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Induction of defence related enzymes and gene expression after resistance induction by rhizobacteria and silicon against *Ralstonia solanacearum* in tomato (*Solanum lycopersicum*)

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INTRODUCTION

Plants have evolved a complex and varied defense mechanisms to protect themselves against pathogen attack. These mechanisms may be constitutive or induced but can fail when a plant is infected by a virulent pathogen, as the pathogen avoids triggering resistance reactions (Pieterse and van Loon, 1999).

Plant growth promoting rhizobacteria strains and silicon are known to elicit plant-mediated resistance referred to as induced systemic resistance (ISR) and acquired systemic resistance (ASR), respectively (Pieterse and van Loon, 1999; Fauteux et al., 2006). Studies on Si-rhizobacteria-mediated ASR and ISR indicated the role of defense enzymes such as peroxidase (POD), phenylalanine ammonia lyase (PAL) and lipoxygenase (LOX) in the induction of systemic resistance (Jetiyanon, 2007; Cai et al., 2009). Therefore, the present investigation was undertaken to evaluate the effect rhizobacteria strain *B. pumilis* and silicon alone or in combination, on bacterial wilt reduction, activity of defense-related enzymes POD, LOX and PAL and high throughput gene expression profiling of induced resistance in tomato genotypes.

MATERIALS AND METHODS

Roots of four-week-old tomato genotypes King Kong 2 (moderately resistant) and L390 (susceptible) to bacterial wilt were immersed in suspensions of *B. pumilis* & transplanted. 2 days after each plant was artificially wounded and inoculated with

Ralstonia solanacearum. 5 days post inoculation bacterial multiplication in mid-stems of tomato was determined with selected symptomless plants & wilt disease development recorded. Total silicon content in the stems & roots of the same plant sample were determined. The activity of LOX, PAL and POD were measured following the standard procedure. Transcriptome analysis of tomato stem tissue was performed to elucidate silicon-rhizobacteria mediated gene expression profiling.

RESULTS AND DISCUSSION

Application of silicon and rhizobacteria significantly reduced bacterial wilt incidence by 50.7% & 26.7%, respectively, in King Kong 2 & by 31.1% & 22.2%, respectively, in L390 genotypes, compared to the pathogen inoculated control (Table.1). However, their combined application reduced wilt incidence by 16.9% in King Kong 2 and by 13.2% in L390. Application of each elicitor also reduced bacterial populations in the mid-stems of tomato, but not their dual application. Our results indicated combined application of elicitors did not result in an additive effect on the suppression of bacterial wilt rather an antagonistic one. This might be due to elicitation of different signaling pathways by each elicitor which might interact in an antagonistic manner. Also Ishida et al. (2008) found non synergistic effect on the suppression of bacterial blight caused by *X. axonopodis* pv. *malvacearum* in cotton when Acibenzolar-S Methyl and *B. cereus* were applied simultaneously. The silicon quantification result indicated that, silicon content in the roots was higher than in the stems of both genotypes amended with silicon which is typical for non-silicon accumulator plants. The activity of LOX was significantly decreased in pathogen inoculated and silicon amended treatment, but increased in the rhizobacteria treatment. In simultaneous application of silicon and rhizobacteria, the activity of POD and PAL, LOX dropped significantly. In contrast non-significant increases of POD and PAL activity were observed in the individual treatments of each elicitor upon inoculation with *R. solanacearum*. Silva et al. (2004) also reported induction of systemic reaction by the increased activity of LOX. Products of lipid membrane peroxidation by LOX contribute to defense reactions by inhibiting pathogen growth and development, as precursors for jasmonic and methyl jasmonate. However, in Silicon amended plant activity of

LOX was significantly declined, this might be due to the ameliorative effect of Si on membrane integrity.

