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Foliar Spray of Elicitors on Physiology and Quality Responses in Turmeric (*Curcuma longa* L.)

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ABSTRACT

Background: In the presented work, we studied the effect of foliar spray of elicitors namely chitosan, phenylalanine and salicylic acid sans any stress treatment on physiological and quality parameters in turmeric genotypes.

Methods: Feld experiment was undertaken under rainfed condition using four genotypes. The elicitors were sprayed at rhizome development stage and various measurements namely chlorophylls, carotenoids content and gas exchange parameters were done at different time intervals. The harvested rhizomes were analysed for its curcuminoids content.

Result: The results showed that elicitor treatment increased the leaf chlorophyll and carotenoids content in statistically significant manner. The highest increase in total chlorophyll content (36%) was observed in chitosan treated plants. Likewise, the highest increase in total carotenoids content (33%) was found in phenylalanine treatment. The highest photosynthetic rate increase (76%) was obtained in salicylic acid treatment. The total curcuminoids showed significant increase in only Pragati (16%) and Rajapuri (20%) genotypes. Results suggested that foliar spray of chitosan and salicylic acid at rhizome development stage could be employed to elicit the physiological response and improve the quality of turmeric grown under rainfed condition.

Key words: Chitosan, Curcuminoids, Phenylalanine, Photosynthesis, Salicylic acid.

INTRODUCTION

Elicitors are chemical substances of organic or inorganic nature when introduced at low concentration elicit a response in plants which is similar to environmental stimuli or pathogen infection, affecting the metabolic processes of plants thereby enhancing the synthesis of certain phytochemicals (Ferrari, 2010).

Chitosan (CHT), a polymer with D-glucosamine and N-acetylglucosamine subunits obtained from deacetylation of chitin (Kurita, 2006) is a well-studied elicitor in different crops. Chitosan application as foliar spray or seed treatment was found to increase the chlorophyll and carotenoids content, the photosynthetic rate and induces the plant secondary metabolites synthesis by altering the biosynthetic pathway mainly by inducing phenylalanine ammonia lyase (PAL) and other enzymes in various crops (Farouk and Amany, 2012; Jail *et al.,* 2014; Satyabhama *et al.,* 2016). Like chitosan, application of salicylic acid (SA) was reported to influence stomatal closure, ion uptake, production of free radicals, induction of antioxidant enzymes and increase the synthesis of many defense related compounds in different crops (Khan *et al.,* 2003). Several studies also highlighted the role of SA treatment to increase the secondary metabolite contents in many crop species (Figueroa Perezx *et al.,* 2014; El-Zaeddi *et al.,* 2017). Likewise, Phenylalanine (PHE), an aromatic amino acid involved in the biosynthesis of secondary metabolites is also considered as effective elicitor and biostimulant with positive influence on photosynthesis, plant growth and yield and enhancement of the production of secondary metabolites in commercially important crop species (Govindaraju and Arulselvi, 2018; Samani *et al.,* 2019).

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Turmeric is an important economic crop cultivated in tropical regions of Asia. Apart from culinary purpose, the active ingredient presents in the crop namely curcuminoids has reported to have numerous pharmaceutical applications. Thus, the present work was designed to study the effect of three chemical elicitors namely chitosan, phenylalanine and salicylic acid on photosynthetic and quality parameters in turmeric.

MATERIALS AND METHODS

The experiment was conducted at ICAR - Indian Institute of Spices Research (IISR) experimental Farm, Peruvannamuzhi, Kerala in the year 2019-20. The layout was designed in randomized block design with three replications. The varieties/genotypes for the study were selected based on its difference in growth duration and quality attributes. Pragati, a short-duration dwarf variety of turmeric with stable curcumin; Rajapuri, traded variety from Central Indian region (medium curcumin) and Prathibha (high curcumin), are long duration genotypes and Acc. 849, a genotype having longest growing period with low curcumin content were taken up for the study.

