showed its increased expression after one day of SA treatment.

**Chaperonin**

Chaperonin was expressed in control and 2 days after SA treatment in Roma (Figure 4A) and after 3 days of SA treatment in Riogrande (Figure 4B). Proteomic approach show enhanced expression of Chaperonin in susceptible variety (Pant Bahr) and unchanged expression in medium resistant variety (Roma) after SA treatment (Afroz et al., 2010). While RTPCR analysis show enhanced expression of chaperonin after two days in Roma and after three days in Riogrande of SA treatment.

## DISCUSSION

Bacterial wilt is one of the important bacterial stresses faced by tomato, restraining its production (Hayward, 1991). Different genes had been identified which are expressed in tomato varieties resistant and susceptible to bacterial wilt (Glick et al., 2009; Afroz et al., 2009). SA mediated defense pathway play a key role in response to biotic stresses (Novakova et al., 2014). RTPCR is widely used for confirmation of gene expression followed by proteomics/transcriptomic analysis (Park et al., 2015; Wu et al., 2013). Here also it was done for confirmation of Iron ABC transporter, ATP synthase and chaperonin 60 in 2 varieties of tomato. Anti-oxidant enzymes CAT and POD help to scavenge the stress damage were confirmed in these two varieties primarily. POD and CAT increased basically with decrease just after 2 days of SA treatment in both varieties (Figure 2A; 2B).

Antioxidant activity was more prominent in resistant variety suspects its role to reduce the ROS damage. Higher induction of antioxidant enzymes (POD, PPO) along with generation of $H_2O_2$ were reported in response to SA (1, 1.5, 2 mM) in *Cicer arietinum L.* (War et al., 2011). Antioxidative enzymes POD, thioredoxin, superoxide dismutase, glutathione S-transferase were reported in tomato after SA treatment to protect plant from damage (Wan et al., 2015; Singh et al., 2015). $H_2O_2$ in plants is generated during photorespiration, mitochondrial electron transport and β-oxidation of fatty acids, and its intracellular level are stringently controlled for maintaining homeostasis in the cell (McClung, 1997). Immune response is suppressed by inhibition of host cell's POD activity, thereby inhibiting ROS production (Liu et al., 2015). CAT degrades $H_2O_2$ in to water and is one of the critical antioxidant enzymes that protect the cells against reactive oxygen species induced damage under stressful conditions (Virdi et al., 2015). Contrarily SA had been reported to inhibit CAT and POD in *Arabidopsis* (Manohar et al., 2015).

Leaves became light green after 1, 2 and 3 days of SA treatment in comparison to control in Roma, while remain same as control in Riogrande. Reduction in chlorophyll in semi-resistant variety (Roma) show its more sensitiveness to water stress, as the leaves were treated with aqueous solutions of SA in comparison to control. It is in line with observation for Roma (Afroz et al. 2010). Iron ABC transporter was found to be expressed within one day of SA treatment in both Roma and Riogrande (Figure 3A). It is in line with its upregulation after one day of SA treatment in both Roma and Pant Bahr (Afroz et al., 2010). RTPCR confirmed proteomics data of its expression in all resistant, susceptible and medium resistant cultivars. Iron ABC transporter had a role in stress resistance in plants, and induced in response to bacteria, fungus, heavy metal stress, ET, SA, JA, ABA, resistance to HR, iron homeostasis, cell viability and iron acquisition in plants (Amaral et al., 2007; Song et al., 2010; Singh et al., 2015; Kobae et al., 2006; Borghi et al., 2015). Iron ABC transporter, photosynthesis related gens, defense genes, antioxidant enzymes were induced in response to SA treatment can be used as genetic tools to investigate disease resistance (Zhang et al., 2016; Li et al., 2014). ABC transporter genes reduction indirectly resulted in reduced photosynthesis, and production of stress in apple (Wu et al., 2015b). Iron ABC transporter is also induced in JA dependent pathway in response to infection by bacterial and fungal infections in tobacco (Stukkens et al., 2005). It expression in response to JA, SA, bacterial and fungal pathogens; made it favorite gene responsible for susceptibility or resistance of tomato cultivars Roma and Riogrande.

