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Changes in Foliar Glycoalkaloids Levels of Potato (*Solanum tuberosum*) Triggered by Late Blight Disease Severity

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Abstract

Glycoalkaloids are secondary metabolites found in *Solanum tuberosum* L. and other members of Solanaceae, which have potential role in the defense of host against certain fungi, nematodes, herbivores and other stress conditions. This study was undertaken to investigate the possible relationship between late blight disease severity and foliar glycoalkaloids of potato cv. Desiree. Disease severity and total glycoalkaloids contents determined after 3, 6, 9 and 12 days after inoculation with *Phytophthora infestans* showed no relationship when compared to control. Values of total glycoalkaloids contents of potato foliage with different late blight disease severity were almost consistent with control plants, which were inoculated with sterile distilled water; although TGA levels in diseased plants showed slight but non-significant elevations than control. Results indicated that disease severity had no effect on foliar TGA concentrations; however, age of plant and length of inoculation period corresponded to higher glycoalkaloids contents and disease severity of leaves.

Keywords: Elicitors; Host defense; Phytoalexins; *Phytophthora infestans*; Secondary metabolites

Introduction

Phytophthora infestans (Mont.) de Bary is perhaps the most widely studied plant pathogen which caused the Great Irish Potato Famine in 1840s by destroying potato crop-the then staple food of Irish people (Bourke, 1993). *P. infestans* parasitizes a number of plants belonging to Solanacea (Turkensteen, 1978), however, it adversely affects potato and tomatoes by causing late blight disease (Tosun *et al*., 2007). Late blight of potato is still one of the major threats faced by potato growers throughout the world resulting in direct yield losses of crop in addition to huge monitory expenditures associated with fungicides application for controlling the disease (CIP, 1997; Hijmans *et al*., 2000). Although late blight of potato can be controlled through rigorous fungicides applications, cultivating resistant varieties and integrated disease management strategies; modern research, however focuses on inducing resistant traits in cultivated potato against various pathogens and pests (Lorito *et al*., 1998; Hoy, 1999; Naqvi *et al.*, 2012) through inter-specific hybridization and genetic transformation methods (Esposito *et al*., 2002). There is also dire need to identify the possible of role of secondary metabolites (phytoalexins) present in potato for anti-pathogenic activities. Understanding the relationship between the pathogens and secondary metabolites would be appealing for potato breeders to develop transgenic varieties with enhanced resistance to potential pathogens.

Glycoalkaloids are important secondary metabolites found in some genera of family Solanaceae including cultivated potato (*Solanum tuberosum* L.) in different concentrations at different plant parts (Osman, 1983), which show toxicity to pathogenic fungi, predators, insects and pest (Tingey, 1984; Matthews *et al*., 2005; Friedman, 2006). Principle glycoalkaloids present in potato are α-chaconine and α-solanine (Freidman and McDonald, 1997). Genetic factors, age of plant, stress conditions, pathogenic attacks and herbivory are important determinants of glycoalkaloids levels in potato (Sinden *et al*., 1984; Friedman, 2006). Their concentrations are usually higher in plant parts with high metabolism rate such as young leaves and flowers with subsequent decrease in parts with low metabolic activity upon maturity (Friedman and McDonald, 1997).

Published reports on possible association between foliar/tuber glycoalkaloids of potato and late blight disease resistance are variable (Friedman, 2006). Deahl *et al*. (1973) investigated the association of foliar and tuber glycoalkaloids and late blight disease resistance in fifteen potato clones however, they reported lack of such association. Similarly, Frank *et al*. (1975) reported no relationship between total glycoalkaloids of potato leaves and late blight disease resistance in field experiments. On the other hand, Andreu *et al*. (2001) observed increased level of accumulation of glycoalkaloids and other phytoalexins in leaves and tubers tissues after inoculation with *P. infestans*, but no correlation between the disease

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resistance and glycoalkaloids was established. The aim of this study was to investigate the effects of late blight disease on foliar glycoalkaloids levels and possible relationship between disease resistance and total glycoalkaloids contents of potato foliage.

