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Elicitor: A Tool for Rapid *In-vitro* Screening of Disease Resistance in *Colocasia*

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ABSTRACT

The cell wall glucans were isolated from *Phytopthora colocasiae* that induce defense mechanism in *Colocasia* and the technique for *in vitro* screening of the *Colocasia* varieties for leaf blight resistance using the elicitor has been standardized. *Colocasia*, the important crop used as vegetable is affected by *Phytophthora colocasiae* which causes the leaf blight disease. Cell wall glucans were isolated from the mycelial cell walls of *P. colocasiae.* The elicitor was inoculated on the cut *Colocasia* leaves of ten local cultivars of Odisha to study the hypersensitive reaction. The hypersensitivity reaction in resistant cultivars was detected within 48-72 hours of incubation. The field tolerant cultivars *Dhalasaru, Panisaru* and *Sankhasaru* showed quick hypersensitive reaction by necrosis which was delayed or not expressed distinctly in the susceptible cultivars viz., *Kalasaru, Satyabhama* and *Kujisaru* cultivated in Orissa. Varietal screening for disease resistant in field condition of crop plants are time consuming and expensive. That also partially regulated by other biotic and abiotic factors and environmental conditions. But *in-vitro* screening is very fast, time saving, economic and free of other external factors. To monitor the response in resistant and susceptible hosts, elicitor injection and systemic induction in pot culture is easier than that under field condition. So the elicitor preparations will be useful for screening of *Colocasia germplasm* for resistance against leaf blight.

Key words: *Cell wall glucans, Colocasia, Elicitor, Hypersensitive reaction, Phytophthora colocasiae*

Introduction

Taro (*Colocasia esculenta* (L.) Scott is extensively cultivated in India as well as other South and South East Asian countries like China, Nepal, Myamar, Indonesia, Cambodia, Thialand, Malayasia, Central America and Africa. Usually it is used as vegetable, staple or subsistancial food as well as a medicinal crop (Chandra, 1984). Yield of this crop is highly affected by an important disease, i.e. Leaf Blight caused by *Phytophthora colocasiae.* There is no such fruitful control measure as the disease occurrence is in rainy season and due to the waxy nature of the leaf surface which makes the fungicidal application or fungicide spray value less. The only alternative is to develop resistance against the disease and the pathogen. Induction of resistance through elicitors may be a tool for disease management. Elicitors are the exterior or foreign molecules associated with plant pest, diseases or synergistic organisms. They act as signal compounds at low concentrations providing information for the plant to trigger defense. Elicitors are the molecules that elicit defense mechanisms in plants. They are the primary signal molecules that induce the synthesis and accumulation of anti-microbial compounds in plant cells which re-

(Associate Professor)

sults the host pathogen recognition and interaction. Most of the fungal pathogens, whether compactable or not, are known to have elicitors particularly in their cell wall. A single elicitor protein is also sufficient to trigger multiple defense reactions like necrosis, callus formation etc. in the cell. Plants recognize certain microbial compounds as elicitors of their active defense mechanisms. In several plant species it has been proved that accumulation of phenols, polyphenols, defense related enzymes and pathogenesis related proteins can be locally and systematically elicited by the compounds of pathogen origin viz., fungal cell wall polysaccharides (either free or engaged in glycoproteins), enzymes that hydrolyze pectic-polysaccharides of plant cell walls, fungal lipids etc. The frequent feature of resistance expressed in plants is hypersensitive reaction characterized by rapid necrosis of plant cells. Many defense-eliciting compounds viz., cell wall glucans; elicitins, cryptogein, PMG elicitor etc. have been isolated from different *Phytopthora* species.

Materials and Methods

Cell wall glucan elicitor of *P. colocasiae* was prepared from mycelial mat of the same fungus. *P. colocasiae* was grown on Potato Dextrose Broth medium for 3 days in static condition and then for 15 days in a shaker Incubator at 25-28 °C. The mycelia mat was harvested and washed with sterile distilled water three times. The mycelia mat was blended and filtered through muslin cloth. The residue was homogenized with chloroform and methanol (1:1) followed with acetone. The preparation was autoclaved and centrifuged. The supernatant contain the Cell Wall Glucan Elicitor (Sharp *et al*., 1984).