ATP synthase was found to be expressed within one day in Roma along with control, while in Riogrande it is expressed in control and 3 days after SA treatment (Figure 3B). It is line with observation that in susceptible cultivars ATP synthase is up-regulated and in resistant cultivar it is expressed after 2 days of SA treatment (Kohzuma et al., 2013). Wu et al. (2015 a) via gel free techniques (LC MS/MS) reported phosphoproteins such as ATP synthase and iron ABC transporters are upregulated by SA in maize. ATP synthase is expressed in response to biotic/abiotic stresses for production of resistance in plants by suppressing HR response, along with increased metabolism of glucose (Sugimoto et al., 2004; Santos, 2006). Janda et al. (2012) reported regulatory effects of SA on photosynthetic electron transport processes. Chloroplastic-ATP synthase was found to be important to trigger ET, SA/JA pathway in response to insect herbivory (Schmelz et al., 2007). Transgenic tobacco with ATPase exhibited resistance in response to *P. syringae*, suggesting its role in defense via JA or SA (Lee and Sano, 2007). Upregulation of ATP synthase after 1st day of treatment in susceptible (Pant Bahr) and medium resistant varieties of tomato and after 2 days in resistant variety of tomato implied its importance as defense gene in response to biotic stresses, insect herbivory, and suppression of HR made it a good candidate transgenic tomato with enhanced resistance to bacterial wilt.

Chaperonin was expressed in control and 2 days after SA treatment in Roma and after 3 days of SA treatment in Riogrande (Figure 4A; 4B). Proteomic approach show enhanced expression of Chaperonin in susceptible variety (Pant Bahr) and unchanged expression in medium resistant variety (Roma) after SA treatment (Afroz et al., 2010). So RTPCR show enhanced expression of chaperonin in Roma. Wu et al. (2013) reported that after inoculation with *P. solanacearum*; ATP synthase was expressed in susceptible and chaperonin was expressed in resistant maize cultivar in SA pathway. Moshe et al. (2012) reported up regulation of chaperonin in resistant tomato cultivar in response to tomato leaf curl virus infection in comparison to susceptible cultivar. Proteomics analysis of *Arabidopsis* associate chaperonin-60 with photosynthesis for increasing duration of stress (Salvucci, 2008). Cueto-Ginzo et al. (2016) also reported SA balance photosynthetic reduction during infection of potato virus in tomato by increasing mesophyll conductance. Molecular Chaperone is another

candidate found to be important for resistance in resistant cultivars in response to stresses in comparison to accumulation of ROS species in susceptible cultivars. In this study Chaperone was expressed in both resistant, medium resistant and in susceptible cultivars previously. But expression of chaperone was more prominent in resistant variety.

**Conclusion**

Proteomics technique was used for identification of iron ABC transporter, ATP synthase and chaperonin in resistant and susceptible varieties of tomato (Afroz et al. 2010). Proteomics analysis data show chaperonin, ATP synthase, iron ABC transporter which were upregulated/remain unchanged in one variety in comparison to other in response to application of SA are candidates proteins that can be responsible for susceptibility or resistance of tomato cultivars. RTPCR show iron ABC transporter expression remain same in resistant and medium resistant cultivars. While chaperonin expression was important; as expressed strongly after 3 days of SA application in resistant cultivars also supported by previous reports. Similarly ATP synthase expression was after 2 days in Riogrande. CAT and POD enzymatic activity was increased in both cultivars after one and more after 3 days of SA treatment little more in Riogrande. Genes encoding proteins related to protein folding and photosynthetic genes are targets for biotic stress related pathway in tomato. These genes could be candidate for bacterial wilt resistant tomato cvs.

