

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.608.054>

Induced Resistance in Potato against Late Blight Caused by *Phytophthora infestans* (Mont.) De Bary, Through Calcium Chloride

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ABSTRACT

Keywords

Resistance,
Potato, Late
blight, Calcium
Chloride.

Article Info

Accepted:
04 June 2017
Available Online:
10 August 2017

An experiment was conducted to evaluate calcium chloride (CaCl_2) as inducers in induce resistance against late blight of potato caused by *Phytophthora infestans*. Two per cent CaCl_2 used in 9 treatments for different time durations (1/2h, 1h, 2h, 4h, 6h, 8h, 10h, 12h and 0h) and foliar spray and resulted that CaCl_2 have ability to reduce disease severity from 22.65 to 48.00 per cent against 57.00 per cent in case of control. Biomolecules changes due to effect of CaCl_2 revealed that increase content of soluble protein and total phenol content with the minimum in T_8 treatment representing 32.5, 34.5, 34.5, 34.5, 33.1 mg/g fresh leaf of soluble protein, and 2.57, 2.62, 2.74, 2.87 and 2.78 mg/g fresh leaf of total phenol at 2, 4, 6, 8 and 10 days of pathogen inoculation. The correlation coefficient between disease severity with total soluble protein and total phenol content showed negative correlation in both cases.

Introduction

Potato (*Solanum tuberosum* L.) is the most important and useful vegetable crop in the world, belonging to the family *Solanaceae*. It is the world's fourth-largest food crop, following maize, wheat and rice. China stands top at the list with around 23% of the world's potato produces that is around 322 million metric tons. In world scenario, India became the 3rd largest producer of potato (Scott and Suarez, 2011).

The production and productivity of potato in India is impressive. However, in the background of increasing population pressure, there is a need to increase productivity from

same piece of land. But the main reason of low productivity is diseases like, early blight, late blight, leaf spot, dry rot, charcoal rot, black scurf, common scab, soft rot, leaf roll etc. Among them, late blight caused by *Phytophthora infestans* (Mont.) de Bary is most destructive disease that had led to most in-famous catastrophe in Ireland (England) during 1840-1845. The pathogen is mainly perpetuated in tuber and soil through production of oospores. The management of the disease can be done through host resistance, cultural adjustments, biological and use of fungicides. But most of the conventional chemicals, biological and use of

resistance varieties tend towards the direct control of pathogens by their elimination. Sometimes these practices raise problem due to development of resistant strains of the pathogen which may become very difficult to control. Induced resistance is a new area of extensive work aiming at developing new strategies for disease control meeting the requirements for safe application in greenhouse and fields conditions, namely: no direct toxicity to pathogens; no toxicity to plants and animals; no negative effects on plant growth, development and yield; broad spectrum of defense; low loading amount; long lasting protection; low economical cost for 64 Aglika Edreva farmers; good profit for producers (Kuc, 2001). Several inorganic chemicals, bio agents, plant extract, secondary metabolites etc. have been used as inducers in management of several diseases in different crops (Biswas *et al.*, 2012, Rajik *et al.*, 2012 and Arzoo *et al.*, 2012).

The mechanism of resistance was found in association of some defense compounds like soluble protein, total phenol and synthesis of some new proteins due to the effect of biotic inducers (Rajik *et al.*, 2012). Biswas *et al.*, (2003) found that pre-application of crude extract of *Chaetomium globosum* provided induced resistance in wheat against spot blotch and also sensitized the plant to produce evaluated level of total phenol soluble protein and some new proteins of different molecular level. Considering that above point on view the study was undertaken in the present investigation as “Induced resistance in potato against late blight disease caused by *Phytophthora infestans* (Mont.) de Bary, through calcium chloride”.

Materials and Methods

Isolation and purification of pathogen

Infected plant sample showing typical late blight symptoms was collected from

Vegetable Research Farm, CSAUA&T, Kanpur for isolation and purification of pathogen. Small pieces of infected leaf along with some green healthy tissues were cut and dipped in HgCl₂ (0.1%) solution for 30 seconds. The pieces were then rinsed in sterilized distilled water thrice and dried off with sterilized filter paper. These pieces were placed on tomato extract based medium (selective medium) in Petri plates and then incubated at 18±1°C. The Petri plates were observed daily to notice the presence of mycelium around the leaf bit. As soon as the mycelia growth is noticed around the bits, pathogen was purified by hypha tip culture method and characterized based on cultural and morphological characteristics.

Preparation of CaCl₂ solution

Twenty gram of CaCl₂ was mixed in a beaker along with 1000ml of distilled water to prepare 2% concentration solution of CaCl₂. The solution was then used for further study as tuber treatment and foliar spray.