Materials and Methods

Collection and Culturing of *Phytophthora infestans*

Culturing procedure for *P. infestans* was carried out at Plant and Environmental Protection unit, National Agriculture Research Centre (NARC), Islamabad. Infected tissues of previously collected leaves were placed in petri-dishes containing rye agar medium supplied with antibiotic 100 µL/mL vancomycin, 100 µL/mL pimaricin and 50 µL/mL rifamycin (Caten and Jinks, 1968). In order to promote sporulation, petridishes were incubated for 4 days at 18ºC in dark. Freshly formed sporangia were transferred by sterilized glass rod to fresh rye agar medium without antibiotics and were re-incubated at 18ºC for 14 days in dark. Freshly formed sporangia were dislodged by sterile glass rod and adding 10 mL of distilled water to each petridish. For separating sporangia from mycelia fragments, sporangial suspensions were filtered through a double layer of cheesecloth (pore size = 60 µm; 14 x 12 threads centimeter^{-1}) and concentrations were adjusted by haemo-cytometer to 60000 sporangia/mL. The sporangia were incubated at 4ºC for two h to induce germination (Mukalazi *et al*., 2001; Pliakhnevich and Ivaniuk, 2008).

Determination of Foliar Glycoalkaloids

For determination of foliar glycoalakaloids, Potato seed tubers (cv Desiree) were grown in four row plots, 3 meter long with spacing of 70 cm between rows and 30 cm within rows at Botany Department, Hazara University Mansehra in July, 2011. Experiment was laid out in a randomized complete block design (RCBD) with four replications. Leaves were inoculated with 20 µL sporangial suspensions at the center 45 days after planting. Control plants were inoculated with sterile distilled water. In order to prevent *Phytophthora* infection, control plants were sprayed with contact fungicide Mandy Prompide (Revus) at three day interval. After inoculation, disease severity (% leaflet infection) was calculated at 3, 6, 9 and 12 days after inoculation (Lebreton *et al*., 1999). Total glycoalkaloids of potato leaves (control and infected at different days of inoculation) were determined following the method of Dao and Friedman (1996) with minor modifications. Healthy and infected leaves were randomly collected from the middle parts of plants from control (water inoculated) and diseased plots (infected with *P. infestans*) at 0, 3, 6, 9 and 12 after inoculation. A single, large and fully expanded leaf (weighing 300 mg) was considered as a single experimental unit replicated four times for each treatment. Each leaf sample was blended in 20 mL aqueous acetic acid (5%) to make a homogenate. The homogenate was then filtered through Whatman filter paper no. 4 and pH of the filtrate was maintained by ammonium hydroxide to 10. The filtrate from control and diseased leaves was used for total glycoalkaloids contents determination by HPLC technique at PCSIR Laboratory complex, Peshawar using HPLC Shimadzu SC – 6A System. For reducing biasedness of results, the procedure was repeated four times.

One way ANOVA was used to analyze disease severity while for finding association between means of glycoalkaloids and disease severity, two-way ANOVA was applied to collected data. Means were separated by LSD at p= 0.05.

Results

The objective of this study was to investigate the relationship between late blight disease severity and total glycoalkaloids of potato leaves. Results indicated that days after inoculation (DAI) had significant effect on the disease severity (Table 1). At 3rd DAI, disease severity was recorded as 5.263% with increasing tendency as DAI progressed; 12th DAI revealed maximum disease severity 70.135 % (Fig. 1).

In order to determine the effect of disease severity on glycoalkaloids, foliar total glycoalkaloids of both diseased plants and control were determined at 3, 6, 9, and 12th day of inoculation. Foliar TGA values of diseased plants were compared with control at each assessment period. Results showed that TGA determined before inoculation of leaves with late blight pathogen and sterile distilled water (in control) was 29.43 mg/100 g fresh weight (Fig. 2). TGA contents increased significantly with age of plant. Increase in TGA levels in both control and diseased plants were almost consistent, although diseased plants showed slightly higher values of TGA than control but these results were insignificant. TGA determined after three days of inoculation in diseased plants were 43.08 mg/100 g fresh weight, whereas in control (inoculated with sterile distilled water) plants 42.14 mg/100 g fr. wt. of TGA were recorded. Likewise, slight but insignificant increase in glycoalkaloids levels was also recorded at 6, 9 and 12 DAI (50.80, 52.81 and 75.67 mg/100 g fr. wt.) when compared to control, where TGA values were 49.33, 52.18 and 74.28 mg/100 g fr. wt. respectively (Fig. 2). ANOVA did not reveal any correlation between disease severity and TGA of leaves determined at different assessment periods however, impact of days after inoculation (DAI) were significant on glycoalkaloids levels in both control and diseased plants (Table 3). At 3rd day of inoculation, foliar TGA in diseased plants increased by 2.230% than control. Maximum increase (2.970%) was recorded at 6th DAI followed by 9th DAI (1.207%) and 12th DAI (1.870%) respectively (Table 2). Contrarily, TGA levels in both diseased and control plants significantly increased with the increase in days after inoculation (DAI) i.e., TGA levels were higher in both

diseased and control at 12 DAI followed by 9 DAI, 6 DAI and 3 DAI respectively (Fig. 2). Comparing TGA levels of diseased plants with control at each DAI, result clearly demonstrated that disease severity had no effect on the TGA levels. On the other hand, TGA levels in diseased and control plants were significantly increased by increase in DAI i.e., age of the plants (Table 3).