For in-vitro screening 10 local Colocasia cultivars of Odisha state were selected. Twenty numbers of fifteen days old leaves of such cultivars were collected and cleaned with sterile distilled water. Ten leaves of each cultivar were treated with cell wall glucan elicitors. Another set was treated with elicitor with pin pricking. Then the leaves were incubated inside a transparent poly bags and kept at 25-28 °C with adequate moisture. Observations for hypersensitivity reaction (development of lesion) were recorded after 24 hours of application of elicitors on leaf surfaces up to one week.

To study the effect of systemic application of elicitor on disease development (*Phytophthora colocasiae* infection) in a susceptible local cultivar Satyabhama was planted in 90 pots. After 45 days of planting elicitor was injected (100 ml/ plant) in the leaf sheath (30 pots) and sprayed on leaf surface of another group (30 pots). Separate pots (30) were maintained without injection or spraying as control. Ten pots of each group were inoculated with *Phytophthora* inoculums (10000 cfus/ml) at 5, 10 and 15 days after elicitor treatment. Percentage of infection in terms of % leaf area damage was calculated and recorded (Birader, 1978).

Biochemical changes in leaves of Satyabhama cultivar during this period were studied. Phenol and Carbohydrate content in leaves of different treatment were analyzed. Phenol content in leaves was measured by the methods of Bray and Thorpe (1954). One gram of fresh leaf sample was collected and grinded with mortar and pestle in 10 ml of 80% ethanol. The homogenate was centrifuged at 10000 RPM for 20 minutes. The supernatant was re-extracted with same 80% ethanol and evaporated to make it dry. The residue was dissolved in 5ml distilled water. 0.2 to 2.0 ml of different aliquots was taken in different test tubes followed with 0.5 ml of Folin-Ciocalteau reagent. After 3 minutes, 2 ml of 20% Na_2CO_3 solution was added to all the tubes, mixed thoroughly and placed in boiling water bath for one minute. Then cooled and absorbance was measured at 650 nm against blank. Phenol content was calculated against standard curve of different concentrations of Catechol.

Total carbohydrates content in leaves of different treatment were estimated by the methods described by Scott and Melvin (1953). 100 mg fresh leaf sample was hydrolyzed in boiling water bath with 5 ml of 2.5 N HCl for 3 hours, cooled to room temperature. Then it was fully neutralized with solid Sodium Carbonate, volume was made-up to 100ml and centrifuged. One ml supernatant was added to 4 ml of anthrone reagent and heated for 8 minutes in a boiling water bath. Cooled to room temperature and absorbance was recorded at 630 nm. Carbohydrate was estimated from the standard curve by taking 0.0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard.

Results and Discussion

For rapid screening of Colocasia cultivars against leaf blight disease caused by *Phytophthora colocasiae* in relation to hypersensitive reaction was tested with detached leaves of 10 local cultivars of Odisha state, India. When detached leaves of all the culti-

vars were subjected to cell wall glucan elicitor, the hypersensitive reaction detected in 48 hours of inoculation. Application of elicitor with pricking is more effective than normal application. Hypersensitive reaction was more prominent in tolerant or resistant varieties. Hypersensitive reaction was highest (70% in normal and 80% with pricking) in case of Sankhasaru after 48 hours of incubation. The tolerant varieties like Dalasaru, Panisaru, Sankhasaru and Manasaru showed 100% hypersensitive reaction after 4th day. Cultivars like Kendrapada local, Kalasaru and Satyabhama showed no hypersensitive reaction within this period and after that showed very low HR. Nayagarh local, Kujisaru and Telia showed moderate hypersensitive reaction (Table 1). The detached leaves of resistant cultivars treated with cell wall glucan elicitor of *Phytophthora colocasiae* hypersensitivity reaction was induced early in resistant or tolerant cultivars (Sankhasaru and Panisaru). Whereas, it is absent or delayed in case of susceptible varieties taken in this test (Satyabhama and Kalasaru). The same trend was also observed in case of Telia and Muktakeshi (Sriram *et al*., 2003). This implies that in susceptible varieties at the time of pathogen infection, the colonization in the host tissue is faster than the resistant or tolerant varieties.