Low molecular weight (>90% degree of deacetylation) chitosan (CHT) solution was prepared at 2000 ppm concentration by dissolving in 0.15 M acetic acid. The salicylic acid (SA) and phenylalanine (PHE) solution was prepared at 100 mM concentration each. The pH of the stock solutions was maintained at 6.5-7.0. The dilutions of 200 ppm of CHT and 1 mM each of PHE and SA were prepared with 0.02% Tween 20 freshly before the spray schedule. Control plants were sprayed with 0.02% Tween 20. The elicitors were sprayed at rhizome development stage, *i.e.*, 120-150 DAP depending upon the growth duration of the genotypes. The leaf gas exchange parameters were measured before the spray and one week after the spray for comparing the effect of treatment. The leaf samples (one sample before the spray and two samples after the spray at one week interval) were collected for the measurement of photosynthetic pigments. The chlorophyll content of samples was extracted using 80% acetone and estimated as per the protocol described by Arnon, 1949. The carotenoids content of the sample was measured using the formula suggested by Yang *et al*. (1998). Gas exchange measurements were taken in the fully expanded third leaf from the top using portable photosynthesis system (LC pro-SD Advanced Photosynthesis Measurement System, England) as described by Alagupalamuthirsolai *et al.* (2020). The parameters measured were net photosynthetic rate (Pn, ìmol m^{-2} s^{-1}), stomatal conductance (gs, mol m^{-2} s^{-1}) and transpiration rate (E, mmol m^{-2} s^{-1}). The processed, dried rhizomes were powdered and curcuminoids were extracted with acetone and total curcuminoids (TC) content was calculated following ASTA method 10.2 (ASTA, 2014) using UV-Vis Spectrophotometer. The data were analyzed statistically and multiple means comparisons of treatment groups were done using SAS tool.

RESULTS AND DISCUSSION

Effect of elicitors on photosynthetic pigments

The chlorophyll and carotenoid content of leaves was measured at three intervals. First sample was collected before elicitors spray and next two samples were collected at 7 days intervals after foliar spray. The readings obtained before spray was taken to measure the inherent variation in the treatment plot and also to compare the changes happened within plot after elicitor spray. In the genotype Pragati, the total chlorophyll content was statistically on par with control and PHE treatment plants showed lower leaf chlorophyll content. But after spray, the PHE, CHT and SA treatments showed 35%, 25% and 17% increase ($p<0.01$) respectively in the sample collected one week after the spray. The total chlorophyll content was reduced in elicitor treated plants in the second sample collected after spray as compared to control (Fig 1). Being the short duration genotype, elicitors spray at 120 DAP in Pragati did not influence the chlorophyll sustenance for longer time as they were nearing their vegetative life cycle. In the genotype Rajapuri, the total chlorophyll content measured before spray was not significant among each other. In first sampling

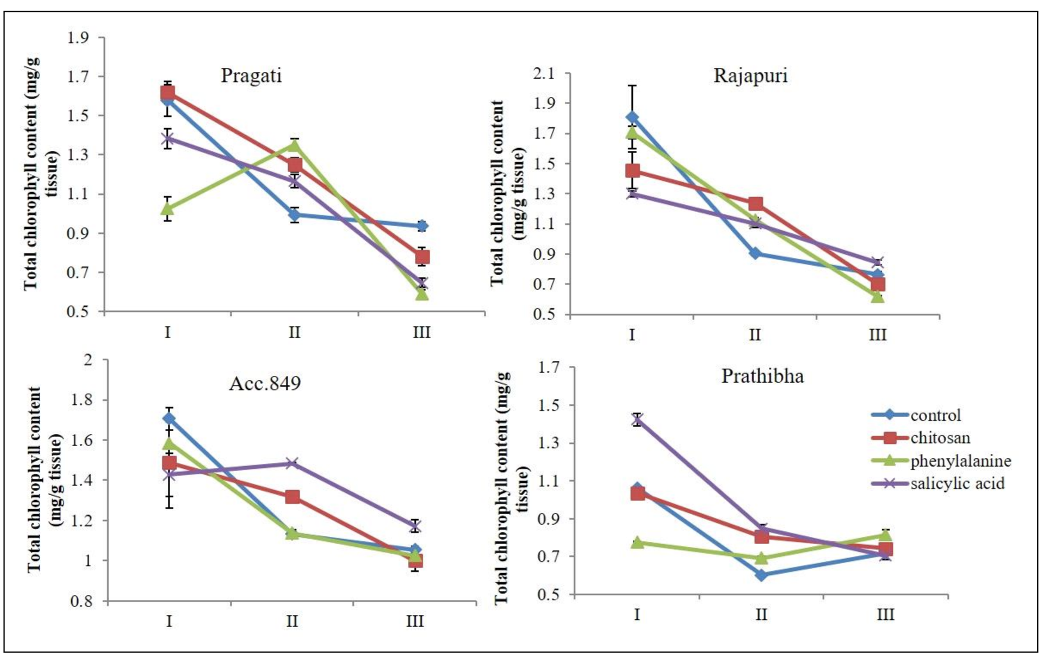

Fig 1: Effect of elicitors treatment on total chlorophyll content of turmeric genotypes. I- Samples collected before spray; II- Samples collected next day after spray; III- Samples collected one week after spray.