Experiment

The experiment was conducted in the wire house complex, Department of Plant Pathology, C. S. Azad University of Agriculture and Technology, Kanpur. The seed tuber of potato variety ‘*Kufri Lalima*’ was treated with solution of CaCl₂ and spraying on plants with the same at 45 days age of plant. The details of treatment are given in table 1

The treated tubers were sown in 30cm earthen pots which were previously filled with a mixture of sterilized sandy loam soil and farm yard manure in the ratio of 2:1. In each pot, one seed tuber was sown and watered as per need base. The experimental design was laid out in simple CRD. Three replications per treatment and three pots were sown with untreated seed tubers served as control. The

observations pertaining to the effect of different treatments were taken on disease severity and biochemical changes occur during pathogenesis.

Effect of calcium chloride (CaCl₂) on disease development

Spraying of calcium chloride

In order to determine the efficacy of Calcium chloride as seed treatment and foliar spray in controlling disease, at 45 days age, the plants were sprayed first with solution of calcium chloride separately.

Inoculation of the crop

After 48 hours, plants were inoculated with spore suspension of *P. infestans*. The concentration of spore was maintained at 10⁶ spore/ml.

The spore suspension was prepared from seven days old culture of the pathogen. The homogenized, spore suspension were inoculated on the foliage of each plant. The inoculated plants were then kept on the bench of wire house. After 2, 4, 6 and 10 days, observations were taken on disease severity.

Measurement of disease severity

Observations for measuring the disease severity were taken after 5 days of pathogen inoculation. The disease severity was recorded on a 0-9 scale as described by Malcolimson, (1976). Ten leaves were randomly selected from the pot for measurement of disease severity. The leaves with 1-9% infection received 1, 10% infection received 2, 11-25% infection received 3, 26-40% infection received 4, 41-60% infection received 5, 61-70% infection received 6, 71-80% infection received 7, 81-90% infection received 8, 91-100% infection received 9 (Malcolimson, 1976).

The disease severity of individual plants was calculated by following formula:-

$$\text{Disease severity PDI} = \frac{\sum \text{Sum of numerical rating}}{\text{Total number of leaves examined} \times \text{Maximum rating}} \times 100$$

Effect of CaCl₂ on changes of biomolecules in potato during pathogenesis

In order to find out the changing biomolecules to effect of CaCl₂ the fresh potato leaves were collected from different treatments and the changes in the content of soluble protein and total phenol in leaves were estimated at 2, 4, 6, 8 and 10 days after pathogen inoculation.

Soluble protein estimation

Total soluble protein content was determined as per method described by Lowery *et al.*, (1951).

Total phenol estimation

The accumulation of phenols in potato plants after treatment with CaCl₂ followed by inoculation of pathogen was estimated following procedure developed by Bary and Thrope (1954). In this method, the total phenol estimation was carried out with FCR which was measured at 650 nm radiation calorimetrically.

Correlation coefficient and regression equation

The bio molecule changes in potato leaves under different treatments and disease severity of the corresponding date showed that reduced disease severity was associated with increased soluble protein and total phenol content. However, to determine the level of association correlation coefficients (r)

between soluble protein and disease severity as well as between total phenol and disease severity were calculated by standard statistical calculation.

Simple regression equation ($Y = a + bx$) were also developed for both the variable (protein and phenol) separately to understand their relation with disease severity.

Results and Discussion

Effect of calcium chloride as inducer on severity of late blight of potato

The effect of tuber treatment and foliar spray with calcium chloride as inducers significantly reduced disease severity of late blight of potato as compared to control (Table 3).

Among the treatments, the minimum disease severity with 15.90%, 20.70% and 22.65% were recorded in T₈ treatment at 2, 6 and 10 days after pathogen inoculation, respectively followed by T₇ treatment as 16.70%, 21.87% and 23.35% and T₆ treatments 16.95%, 22.68% and 24.08%.

From the table, it is also cleared, that all the inducer treated potato plants were showing comparatively low disease severity over control.

The decrease in disease severity might be the activity of calcium chloride as inducer which stimulates to synthesis of some defence related compounds in potato plant against *P. infestans*. Several workers also reported that application of biotic and abiotic inducers reduce disease severity in rice (He *et al.*, 2002, Selein *et al.*, 2011, Girdhari *et al.*, 2010), in tomato against *Fusarium* wilt (Arzoo *et al.*, 2010, Biswas *et al.*, 2012), in wheat against Spot blotch (Mishra *et al.*, 2011) (Table 2).

Biochemical changes associated with the effect of calcium chloride as inducer during pathogenesis at different days

Biochemical changes like total soluble protein, total phenol content due to effect of calcium chloride as inducers were studied as pre inoculation method and changing bio-molecules were estimated at 2, 4, 6, 8 and 10 days after pathogen inoculation.