Discussion

Previously Deahl *et al*. (1973) and Frank *et al*. (1975) investigated the effect of late blight disease and some other pathogenic fungi on total glycoalkaloids of different potato clones but were unable to find association between late blight disease and leaf TGA contents. Andreu *et al.* (2001) reported that glycoalkaloids, phenolic compounds and phytoalexins slightly but insignificantly increased in potato leaves after inoculation with *P. infestans*; however, association between disease severity and glycoalkloids could not be established in their studies.

Glycoalkaloids are important secondary metabolites of members of family Solanacea including potato. Principle glycoalkaloids present in potato are α-chaconine and α-solanine (Freidman and McDonald, 1997). In potatoes, like other Solanacea members, concentrations of these compounds are dependent on several factors like age of plant, climate, varieties, biotic and abiotic stresses (Sinden *et al*., 1984; Friedman, 2006; Khan *et al*., 2013). Young tissues of plant have maximum levels of glycoalkaloids

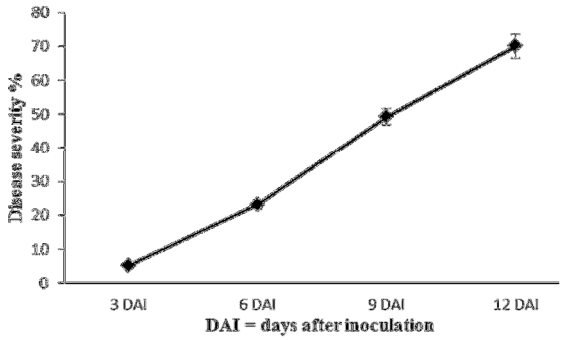

Fig. 1: Foliar disease severity (% leaflet area infected) measured after different days of inoculation with *Phytophthora infestans*

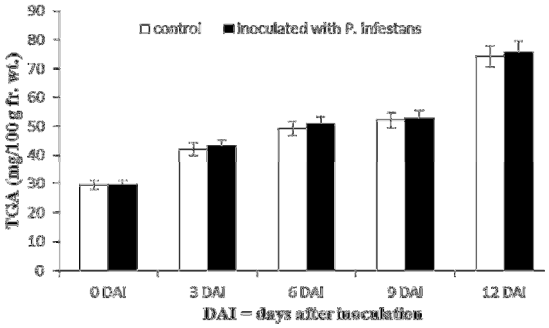

Fig. 2: Total glycoalakloids (TGA) of leaves determined after different days after inoculation with *P. infestans*. Control plants were inoculated with sterile distilled water

Table 1: ANOVA table of mean square for disease severity (%)

Source	Degrees of freedom	Sum of squares	Mean square	F-value	Prob.
Replications	3	0.01	0.003	4.95	0.0268
Treatment	3	9795.06	3265.020*	5966131.65	0.0000
Error	9	0.00	0.001		
Total	15	9795.07			

*Significantly different

Table 2: Effect of disease severity on foliar total glycoalkaloids of potato determined at different inoculation periods. Values in parenthesis in the third column represent percent increase in TGA to control

Disease severity (%)	Days after inoculation (DAI)	Foliar TGA (mg 100 g^{-1} fresh wt.)		LSD value at p=0.05
		Inoculated with *P. infestans*	Control (inoculation with distilled water)	
0	0	29.43[a]	29.43[a]	-
5.263	3	43.08[b] (2.230)	42.14[b]	1.204
23.14	6	50.8[c] (2.970)	49.33[c]	2.86
49.308	9	52.81[c] (1.207)	52.18[c]	0.987
70.135	12	75.67[d] 1.870)	74.28[d]	2.023
LSD value at p=0.05		5.129	4.71	-

Table 3: Effect of days after inoculation (DAI) on Total Glycoalkaloids (TGA) of potato leaves

K value	Source	Degrees of freedom	Sum of squares	Mean squares	F value	Prob
1	Replications	3	1.858	0.619	0.9438	
2	TGA	1	1.069	1.069	1.6292	0.212[ns]
4	DAI	4	8859.782	2214.945	3374.7968	0.0000*
6	TGA x DAI	4	9.690	2.423	3.6911	0.0160*
-7	error	27	17.721	0.656		
	Total	39	8890.120			

ns= non-significant; *=significant at p=0.05
Coefficient of variation 1.62%

concentrations, which increase with plant maturity and become even higher during flowering stage (Friedman, 2006). Similarly environmental stresses and pathogenic attacks may also contribute to variation in glycoalkaloids levels indicating their possible role in defense of host and disease resistance (Andreu *et al.,* 2001).