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Table 1. Details of the protein sequences whom coding genes primers were designed taken from Afroz et al. (2010).

| Sr. No | Protein Name/ Accession Number[a] | Functional category |
|---|---|---|
| 1 | Iron ABC transporter/gi57504871 | electron transport and photosynthesis |
| 2 | ATP synthase epsilon chain/P00834 | glycolysis pathway |
| 3 | 60 kDa chaperonin/Q42694 | Protein destination and storage |

a) Accession No. means NCBI accession number for MS analysis and Swiss-Prot accession number for protein sequencer analysis.

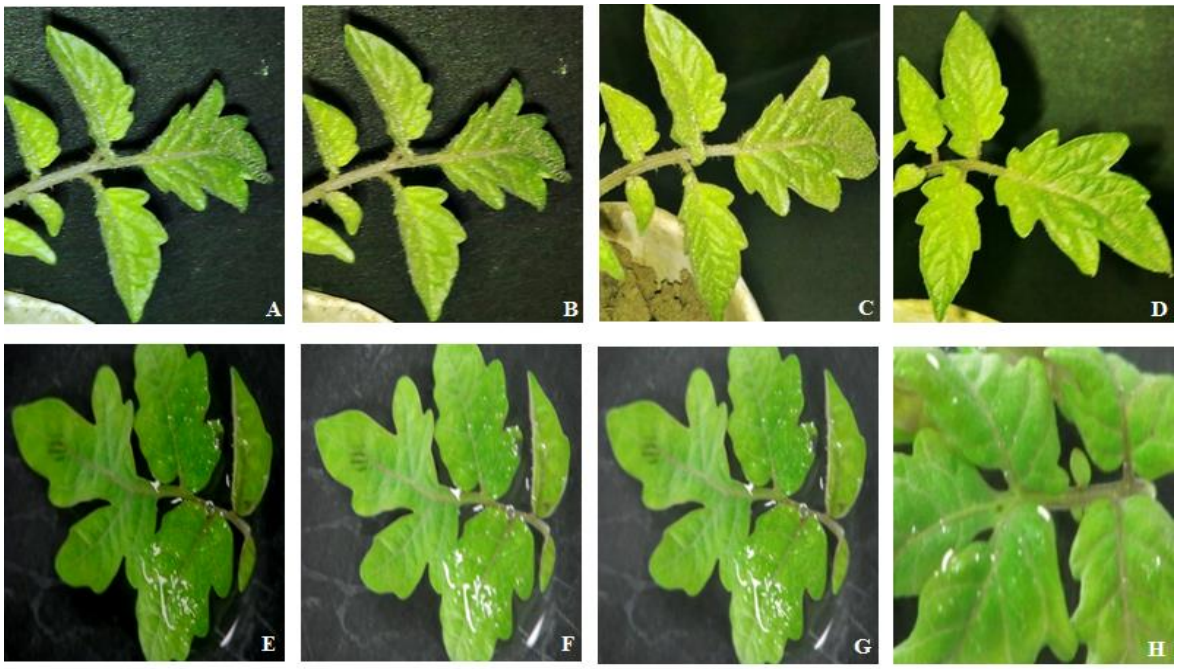


**Fig 1**

Fig 1. Morphological variation of *Solanum lycopersicum* cvs Roma (a, b, c and d) and Riogrande (e, f, g & h) in control and 1, 2 and 3 days after SA treatment.