Elicitor was injected inside the petiole of 45 days old susceptible cultivar (Satyabhama). After 5, 10, 15 days of elicitor treatment pathogen was inoculated (sprayed) on leaves and observation was recorded after one week up to 6 weeks. Percentage of infection varied from 20 to 40% when pathogen was inoculated five days after elicitor treatment. But no infection was observed when pathogen was applied after 10 and 15 days of elicitor injection. The corresponding result in case of elicitor spraying was varied 80-100%, 70-100% and 60-100% respectively. The control plants without elicitor treatment showed 100% infection by pathogens (Table 2). In between elicitor injection and elicitor spraying, elicitor injection is more effective than elicitor spraying. Colocasia leaves have waxy substances which make the fungicidal spraying ineffective. But elicitor injection was systemic and highly effective against leaf blight diseases. As elicitor was injected in leaf sheath, resistance was induced in the whole plant after 5 to 10 days of inoculation. Some elicitors do not act or show little activity on susceptible cultivars, although they induce accumulation of defensive chemicals in the incompatible hosts. Galactoglucomannan elicitor isolated from *Colletotrichum lindemuthianum* also has cultivar specificity (Tepper and Anderson, 1986).

Table 1. Hypersensitive reaction in *Colocasia* cultivars leaves treated with *Phytophthora colocasiae* elicitor.

Sl. No.	Cultivars	Treatment	Leaves showing HR (%) at different days				
			2 days	3 days	4 days	5 days	6 days
1	Nayagarh Local	E	0	0	40	40	50
		E+P	10	20	70	80	80
2	Dhala Saru	E	30	60	70	70	70
		E+P	70	90	100	100	100
3	Pani Saru	E	60	80	90	100	100
		E+P	70	90	100	100	100
4	Sankha Saru	E	70	100	100	100	100
		E+P	80	100	100	100	100
5	Mana Saru	E	50	100	100	100	100
		E+P	70	100	100	100	100
6	Kendrapada local	E	0	0	0	30	40
		E+P	0	10	10	20	30
7	Kalasaru	E	0	0	0	10	20
		E+P	0	0	10	30	40
8	Satyabhama	E	0	0	0	0	0
		E+P	0	0	0	10	20
9	KujiSaru	E	10	10	20	20	30
		E+P	20	30	40	40	50
10	Telia	E	20	50	60	60	70
		E+P	30	60	70	70	80

*E= Elicitor, E+P=Elicitor with pricking

Some bio-chemical changes were also observed in the susceptible Colocasia plant (leaf) when elicitor was either injected or sprayed. The phenol content in leaves of plant of 5days after elicitor treatments (injected) varied from 26.0 to 38.0 mg^{-1} and plants with elicitor sprayed, phenol content was 28 to 31.2 mg^{-1}. The control plant without elicitor treatment was 25 to 29 mg^{-1}. The corresponding value for 10 days elicitor treated plants 39-56, 29-30 and 26-32 mg^{-1} respectively. The values for plants inoculated with pathogen after 15 days of elicitor treatment were 38-56, 30-38 and 26.6-38 mg^{-1} respectively (Table 3). Elicitors never attack and kill the pathogens; they only create and enhance the plant defense mechanism by various ways. One of them is to increase the level of phenol and phenolic compounds in the plant body. Phenol or phenolic compounds are important for plants. They protect the plants from biotic and abiotic stress factors. Phenolic compounds are only induced and involved in defense mechanism and are synthesized after pathogen attack (Cantos *et al*., 2003). Elicitors were initially used to increase the plant resistance against pathogens but later if was proved that elicitors increased the poly-phenol levels (Yolanda and Encarna, 2013). So elicitors are considered as the alternative tool for increasing plant resistance by increasing the phenol content in a plant. Disease resistance in plants is dependent on both pre-existing physical or chemical barriers, i.e. thick cell wall, lignin or tannins and induced defense mechanism in plants (Beckers and Spoel, 2006). In this study phenol content increased with systemic application (leaf sheath injection) of elicitor than external spraying of elicitor and with increase of time of elicitor application. Depending on the type of pathogen attack, the plant activates different signaling pathway to synthesize a specific set of defensive compounds. Phenolic compounds are one of the important groups of secondary metabolites that involved in the resistance to pathogens. Elicitors also stimulated the accumulation of soluble phenolic compounds in cucumber leaves (Farouk *et al*., 2008).