after spray CHT, PHE and SA treatment increased (p<0.01) its content to 36%, 24% and 22% respectively. In second samples after spray, only SA could maintain its increase (p<0.01) to 10% (Fig 1). In the genotype Prathibha, the first sample collected after spray, SA, CHT and PA treatment showed 40%, 33% and 14% increase (p<0.01) respectively in total chlorophyll content, whereas in the second samples after spray, only PHE treatment showed significant increase (p<0.01) of 13% (Fig 1). In Acc. 849, the first sampling after spray showed 30% and 16% increase in total chlorophyll content (p<0.01) in SA and CHT treated plants respectively. Only SA treated plants, maintained higher (p<0.05) content in the second sampling done after spray (Fig 1).

The total carotenoids content was increased (p<0.01) to 33% and 24% in PHE and CHT treated plants respectively in the first sampling after spray in Pragati. The second

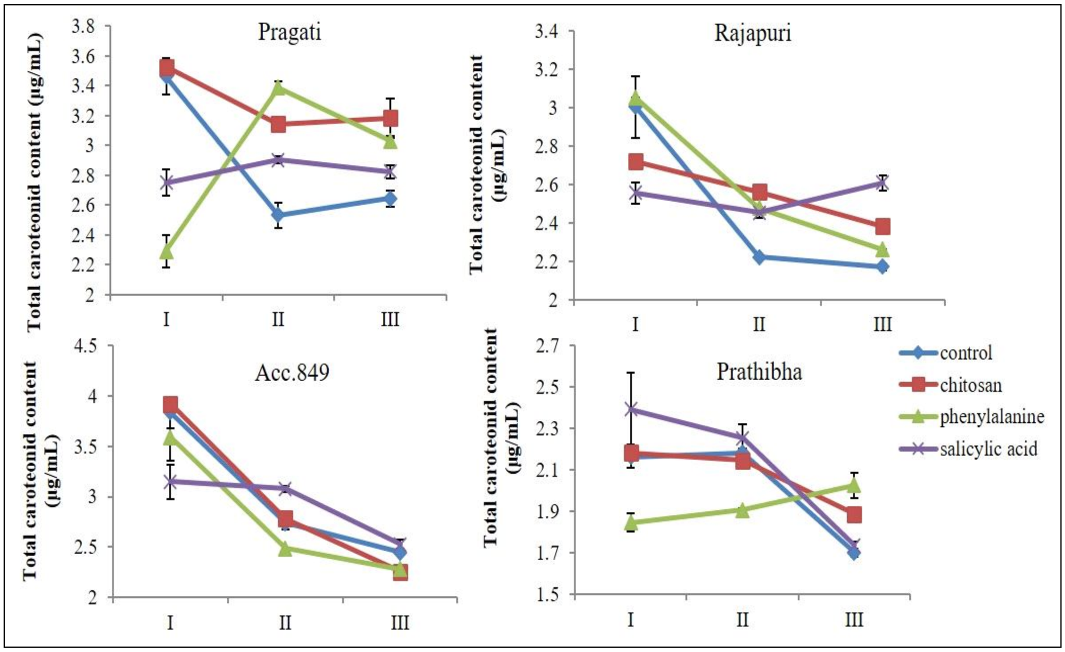

Fig 2: Effect of elicitors treatment on total carotenoids contents of the turmeric genotypes. I- Samples collected before spray; II- Samples collected next day after spray; III - Samples collected one week after spray.

Table 1: Effect of elicitors on gas exchange parameters of turmeric genotypes.