Soluble proteins

The data presented in table 4 showed that the maximum amount of soluble protein contents was found in T₈ treatment, representing the value 32.50 mg/gm of fresh leaves, 34.53mg/gm of fresh leaves, 34.50mg/gm of fresh leaves, 34.68mg/gm of fresh leaves, 33.10 mg/gm of fresh leaves of fresh leaves at 2,4,6,8 and 10 days of pathogen inoculation. The soluble protein contents in control plant were 21.87 mg/gm of fresh leaves, 23.75mg/gm of fresh leaves, 23.85 mg/gm of fresh leaves, 24.15 mg/gm of fresh leaves, and 23.44mg/gm of fresh leaves at 2,4,6,8 and 10 days of pathogen inoculation. The T₇ treatment representing the value of soluble protein as 32.42 mg/gm of fresh leaves, 34.33 mg/gm of fresh leaves, 34.41mg/gm of fresh leaves, 34.58 mg/gm of fresh leaves and 33.05 mg/gm of fresh leaves at 2,4,6,8 and 10 days of pathogen inoculation which is second highest among the treatments. The per cent increased soluble protein content was ranges 37.96 to 41.21, indicating all the treated plants were able to synthesized soluble protein in plant. Antoniew *et al.*, (1980) considered that pathogen related proteins (PR protein) in the form of chitinase, PR-1, peroxides, β -glycosidase etc. are involved in plant defence response to pathogens. Biswas *et al.*, (2012) found that pre-foliar spray with inorganic chemicals increases soluble protein content in tomato resulted decline in disease incidence of wilt. Singh and Prithviraj (1977)

also found that neemazol, a product of neem increased protein in pea leaves against *Erysiphe pisi*. Chakraborty and Sharma

(2007) reported that neem leaf extract caused accumulation of polyphenol and PR-protein (Satesh Kagale *et al.*, 2004).

Table.1 Details of treatment

T ₁	Seed tuber treatment with CaCl ₂ solution @2.0% for 1/2h + spraying with CaCl ₂
T ₂	Seed tuber treatment with CaCl ₂ solution @2.0% for 1h + spraying with CaCl ₂
T ₃	Seed tuber treatment with CaCl ₂ solution @2.0% for 2h + spraying with CaCl ₂
T ₄	Seed tuber treatment with CaCl ₂ solution @2.0% for 4h + spraying with CaCl ₂
T ₅	Seed tuber treatment with CaCl ₂ solution @2.0% for 6h + spraying with CaCl ₂
T ₆	Seed tuber treatment with CaCl ₂ solution @2.0% for 8h + spraying with CaCl ₂
T ₇	Seed tuber treatment with CaCl ₂ solution @2.0% for 10h + spraying with CaCl ₂
T ₈	Seed tuber treatment with CaCl ₂ solution @2.0% seed for 12h + spraying with CaCl ₂
T ₉	No seed tuber treatment (Control)

Table.2 Effect of calcium chloride as inducer on disease severity of late blight of potato

Treatment	Concentration	Disease severity at different days of intervals after inoculation (%)		
		2 Days	6 Days	10 Days
T ₁	2%	38.06	40.90	48.00
T ₂	2%	31.43	35.85	40.71
T ₃	2%	25.01	30.90	36.87
T ₄	2%	19.70	24.55	28.10
T ₅	2%	17.75	23.40	25.30
T ₆	2%	16.95	22.68	24.08
T ₇	2%	16.70	21.87	23.35
T ₈	2%	15.90	20.70	22.65
T ₉		43.02	48.65	57.00
SE		4.324	3.240	8.663
C.D.P=(0.05)		3.600	3.116	5.095

Table.3 Effect of calcium chloride as inducer on total soluble protein in potato leaves at different Days of interval after inoculation during pathogenesis (mg/g of fresh leaves)

Treatments	Concentrate	Soluble protein content at different days of interval (mg/g of fresh leaves)					% increase over control (at 10 days)
		2 Days	4 Days	6 Days	8 Days	10 Days	
T ₁	2%	31.75	7	33.80	33.97	32.34	66.03
T ₂	2%	31.83	33.74	33.91	34.10	32.44	65.90
T ₃	2%	31.92	33.83	34.02	34.22	32.56	65.78
T ₄	2%	32.10	33.92	34.17	34.31	32.69	65.69
T ₅	2%	32.13	34.06	34.23	34.43	32.79	64.94
T ₆	2%	32.40	34.18	34.32	34.51	32.90	65.49
T ₇	2%	32.42	34.33	34.41	34.58	33.05	65.42
T ₈	2%	32.50	34.53	34.50	34.68	33.10	65.32
T ₉		21.87	23.75	23.85	24.15	23.44	-
SE		2.584	3.051	2.677	2.616	1.188	-
C.D (0.05%)		2.783	3.107	2.832	2.800	1.887	-

Table.4 Effect of calcium chloride as inducer on total phenol in potato leaves at different days of Intervals during pathogenesis (mg/g of fresh leaves)