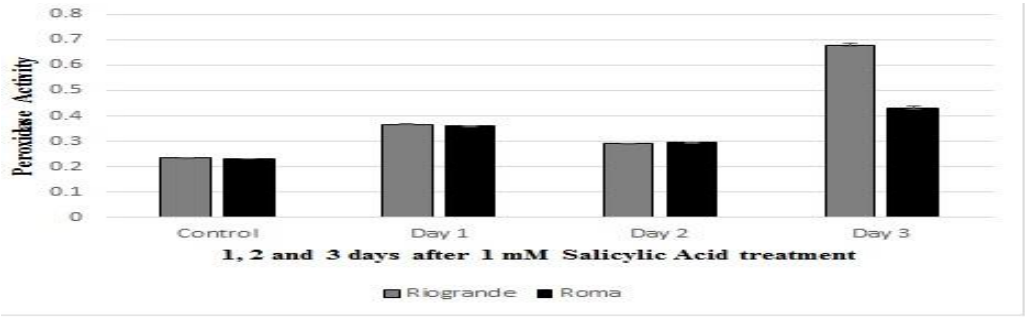


Fig 2a

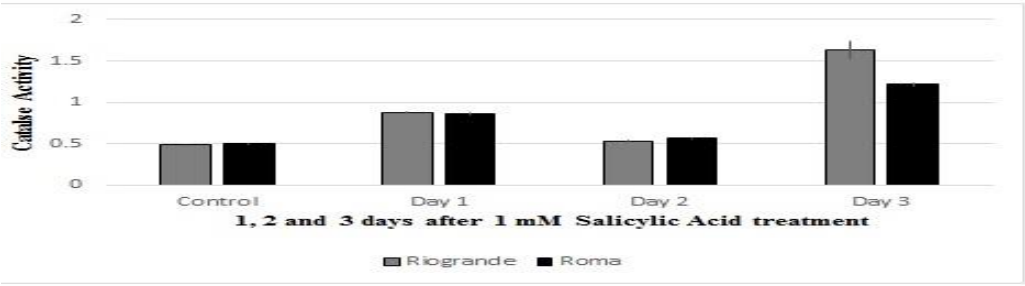


Fig 2b

Fig 2a.
Peroxidase activity in *S. lycopersicum* cvs Roma and Riogrande 1, 2 and 3 days after 1 mM SA treatment. (2b) Catalase activity in tomato cvs Roma and Riogrande 1, 2 and 3 days after 1 mM SA treatment.

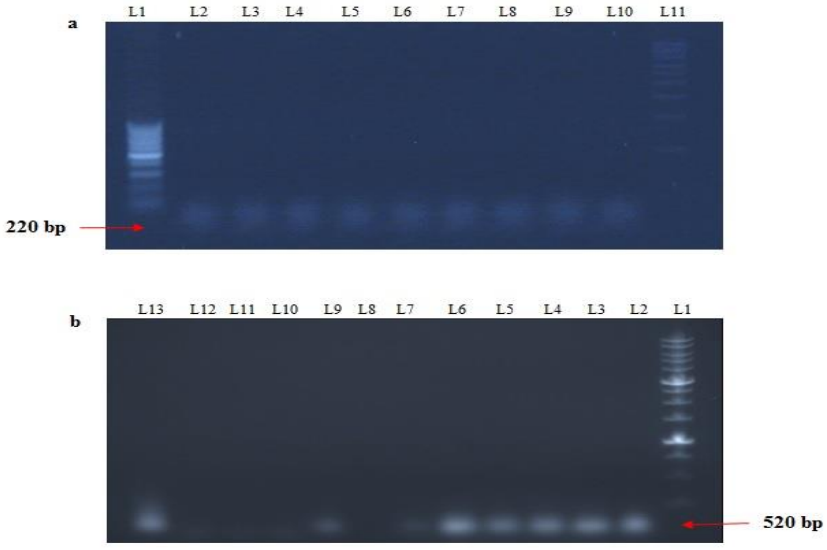


Fig 3

Fig 3. 3a. RTPCR of 3-W-Old *S. lycopersicum* leaves after 1, 2 and 3 day treatment of SA using ABC transporter primer (220 bp). L 2-5 containing PCR products of Roma control, 1, 2 & 3 days after treatment of SA. L 6-9 containing PCR products of Riogrande control, 1, 2 & 3 days after treatment of SA. L1 100 bp marker.
3b. RT PCR of 3-W-Old *S. lycopersicum* leaves after 1, 2 and 3 day treatment of SA using ATP Synthase primer (520 bp). L 1: 1 Kb ladder, L2-L5: PCR product of control, 1, 2 & 3 days after treatment of SA of Roma respectively, L6-L9 contain PCR product of control, 1, 2 & 3 days after treatment of SA of Riogrande respectively, L11- 13 contain PCR product of Riogrande 1, 2 & 3 days after SA treatment respectively as repeat.