Genotype	Treatments	Pn (µmol m^{-2} s^{-1})		E (mmol H_2O m^{-2} s^{-1})		gs (mol m^{-2} s^{-1})	
		Before spray	After spray	Before spray	After spray	Before spray	After spray
Pragati	Control	8.76 ±0.27^{a}	5.15±0.32^{c}	3.48±0.08^{ab}	1.68±0.18^{c}	0.28±0.02^{a}	0.13±0.01^{c}
	CHT	7.14±0.32^{b}	7.58±0.38^{b}	3.17±0.05^{bc}	2.42±0.05^{b}	0.20±0.01^{b}	0.24±0.01^{b}
	PHE	9.13±0.35^{a}	8.92±0.25^{a}	3.59±0.15^{a}	2.71±0.06^{b}	0.29±0.03^{a}	0.26±0.01^{ab}
	SA	7.42±0.18^{b}	9.04±0.39^{a}	2.85±0.10^{c}	3.37±0.04^{a}	0.20±0.01^{b}	0.28±0.01^{a}
Rajapuri	Control	8.39±0.11^{b}	8.71±0.42	4.27±0.19	3.13±0.01^{ab}	0.24±0.01^{b}	0.21±0.01^{ab}
	CHT	8.42±0.23^{b}	9.78±0.26	5.22±0.14	2.99±0.06^{b}	0.31 ±0.02^{a}	0.18±0.01^{b}
	PHE	9.45±0.19^{a}	9.36±0.27	5.16±0.37	3.07±0.11^{b}	0.31±0.02^{a}	0.20±0.01^{b}
	SA	8.83±0.19^{ab}	9.43±0.21	4.78±0.31	3.41±0.07^{a}	0.33±0.01^{a}	0.23±0.01^{a}
Prathibha	Control	5.41±0.09^{a}	3.03±0.35^{b}	3.04±0.16^{a}	1.45±0.20^{ab}	0.12±0.003^{a}	0.05±0.005^{b}
	CHT	1.06±0.14^{d}	4.70±0.34^{a}	1.15±0.07^{b}	1.83±0.09^{a}	0.03±0.003^{b}	0.09±0.01^{a}
	PHE	3.84±0.37^{b}	2.38±0.26^{b}	2.73±0.16^{a}	1.13±0.14^{b}	0.10±0.01^{a}	0.04±0.006^{b}
	SA	2.24±0.38^{c}	4.22±0.17^{a}	1.47±0.24^{b}	1.65±0.08^{ab}	0.05±0.01^{b}	0.08±0.003^{a}
Acc.849	Control	10.66±0.33	7.70±0.30^{c}	3.66±0.07^{b}	4.05±0.10^{b}	0.32±0.02	0.19±0.002^{b}
	CHT	11.3±0.52	8.48±0.36^{bc}	4.08±0.14^{a}	4.11±0.10^{ab}	0.28±0.03	0.19±0.003^{b}
	PHE	10.6±0.60	9.94±0.52^{a}	4.01±0.06^{a}	4.59±0.21^{a}	0.3±0.01	0.23±0.01^{a}
	SA	10.01±0.20	9.18±0.19^{ab}	3.56±0.07^{b}	3.81±0.10^{b}	0.29±0.03	0.19±0.003^{b}

The data is represented as mean (±SE) from three replicates for each treatment. Means accompanied by the different superscript are significantly different at P<0.01 and means accompanied by at least one common superscript are significantly different at P<0.05.

sampling after spray showed only moderate increase (p<0.05) of 14% and 20% in total carotenoids content as compared to control in the same treatments. In case of Rajapuri, the highest increase (p<0.01) of total carotenoids content over control was found in CHT treatment (15%) in the first sampling after spray. In second samples collected after spray, the highest increase (p<0.01) of total carotenoids content was found in SA treated plants (20%) followed by CHT treatment (10%). Only SA produced significant increase (p<0.01) in total carotenoids content in both first and second sampling in Acc. 849 genotype (Fig 2). In the genotype Prathibha, PHE and CHT produced significant increase (p<0.01) of 19% and 10% in samples collected second week after spray. Our results are in consonance with earlier reported results (Farouk and Amany, 2012; Jail *et al*., 2014). Though numerous studies on chitosan application with stress treatments are available in different crops, only few studies were done to study its effect without any stress treatments (Salimi *et al.,* 2019; Satyabhama and Manikandan, 2021) and our study brought out the useful information that even single foliar spray of elicitors are effective in enhancing the content of photosynthetic pigments in turmeric genotypes without any stress treatments. Like our results, in many field crops, foliar application of salicylic acid (Nazar *et al.,* 2015; Ma *et al.,* 2017) and phenylalanine (Samani *et al.,* 2019) was found to produce positive effect on its growth and physiology by enhancing the activity of chlorophyll synthesizing enzymes. The results of our study showed that all the elicitors were efficient in inducing the chlorophyll and carotenoids accumulation in leaves of turmeric genotypes whereas only SA was found to maintain their increase even one week after the spray. Being a hardy crop, the increase in photosynthetic pigments by elicitors as observed in our study in highly encouraging.