The mechanism of the potential role of glycoalkaloids in disease resistance and host defense is not well understood. However, it is assumed that changes in glycoalkaloids or other phytoalexins after infection by a pathogen is triggered by either chemicals released from the pathogen or it may be due to the host and pathogen interaction. Defense response of the host to the pathogenic attack results in the production or induction of changes (increase or decrease) in phytoalexins which may correlate with the pathogen in a positive or negative way (Hammerschmidt, 1999). The defense response of the host is generally initiated by elicitors- molecules released by the pathogen or produced by the host in response to pathogen interaction (Hammerschmidt, 1999; Sharma *et al.*, 2011). In turn, the host may possibly produce toxins resistant to the pathogen's growth and feeding; or may trigger other metabolites and enzymes of the host for a prompt response to the stress caused by the pathogen. The antifungal and pesticidal activity of glycoalkaloids, particularly α-solanine and α-chaconine, are presumed to be because of their ability to bind with and disrupt cell membranes of the pathogens (fungi, insects, pest) having high sterols (Martin and Douglas, 1997). Sterol binding, destabilization of cell membranes and inhibition of enzymes by glycoalkaloids in different studies have been confirmed (Roddick *et al.,* 1988, 2001). Differences in sensitivity of glycoalkloids of potato to different pathogens may possibly be because of differential concentrations of cell membrane's sterol contents in different pathogens (Martin and Douglas, 1997). Thus one of the possible answers for the lack of correlation between glycoalkloids of potato and late blight disease caused by *P. infestans*, as indicated in many studies, may probably be due to low sterols contents in cell membrane of *P. infestans*; hence low binding and membrane disruption capacity of these compounds with *P. infestans* (Nes *et al.*, 1983; Roddick, 1987).

In conclusion, foliar total glycoalkaloids contents of potato studied at different timing after inoculation with *Phytophthora infestans* did not varied significantly than foliar TGA contents of control plants and thus, no correlation between disease severity levels and glycoalkaloids contents of potato leaves could be established in our study. However, TGA contents in both control and test plants significantly increased at different inoculation periods, revealing that these compounds are dependent on the age of plants.

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References

Andreu, A., C. Oliva, S. Distel and G. Daleo, 2001. Production of phytoalexins, glycoalkaloids and phenolics in leaves and tubers of potato cultivars with different degrees of field resistance after infection with *Phytophthora infestans. Potato Res*., 44: 1–9

Bourke, A., 1993. '*The Visitation of God*'? The potato and the great Irish famine. Lilliput Press, Ltd., Arbour Hill., Dublin, Ireland

Caten, C.E. and J.L. Jinks, 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. *Can. J. Bot*., 46: 329–348

CIP, 1997. *The International Potato Center Annual Report*, p: 59. International Potato Centre, Lima

Dao, L. and M. Friedman, 1996. Comparison of glycoalkaloid content of fresh and freeze-dried potato leaves determined by HPLC and Colorimetry. *J. Agric. Food Chem*., 44: 2287–2291

Deahl, K.L., R.J. Young and S.L. Sinden, 1973. Relation of late blight resistance to glycoalkaloid content in fifteen potato clones. *Amer. Potato J*., 50: 248–253

Esposito, F., V. Fogliano, T. Cardi, D. Carputo and E. Filippone, 2002. Glycoalkaloid content and chemical composition of potatoes improved with nonconventional breeding approaches. *J. Agric. Food Chem.*, 50: 1553–1561

Frank, J.A., J.M. Wilson and R.E. Webb, 1975. The relationship between glycoalkaloids and disease resistance in potatoes. *Phytopathology*, 65: 1045–1049

Friedman, M. and G.M. McDonald, 1997. Potato glycoalkaloids: Chemistry, analysis, safety, and plant physiology. *Crit. Rev. Plant Sci*., 16: 55–132

Friedman, M., 2006. Potato Glycoalkaloids and Metabolites: Roles in the plant and in the diet. *J. Agric. Food Chem*., 54: 8655–8681