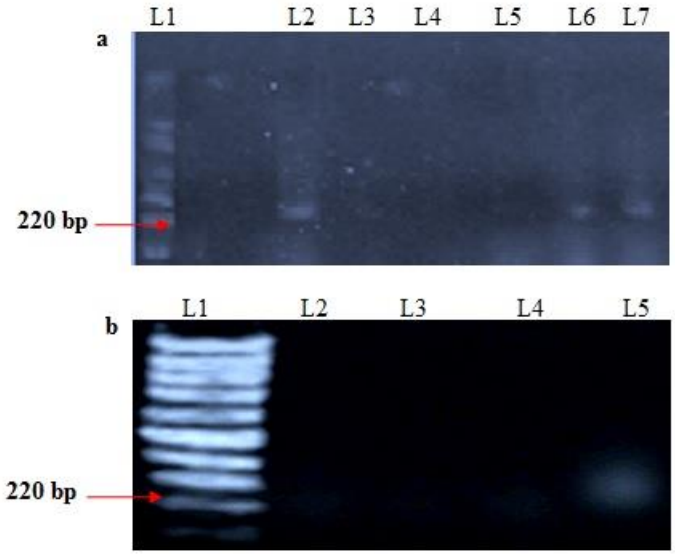


**Fig 4**

Fig 4. 4a. PCR of 3-W-Old *S. lycopersicum* leaves after 1, 2 and 3 day treatment of SA using Chaperonin primer (220 bp). L 1: 100 bp ladder, L2: PCR product of control, L5, L6, L7 1, 2 & 3 days after treatment of SA of Roma respectively. Fig 4b. PCR of 3-W-Old *S. lycopersicum* leaves after 1, 2 and 3 day treatment of SA using Chaperonin primer (220 bp). L 1: 100 bp ladder, L2: PCR product of control, L3, L4, L5 1, 2 & 3 days after treatment of SA of Riogrande respectively.

**References**

[1]. Abu Qamar S, Luo H, Laluk K, Mickelbart MV, Mengiste T. Crosstalk between biotic and abiotic stress responses in tomato is mediated by the AIM1 transcription factor. The Plant Journal 2009;58(2):347-50.

[2]. Aebi H. Catalase in vitro. Methods in Enzymology 1984;105:121-6.

[3]. Afroz A, Khan MR, Ahsan N, Komatsu S. Comparative proteomic analysis of bacterial wilt susceptible and resistant tomato cultivars. Peptides 2009;30(9):1600-7.

[4]. Afroz A, Khan MR and Komatsu S. Determination of proteins induced in response to jasmonic acid and salicylic acid in resistant and susceptible cultivars of tomato. Protein and Peptide Letters 2010;17(7):836-46.

[5]. Amaral AMD, Saito D, Formighieri EF, Rabello E, de Souza AN, Silva-Stenico ME, Tsai SM. Identification of citrus expressed sequence tags (ESTs) encoding pleiotropic drug resistance (PDR)-like proteins. Genetics and Molecular Biology 2007;30:857-65.

[6]. An C, Mou Z. Salicylic acid and its function in plant immunity. Journal of Integrative Plant Biology 2011;53:412-28.

[7]. Borghi L, Kang J, Ko D, Lee Y, Martinoia E. The role of ABCG-type ABC transporters in phytohormone transport. Biochemical Society Transactions 2015;43:924-30. doi:10.1042/BST20150106.