Treatment	Con.	Total phenol content at different days of interval(mg/g of fresh leaves)					Per cent increased over control (at 10 days)
		2 Days	4 Days	6 Days	8 Days	10 Days	
T ₁	2%	2.18	2.20	2.31	2.43	2.35	49.68
T ₂	2%	2.25	2.30	2.43	2.51	2.48	57.96
T ₃	2%	2.30	2.36	2.48	2.27	2.52	60.50
T ₄	2%	2.35	2.41	2.54	2.65	2.60	65.60
T ₅	2%	2.39	2.47	2.51	2.72	2.70	71.97
T ₆	2%	2.46	2.50	2.64	2.75	2.71	72.61
T ₇	2%	2.55	2.58	2.72	2.86	2.77	76.43
T ₈	2%	2.57	2.62	2.74	2.87	2.78	77.07
T ₉		1.12	1.18	1.45	1.68	1.57	-
SE		0.108	0.132	0.142	0.121	0.126	-
C.D.P=(0.0)		0.570	0.629	0.652	0.601	0.616	-

Table.5 Correlation of disease severity with total soluble protein and phenol content of potato leaves

Biochemical parameters	Days after Pathogen inoculation	Correlation Coefficient (r) With disease	Regression Equation
Total soluble Protein	2 days	-0.5087	Y= 40.2134
	4 days	-0.5095	Y= 39.1227
	6 days	-0.5102	Y= 39.3198
	8 days	-0.5279	Y= 39.5485
	10 days	-0.5105	Y= 39.6303
Total phenol	2 days	-0.9879	Y= 3.9539
	4 days	-0.9887	Y= 3.9568
	6 days	-0.9926	Y= 3.9716
	8 days	-0.9946	Y= 3.9797
	10 days	-0.9766	Y= 3.9064

Total phenol

The result presented in table 5 shows that all the treatment significantly increased the total phenol content as compared to control at 2, 4, 6 and 8 days of pathogen inoculation. The maximum amount of total phenol contents was found in T₈ treatment, representing the value 2.57mg/g of fresh leaves, 2.62 mg/g of fresh leaves, 2.74mg/g of fresh leaves, 2.87 mg/g of fresh leaves and 2.78 mg/g of fresh leaves of fresh leaves at 2,4,6,8 and 10 days of pathogen inoculation. Among the treatments, the minimum amount of total phenol contents was found in T₁ treatment, representing the value 2.18 mg/g of fresh

leaves, 2.20 mg/g of fresh leaves, 2.31 mg/g of fresh leaves, 2.43 mg/g of fresh leaves and 2.35 mg/g of fresh leaves at 2,4,6,8 and 10 days of pathogen inoculation. The per cent increase of total phenol over control was highest in T₈ treatment (77.07), followed by T₇ treatment (76.43). Phenols are well known antifungal, antibacterial and antiviral compound. Matern and Kneusal (1988) suggested that the first stage of defense mechanism involve a rapid a rapid accumulation of phenol at the infection site which restricts or slow the growth of the pathogen. Gridhr *et al.*, (2008) reported that phenol was found increase in rice leaf after treatment with biotic inducer. Mishra *et al.*,

(2010) also reported that increased total phenol content was found on varieties of wheat showing resistance to spot blotch.

Correlation co-efficient and regression equation

The biomolecules changes in potato leaves at different days and disease severity of corresponding day showed that reduced disease severity was associated with increase soluble protein and total phenol content. A negative correlation (r) -0.5087, -0.5095, -0.5102, -0.5279 and -0.5105 was found between disease severity and soluble protein content. Similarly, correlation between disease severity and total phenol content also showed negative correlation. The corresponding simple regression equation also showed the negative relation between total protein and disease severity as well as total phenol and disease severity. Similar observations were also found in rice against brown leaf spot (Girdhari *et al.*, 2010) in tomato against Fusarium wilt (Arzoo *et al.*, 2010, Biswas *et al.*, 2012), in wheat against spot blotch (Mishra *et al.*, 2011).

It may be concluded from the present finding that CaCl₂ have inducing effect of reduce disease severity of potential to control Late blight of potato. It has also ability to synthesize some defense compound like total soluble protein total phenol etc. resulted defense response in plant. Thus it may be a better alternative of disease management in sustainable agriculture.

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How to cite this article:

Surjeet Kumar, S.K. Biswas, Virendra Kumar, Kishan Lal, Anuj Bansal and Tilak Chowdary, V. 2017. Induced Resistance in Potato against Late Blight Caused by *Phytophthora Infestans* (Mont.) De Bary, Through Calcium Chloride. *Int.J.Curr.Microbiol.App.Sci.* 6(8): 410-417. doi: <https://doi.org/10.20546/ijcmas.2017.608.054>