Hammerschmidt, R., 1999. Phytoalexins: what have we learned after 60 years?. *Ann. Rev. Phytopathol*., 37: 285–306

Hijmans, R.J., G.A. Forbes and T.S. Walker, 2000. Estimating the global severity of potato late blight with GIS linked disease forecast models. *Plant Pathol*., 49: 697–705

Hoy, C.W., 1999. Colorado potato beetle resistance management strategies for transgenic potatoes. *Amer. J. Potato Res*., 76: 215–219

Khan, M.S., I. Munir and I. Khan, 2013. The potential of unintended effects in potato glycoalkaloids. *Afr. J. Biotechnol*., 12: 754–766

Lebreton, L., J. Lucas and D. Andrivon, 1999. Aggressiveness and competitive fitness of *Phytophthora infestans* isolates collected from potato and tomato in France. *Phytopathology*, 89: 679–686

Lorito, M., S.L. Woo, I. Garcia, G. Colucci, G.E. Harman, J.A. Pintor-Toro, E. Filippone, S. Muccifora, C.B. Lawrence, A. Zoina and F. Scala, 1998. Genes from mycoparasitic fungi as a source for improving plant resistance to fungal pathogens. *Proc. Natl. Acad. Sci. USA,* 95: 1–6

Martin, L.C. and J.L. Douglas, 1997. Antifungal activity of Solanum fruit glycoalkaloids: implications for frugivory and seed dispersal. *Ecology*, 78: 799–809

Matthews, D., H. Jones, P. Gans, S. Coates and L.M.J. Smith, 2005. Toxic secondary metabolite production in genetically modified potatoes in response to stress. *J. Agric. Food Chem.*, 53: 7766–7776

Mukalazi, J., E. Adipala, T. Sengooba, J.J. Hakiza, M. Olanya and H.M. Kidanemariam, 2001. Metalaxyl resistance, mating type and pathogenecity of *Phytophthora infestans* in Uganda. *Crop Prot.,* 20: 379–388

Naqvi, S.F., M. Inam-ul-Haq, M.I. Tahir and S.M. Mughal, 2012. Screening of sesame germplasm for resistance against the bacterial blight caused by *Xanthomonas campestris* pv. sesami. *Pak. J. Agric. Sci.,* 49: 131–134

Nes, W.D., G.A. Saunders and E. Heftmann, 1983. A reassessment of the role of steroidal alkaloids in the physiology of *Phytophthora*. *Photochemistry*, 22: 75–78

Osman, S.F., 1983. Potato glycoalkaloids. *Food Chem*., 11: 235–247

Pliakhnevich, M. and V. Ivaniuk, 2008. Aggressiveness and metalaxyl sensitivity of *Phytophthora infestans* strains in Belarus. *Zemdirbyste-Agriculture*, 95: 379–387

Roddick, J.G., 1987. Antifungal activity of plant steroids. *In:* Fuller, G. and W.D. Nes (eds.). *Ecology and Metabolism of Plant Lipids*, pp: 286–303. American Chemical Society, Washington, DC, USA

Roddick, J.G., A.L. Rijnenberg and S.F. Osman, 1988. Synergistic interaction between potato glycoalkaloids α-solanine and α-chaconine in relation to destabilization of cell membranes: Ecological implications. *J. Chem. Ecol*., 14: 889–902

Roddick, J.G., M. Weissenberg and A.L. Leonard, 2001. Membrane disruption and enzyme inhibition by naturally-occurring and modified chacotriose-containing Solanum steroidal glycoalkaloids. *Photochemistry,*56: 603–610

Sharma, M., A. Sharma, A. Kumar and S.K. Basu, 2011. Enhancement of secondary metabolites in cultured plant cells through stress stimulus. *Amer. J. Plant Physiol*., 6: 50–71

Sinden, S.L., W.W. Cantelo and R.E. Webb, 1984. Genetic and environmental control of potato glycoalkaloids. *Amer. Potato J*., 61: 141–156

Tingey, W.M., 1984. Glycoalkaloids as pest resistance factors. *Amer. Potato J*., 61: 157–167

Tosun, N., A. Yildrim, H. Turkusay and B. Tanyolac, 2007. Genetic variation among *Phytophthora infestans* (Tomato blight) isolates from western Turkey revealed by inter simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers. *Pak. J. Bot*., 39: 897–902

Turkensteen, L.J., 1978. *Phytophthora infestans*: Three new hosts and a specialized form causing a foliar blight of *Solanum muricatum* in Peru. *Plant Dis. Rep*., 62: 829

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