[8]. Chen Z, Malamy J, Henning J, Conrath U, Sanchez-Casas P, Silva H, Ricigliano J, Klessig DF. Induction, modification, and transduction of the salicylic acid signal in plant defense responses. Proceedings of the National Academy of Sciences USA 1995;92:4134-37.

[9]. Clarke JD, Volko SM, Ledford H, Ausubel FM, Dong X. Roles of salicylic acid, jasmonic acid, and ethylene in cpr-induced resistance in *Arabidopsis*. The Plant Cell 2000;12(11):2175-90.

[10]. Cueto-Ginzo AI, Serrano L, Bostock RM, Ferrio JP, Rodriguez R, Arcal L, Achon MA, Falcioni T, Luzuriaga WP, Medina V. Salicylic acid mitigates physiological and proteomic changes induced by the SPCP1 strain of Potato virus X in tomato plants. Physiological and Molecular Plant Pathology 2016;93:1e11.

[11]. Da Cunha L, McFall AJ, Mackey D. Innate immunity in plants: a continuum of layered defenses. Microbes and Infection 2006;8(5):1372-81.

[12]. Dong CJ, Wang XL, Shang QM. Salicylic acid regulates sugar metabolism that confers tolerance to salinity stress in cucumber seedlings. Scientia Horticulturae 2011;129(4):629-36.
[13]. Eichhorn H, Klinghammer M, Becht P, Tenhaken R. Isolation of a novel ABC-transporter gene from soybean induced by salicylic acid. Journal of Experimental Botany 2006;57:2193-201. doi:10.1093/jxb/erj179.
[14]. Glick E, Levy Y, Gafni Y. The viral etiology of tomato yellow leaf curl disease-a review. Plant Protection Science 2009;45(3):81-97.
[15]. Hayward AC. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annual Review of Phytopathology 1991;29:65-87.
[16]. Horvath E, Szalai G and Janda T. Induction of abiotic stress tolerance by salicylic acid signaling. The Journal of Plant Growth Regulation 2007;26(3):290-300.
[17]. Janda K, Hideg E, Szali G, Kovacs L, Janda T. Salicylic acid may indirectly influence the photosynthetic electron transport. Journal of Plant Physiology 2012;169(10):971-8.
[18]. Kimura S, Sinha N. Tomato (*Solanum lycopersicum*): a model fruit-bearing crop. Cold Spring Harb Protoc 2008;11:1-9.
[19]. Kissoudis C, Chowdhury R, van Heusden S, de Wiel CV, Finkers R, Visser RGF, Bai Y, Linden GVD. Combined biotic and abiotic stress resistance in tomato. Euphytica 2015;202(2):317-32.
[20]. Kobae Y, Sekino T, Yoshioka H, Nakagawa T, Martinoia E, Maeshima M. Loss of AtPDR8, a plasma membrane ABC transporter of *Arabidopsis thaliana*, causes hypersensitive cell death upon pathogen infection. Plant and Cell Physiology 2006;47:309-18.
[21]. Kohzuma K, Dal Bosco C, Meurer J, Kramer DM. Light-and metabolism-related regulation of the chloroplast ATP synthase has distinct mechanisms and functions. Journal of Biological Chemistry 2013;288(18):13156-63.