Effect of elicitors on gas exchange parameters

The gas exchange parameters were measured at two time intervals; the first reading was taken before the treatment and second reading was taken two week after the spray to compare the effect of treatment on increase or decrease in photosynthetic parameters in the four genotypes. In Pragati CHT, PHE and SA treatment has produced 47%, 73% and 76% increase (p<0.01) in net photosynthetic rate (Pn) respectively. This was accompanied by increase in transpiration rate (E) and stomatal conductance (gs). In the genotype Rajapuri, treatments did not give significant change in Pn and other parameters as compared to control though we found increase in the photosynthetic pigments. In Acc. 849, CHT, PHE and SA treatment produced 10%, 29% and 19% increase (p<0.01) in Pn. On the other hand, in Prathibha, the CHT and SA treatment increased (p < 0.01) Pn to 55% and 39% respectively. When comparing the values taken before and after spray, the trend emerged was that only CHT and SA increased the Pn in all the genotypes except Acc. 849 (Table 1).

Table 2: Effect of elicitors total curcuminoids (TC) of turmeric genotypes.

Treatments	Total Curcuminoids (%)			
	Pragati	Rajapuri	Acc.849	Prathibha
Control	3.28±0.09[b]	1.60±0.02[b]	1.54±0.01	3.93±0.16
CHT	3.82±0.1[a]	1.62±0.06[b]	1.60±0.04	3.91±0.07
PHE	3.59±0.05[ab]	1.38±0.01[b]	1.52±0.07	3.59±0.09
SA	3.54±0.07[ab]	1.93±0.1[a]	1.42±0.01	4.03±0.16

The data is represented as mean (±SE) from three replicates for each treatment. Means accompanied by the different superscript are significantly different at P<0.01 and means accompanied by at least one common superscript are significantly different at P < 0.05.

The increased photosynthesis rate observed in elicitor treated samples in our study were corroborated by previous studies where chitosan spraying increased Pn in soybean and maize (Khan *et al.,* 2002) and rice (Phothi and Theerakarunwong, 2017) and SA on purslane plant (Saheri *et al.,* 2020) and maize (Khan *et al.,* 2003). Chamnanmanoontham *et al.* (2015) explained that chitosan treatment induces nucleus and chloroplast genes involved in photosynthesis and antioxidant machinery at chromatin level. The conclusion from our study is that application of chitosan and salicylic acid produced significant increase in the photosynthetic rate (Pn) of turmeric genotypes irrespective of its different growth nature. This would be the first report on the effect of PHE on photosynthetic parameters in turmeric.

Effect of elicitors on curcuminoids content

Among all the genotypes, chitosan treatment showed 16% increase (p<0.01) in total curcuminoids content only in the genotype Pragati (Table 2). In the study, SA treatment also showed significant increase (p<0.05) of 9% and 20% (p<0.01) of TC content in Pragati and Rajapuri respectively. (Table 2). There is no significant increase of TC content in the genotypes Acc. 849 and Prathibha in all the treatments (Table 2). Lan *et al*. (2019) reported that SA treatment increased the curcumin accumulation by stimulating the genes involved in its biosynthesis in *Curcuma zedoria* cell cultures. The results we obtained in our study was supportive to earlier published results (Anusuya *et al.,* 2016; Satyabhama *et al.,* 2016; Loc *et al.,* 2016) though we found their differential effect on different genotypes. This would be the first report by far our knowledge extends, to show the positive effect of SA treatment in increased curcuminoids accumulation in genotypes dependent manner in turmeric crop.

CONCLUSION

The study concluded that the elicitors like CHT, PHE and SA was found to be efficient in maintaining the higher leaf chlorophyll and carotenoids content in the four turmeric genotypes. The elicitors were also found to increase the photosynthetic rate of the genotypes studied. They increased the TC accumulation in the rhizome of Pragati and Rajapuri genotypes. Phenylalanine did not show holistic improvement in all the parameters analyzed though significant increase in chlorophyll, total carotenoids and Pn was observed in few genotypes. The present work would be the first in many kinds like including varied genotypes differing in their growth pattern and quality in field level experiment. This gave us the understanding that the influence of elicitors used in the study is not uniform and some genotypes performed better under some elicitors. This would be the first study to measure the effect of elicitors on increasing plant pigments and photosynthetic rate in turmeric. The present work could be extended by including more locations and more genotypes to estimate the genotype and environmental influence of elicitor activities on turmeric plants to select location and genotype specific elicitors for quality improvement.

Conflict of interest: None.

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