Table 1: Effect of silicon and rhizobacteria treatment on disease severity and disease incidence in tomato genotypes L390 and King Kong 2 inoculated with *R. solanacearum* strain ToUdk2

	AUDPC			
	Disease severity		Wilt incidence	
Treatments	L390	King Kong 2	L390	King Kong 2
+Si-A+Rs	59.0 ± 11.1 bA	47.8 ± 14.0 cB	620.1 ± 31.8 cA	353.3 ± 38.4 cB
+Si+A+Rs	68.7 ± 12.0 abA	58.8 ± 16.2 aB	781.7 ± 68.1 bA	595 ± 29 abB
-Si+A+Rs	62.4 ± 20.2 abA	53.5 ± 13.2 bB	700.5 ± 26.8 bcA	525 ± 19 bB
-Si-A+Rs	71.5 ± 15.2 aA	62.8 ± 16.2 aB	900.5 ± 73.8 aA	716.7 ± 33.7 aB

Data are means ± SE of three independent trails with three plants per treatments. Small letters vertically refer to comparison with in the same genotype and capital letters horizontally to comparison between genotypes for the same treatment. Means followed by same letters are not significantly different according to Tukey test at α = 5% probability level.

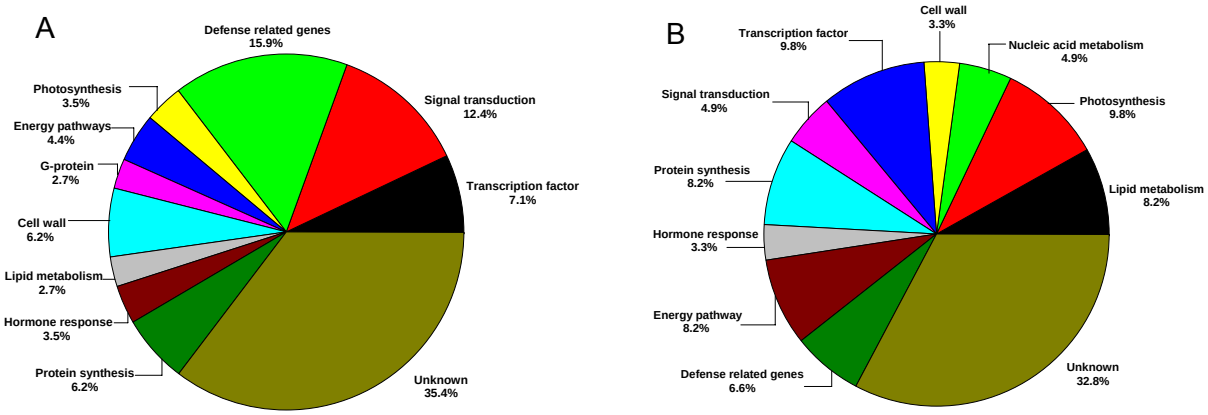

Fig. 1: Pie charts showing the number of up-regulated (A) and down-regulated genes (B) in each functional category

In gene expression profiling anaylsis of tomato stem, a total of 174 genes were differentially regulated, of which 113 were up-regulated and 61 down-regulated Here, Si regulated more defense related genes than *B. pumilis.* However, during simultaneous application of the elicitors antagonistic interaction occurred. Our result was in line with Nickel et al. (2010) who reported up-regulation of defense related genes in silicon amended tomato plants 72 hours post inoculation of *R. solanacerum.* The results demonstrate the ability of rhizobacteria and silicon to differentially trigger expression of a variety of defense related genes, with Si being the stronger inducer.

CONCLUSIONS AND OUTLOOK

The study showed the vital role of the biotic & abiotic elicitors in the induction of defense in tomato against the pathogen. Application of either Si or rhizobacteria alone reduced wilt incidence indicating the induction of systemic resistance which is also supported by activity of defense related enzymes & transcriptome analysis. But, combined application of the elicitors resulted in antagonistic interaction rather than additive which were expressed at phenotypic, biochemical & molecular level. To elucidate the intricate plant-microbe-Si interaction further molecular analyses should be done with mutant tomato genotypes.

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