[22]. Kunkel BN, Brooks DM. Cross talk between signaling pathways in pathogen defence. Current Opinion in Plant Biology 2002;5:325-31. doi:10.1016/S1369-5266(02)00275-3.
[23]. Li T, Hu Y, Du X, Tang H, Shen C, Wu J. Salicylic acid alleviates the adverse effects of salt stress in *Torreya grandis* cv. Merrillii seedlings by activating photosynthesis and enhancing antioxidant systems. PLoS One 2014;9:e109492. doi: 10.1371/journal.pone.0109492.
[24]. Liu P, Zhang H, Yu B, Xiong L, Xia Y. Proteomic identification of early salicylate- and flg22-responsive redox-sensitive proteins in *Arabidopsis*. Scientific Reports 2015;5:8625. doi 10.1038/srep08625.
[25]. Malik CP, Singh MB. Extraction and estimation of amino acids and keto acids. In: Malik CP, Singh MB, editors. Plant Enzymology and Histo-Enzymology. New Delhi, Kalyani Publishers. 1980;p. 286.
[26]. McClung CR. Regulation of catalases in *Arabidopsis* Free Radical Biology and Medicine 1997;23:489-96. doi:10.1016/S0891-5849(97)00109-3
[27]. Moshe A, Pfannstiel J, Brotman Y, Kolot M, Sobol I, Czosnek H, Gorovits R. Stress responses to tomato yellow leaf curl virus (TYLCV) infection of resistant and susceptible tomato plants are different. Metabolomics 2012;S1:006. doi:10.4172/2153-0769.S1-006.
[28]. Novakova M, Sasek V, Dobrev PI, Valentova O, Burketova L. Plant hormones in defense response of *Brassica napus* to *Sclerotinia sclerotiorum*. Reassessing the role of salicylic acid in the interaction with a necrotroph. Plant Physiology and Biochemistry 2014;80:308e317.
[29]. Pangesti N, Pineda A, Pieterse CMJ, Dicke M, VanLoon JJA. Two-way plant-mediated interactions between root associated microbes and insects: from ecology to mechanisms. Frontiers in Plant Science 2013;4:414. doi: 10.3389/fpls.2013.00414.
[30]. Park J-W, Benatti TR, Marconi T, Yu Q, Solis-Gracia N, Mora V, da Silva JA. Cold responsive gene expression profiling of sugarcane and *Saccharum spontaneum* with functional analysis of a cold inducible *Saccharum* homolog of NOD26- like intrinsic protein to salt and water stress. PLoS ONE 2015;10(5):e0125810. doi:10.1371/journal.pone.0125810.
[31]. Peterson DG, Pearson WR, Stack SM. Characterization of the tomato (*Lycopersicon esculentum*) genome using *in vitro* and in situ DNA reassociation. Genome 1998;41(3):346-56.
[32]. Qi PF, Johnston A, Balcerzak M, Rocheleau H, Harris LJ, Long XY, Wei YM, Zheng YL, Ouellet T. Effect of salicylic acid on *Fusarium graminearum*, the major causal agent of fusarium head blight in wheat. Fungal Biology 2012;116(3):413-26.
[33]. Salvucci ME. Association of Rubisco activase with chaperonin-60 β: a possible mechanism for protecting photosynthesis during heat stress.