Total carbohydrate content was varied from 4.52

Table 2. Effect of Elicitor Injection and spraying on *Phytophthora colocasiae* infection in *Colocasia.*

Sl. No.	Treatment		% infection					
			1 week	2 week	3 weeks	4 weeks	5 weeks	6 weeks
1	5 days after Elicitor Treatment	E(I)+P	10	20	40	40	40	40
		E(S)+P	80	90	100	100	100	100
		P	100	100	100	100	100	100
2	10 days after Elicitor Treatment	E(I)+P	0	0	0	0	0	0
		E(S)+P	70	80	90	100	100	100
		P	100	100	100	100	100	100
3	15 days after Elicitor Treatment	E(I)+P	0	0	0	0	0	0
		E(S)+P	60	70	70	70	70	70
		P	100	100	100	100	100	100

*E(I)= Elicitor Injection, E(S)= Elicitor spraying, P= Pathogen

Table 3. Effect of Elicitor Injection and spraying on Phenol content in *Colocasia* leaf during *Phytophthora colocasiae* infection.

Sl. No.	Treatment		Phenol Content in mg^{-1}					
			1 week	2 week	3 weeks	4 weeks	5 weeks	6 weeks
1	5 days after Elicitor Treatment	E(I)+P	26.0	27.0	32.0	38.0	39.5	38.0
		E(S)+P	28.0	30.0	31.0	31.2	30.0	31.0
		P	25.0	26.0	27.0	27.0	29.0	28.5
2	10 days after Elicitor Treatment	E(I)+P	39	39.0	41.8	54.0	57.8	56.0
		E(S)+P	29.0	30.0	31.5	32.0	32.0	33.0
		P	26.0	28.0	28.6	31.0	32.6	32
3	15 days after Elicitor Treatment	E(I)+P	38.0	41.0	43.0	56.0	58.0	57.0
		E(S)+P	30.0	31.0	35.0	37.0	38.0	37.0
		P	26.6	27.0	31.0	33.0	34.0	35.0

*E(I)= Elicitor Injection, E(S)= Elicitor spraying, P= Pathogen

Table 4. Effect of Elicitor Injection and spraying on carbohydrate content in *Colocasia* leaf during *Phytophthora colocasiae* infection

Sl. No.	Treatment		Carbohydrate content (%)					
			1 week	2 week	3 weeks	4 weeks	5 weeks	6 weeks
1	5 days after Elicitor Treatment	E(I)+P	4.52	4.63	4.84	5.21	5.29	5.25
		E(S)+P	3.98	4.21	4.72	4.89	5.11	5.12
		P	3.68	4.09	4.52	4.69	4.94	5.07
2	10 days after Elicitor Treatment	E(I)+P	4.63	4.89	4.99	5.42	5.66	6.62
		E(S)+P	4.49	4.76	4.87	5.31	5.52	6.33
		P	3.74	4.17	4.61	4.79	4.99	5.19
3	15 days after Elicitor Treatment	E(I)+P	4.64	4.92	5.08	5.59	5.91	6.76
		E(S)+P	4.51	4.81	4.97	5.06	5.67	6.41
		P	3.69	4.99	5.15	4.96	5.49	6.27

*E(I)= Elicitor Injection, E(S)= Elicitor spraying, P= Pathogen

to 5.25% in plants with elicitor injection in petiole and pathogen was inoculated after 5 days of elicitor treatment. The corresponding value for pathogen inoculation after 10 and 15 days of elicitor injection were 4.63 to 6.62 and 4.64 to 6.76% respectively. Less amount of carbohydrate was accumulated in leaves of colocasia plant when elicitor was sprayed on leaves instead of elicitor injection in petioles. Carbohydrate content varies from 3.98 to 5.12, 4.49 to 6.33 and 4.51% to 6.41 at 5, 10 and 15 days pathogen inoculation after elicitor spraying. The corresponding results in control (without elicitor treatment) were 3.68 to 5.07, 3.74 to 5.19 and 3.69 to 5.27% respectively. Carbohydrate accumulation in leaves increase with increase of age of the plant (Table 4). In this experiment sugar content increased with application of elicitor may be systemic or foliar. In other studies foliar application of elicitor caused significant increase of total phenols, soluble sugars and total free amino acids (Amin *et al*., 2007). Elicitors also accelerate the sugar accumulation and translocation from leaves to fruits (Sharma *et al*., 1986).

Conclusion

Elicitor plays a greater role in plant defense mechanism. It can induce resistance in both susceptible as well as tolerant cultivars. Elicitor induces hypersensitive reaction (HR) early in resistant variety, either delayed or absent in susceptible variety which may be a tool for in-vitro screening of many plants.

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Conflicts of interest

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