Journal of Experimental Botany 2008;59(7):1923-33. doi:10.1093/jxb/erm343.
[34]. Santos L. Molecular mechanisms of the AAA proteins in plants. Advances in Agricultural and Food Biotechnology 2006;37:1-15.
[35]. Schenk PM, Kazan K, Wilson I, Anderson JP, Richmond T, Somerville SC, Manners JM. Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. Proceedings of the National Academy of Sciences USA 2000;97(21):11655-60.
[36]. Schmelz EA, LeClere S, Carroll MJ, Alborn HT, Teal PE. Cowpea chloroplastic ATP synthase is the source of multiple plant defense elicitors during insect herbivory. Plant Physiology 2007;144(2):793-805.
[37]. Simms D, Cizdziel PE, Chomczynski P. TRIzol: A new reagent for optimal single-step isolation of RNA. Focus 1993;15(4):532-5.
[38]. Singh AP, Dixit G, Mishra S, Dwivedi S, Tiwari M, Mallick S, Pandey V, Trivedi PK, Chakrabarty D, Tripathi RD. Salicylic acid modulates arsenic toxicity by reducing its root to shoot translocation in rice (*Oryza sativa* L.). Frontiers in Plant Science 2015;6:340. doi: 10.3389/fpls.2015.00340
[39]. Song WY, Park J, Mendoza-Cozat DG, Suter-Grotemeyer M, Shim D, Hortensteiner S. Arsenic tolerance in *Arabidopsis* is mediated by two ABCC-type phytochelatin transporters. Proceedings of the National Academy of Sciences USA 2010;107:21187-92. doi:10.1073/pnas.1013964107.
[40]. Stukkens Y, Bultreys A, Grec S, Trombik T, Vanham D, Boutry M. NpPDR1, a pleiotropic drug resistancetype ATP binding cassette transporter from *Nicotiana plumbaginifolia*, plays a major role in plant pathogen defense. Plant Physiology 2005;139:341-52.
[41]. Sugimoto M, Yamaguchi Y, Nakamura K, Tatsumi Y, Sano H. A hypersensitive response-induced ATPase associated with various cellular activities (AAA) protein from tobacco plant. Plant Molecular Biology 2004;56(6):973-85.
[42]. Virdi AS, Singh S, Singh P. Abiotic stress responses in plants: roles of calmodulin-regulated proteins. Frontiers in Plant Science 2015;6:809. doi: 10.3389/fpls.2015.00809
[43]. Vlot AC, Dempsey DMA, Klessing D. Salicylic acid, amulti-faceted hormone to combat disease. Annual Review of Phytopathology 2009;47:177-206. doi: 10.1146/annurev.phyto.050908.135202.
[44]. Wan XL, Zhou Q, Wang YY, Wang WE, Bao MZ, Zhang JW. Identification of heat-responsive genes in carnation (Dianthus caryophyllus L.) by RNA-seq. Frontiers in Plant Science 2015;6:519. doi: 10.3389/fpls.2015.00519
[45]. War AR, Paulraj MG, War MY, Ignacimuthu S. Role of salicylic acid in induction of plant defense system in chickpea (*Cicer arietinum* L.). Plant Signaling & Behavior 2011;6(11):1787-92.
[46]. Wu L, Hu X, Wang S, Tian L, Pang Y, Han Z, Wu L, Chen Y. Quantitative analysis of changes in the phosphor proteome of maize induced by the plant hormone salicylic acid. Scientific Reports 2015a;5:18155. doi: 10.1038/srep18155.
[47]. Wu T, Wang Y, Zheng Y, Fei Z, Dandekar AM, Xu K, Han Z, Cheng L. Suppressing sorbitol synthesis substantially alters the global expression profile of stress response genes in apple (*Malus domestica*) leaves. Plant Cell Physiology 2015b;56(9):1748-61. doi: 10.1093/pcp/pcv092.
[48]. Wu L, Wang S, Chen X, Wang X, Wu L, Zu X, Chen Y. Proteomic and phytohormone analysis of the response of maize (*Zea mays* L.) seedlings to sugarcane mosaic virus. PLoS ONE 2013;8(7):e70295. doi:10.1371/journal.pone.0070295.
[49]. Yasuda M, Ishikawa A, Jikumaru Y, Seki M, Umezawa T, Asami T, Maruyama-Nakashita A, Kudo T, Shinozaki K, Yoshida S, Nakashita H. Antagonistic interaction between systemic acquired resistance and the abscisic acid-mediated abiotic stress response in *Arabidopsis*. The Plant Cell 2008;20(6):1678-92.
[50]. Zandalinas SI, Rivero RM, Martinez V, Gomez-Cadenas A, Arbona V. Tolerance of citrus plants to the combination of high temperatures and drought is associated to the increase in transpiration modulated by a reduction in abscisic acid levels. BMC Plant Biology 2016;16:105.
[51]. Zhang X, Dong J, Liu H, Wang J, Qi Y, Liang Z. Transcriptome sequencing in response to salicylic acid in *Salvia miltiorrhiza*. PLoS ONE 2016;11(1):e0147849. doi:10.1371/journal. pone. 0147849.

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