

Technical Report: Total Glycoalkaloids Analysis in a Late Blight Heat Tolerant (LBHT) Potato Population

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INTRODUCTION

Glycoalkaloids are secondary plant metabolites that serve as natural defenses against pest and diseases (Friedman 2004). They can be toxic for humans when present in high concentrations and can impart a bitter taste to potatoes (Friedman 2006).

Glycoalkaloid levels vary greatly in different potato varieties and may be influenced by factors such as light, temperature, precipitation, and postharvest storage conditions such as mechanical injury and storage (Burgos et al. 2020). They are also influenced by stress conditions such as heat, drought and cold during the plant growth. Hence, increased attention may be needed to evaluate glycoalkaloid concentrations of potato varieties bred for or grown in different abiotic stress conditions.

Glycoalkaloid content in potato tubers should not exceed 20 mg / 100 g FW, because this level is dangerous for human health (Ruprich et al 2009). Due to variations of the glycoalkaloid content according to pre-harvest and post-harvest factors and the individual variations of the toxic dose, it has been proposed that the safe limit must be brought down to a level less than the recommended. According to Van Dam, 2002, glycoalkaloid levels below 7 mg / 100g FW are preferred in new potato varieties.

The International Potato Center (CIP), has developed late blight resistant and heat tolerant potato clones (Gastelo et al., 2019). These clones show adaptation to high temperatures in warm environments, one of the major abiotic factors affecting the production of tubers in potato crop under climate change; and there is the need to monitor the glycoalkaloid concentration of these clones in order to assure that any clone with unsafe levels of glycoalkaloids be removed from the list of late blight resistant and heat tolerant clones recommended for distribution

The main objective of this study was to evaluate the effect of location on the concentration of total glycoalkaloids of thirty-one late blight resistant and heat tolerant clones grown in 3 locations and identify those clones with unsafe levels of glycoalkaloids to remove them from the distribution list.

MATERIAL AND METHODS

Plant material

Thirty-one heat-tolerant CIP-bred clones were planted in three Peruvian locations: 1) Huamachuco, La Libertad 2) San Ramon, Junín, 3) Nueva Independencia, Huánuco. Each clone was planted with three replications in each location.

Sampling and sample preparation

The sample preparation was carried out according to the methodology reported by Porras et al (2014). By each replication and location, ten raw and unpeeled tubers were cut longitudinally into four sections. Two or three slices were taken from each section to obtain a 50 g sample, which was placed in bag plastic and freeze-dried. The dried samples were weighed and ground in a stainless-steel mini mill and stored at -70C in hermetically sealed plastic bags until analysis of total glycoalkaloids was performed.

Glycoalkaloid analysis

Total glycoalkaloid was analyzed using the method described by Hellenas et al. (1986). Small amount freeze-dried and milled potato with peel sample was extracted using methanol and chloroform before concentration at 60°C in a rotary evaporator. The extract was transferred to a 2% acetic acid solution and then purified using ammonium hydroxide at 85 °C and ultracentrifugation at 27,000 rpm. The pellet was reacted with 85% orthophosphoric acid and read at 408 nm. The determination of total glycoalkaloids was achieved against a standard curve of α -chaconine as reference.

RESULTS AND DISCUSSION

Total glycoalkaloids (TGA) in unpeeled tubers of the 31 clones grown in three locations were determined by spectrophotometer and expressed in fresh weight. The TGA concentration of the late blight resistant clones ranged from 1.83 to 49.38 mg / 100 g FW in San Ramon, from 1.55 to 109.60 mg / 100 g FW in Huanuco and from 1.13 to 98.55 mg / 100 g FW in Huamachuco while the TGA concentration of the control varieties ranged from 4.40 to 13.96 mg / 100 g FW in San Ramon, from 1.55 to 3.43 mg / 100 g FW in Huanuco and 1.90 to 3.28 mg / 100 g FW in Huamachuco.

Table 1 shows the 11 late blight resistant and heat tolerant clones that show safe and low TGA concentration (below 10 mg / 100 g FW) in the three locations.

Table 1: TGA concentration (mg/100g FW) in late blight heat tolerant (LBHT) clones with less to 20mg/100 FW obtained in three locations.

	TGA concentration*		
	San Ramon	Huánuco	Huamachuco
CIP312917.096	2.54 ± 0.59	3.19 ± 0.54	1.13 ± 0.11
CIP312925.105	3.03 ± 0.86	1.56 ± 0.17	1.83 ± 0.44
CIP312925.108	3.27 ± 1.02	2.05 ± 0.61	1.40 ± 0.24
CIP312888.048	4.06 ± 0.84	3.29 ± 0.92	5.04 ± 3.72
CIP800048	4.40 ± 0.04	3.43 ± 1.09	3.28 ± 0.66
CIP312917.029	4.79 ± 2.32	1.83 ± 0.25	1.85 ± 0.24
CIP312887.075	5.71 ± 2.49	5.11 ± 0.93	3.41 ± 0.79
CIP312906.102	6.02 ± 0.57	1.84 ± 0.59	1.51 ± 0.16
CIP312927.048	6.22 ± 1.71	3.07 ± 0.96	2.57 ± 0.64
CIP312895.056	6.48 ± 2.77	1.73 ± 0.41	2.48 ± 1.18
CIP312927.017	6.99 ± 2.38	3.06 ± 1.03	4.28 ± 2.05
CIP312923.058	7.25 ± 1.21	2.26 ± 0.11	3.48 ± 0.90

*Data are the average ± standard deviation of three repetition for each sample

Table 2 shows 7 clones that have safe levels of TGZ in the three location (below 20 mg/100 g FW) but at least in one location levels above 10 mg / 100 g FW which can produce a bitter taste in the tubers. Experiments with human taste panels revealed potato varieties with glycoalkaloid levels exceeding 14 mg / 100 g FW tasted bitter (Friedman 2006).

Table 2: TGA concentration (mg/100g FW) in late blight heat tolerant (LBHT) clones with safe levels in the three locations but with with at least one location with levels above 10 mg / 100 g FW

	TGA concentration*		
	San Ramon	Huánuco	Huamachuco
CIP312896.012	1.83 ± 0.84	10.96 ± 1.64	14.93 ± 2.30
CIP312896.133	3.52 ± 1.32	11.89 ± 2.53	12.93 ± 1.11
CIP720201	11.04 ± 3.07	1.97 ± 0.12	1.90 ± 0.66
CIP312895.102	13.89 ± 2.98	7.04 ± 0.43	8.31 ± 1.87
CIP380389.1	13.96 ± 0.74	1.55 ± 0.17	2.60 ± 0.11
CIP312913.022	14.01 ± 5.51	1.92 ± 0.68	4.15 ± 1.09
CIP312903.077	15.27 ± 3.55	6.92 ± 0.26	7.32 ± 2.18
CIP312903.094	16.84 ± 2.13	4.29 ± 0.57	8.74 ± 3.80
CIP312899.078	17.89 ± 4.87	13.03 ± 7.06	11.45 ± 3.94

*Data are the average ± standard deviation of three repetition for each sample

Table 3 shows 10 clones with levels of TGA that are non-safe for consumption (above 20 mg / 100g FW) at least in one location. These clones should be removed from the list of late blight resistant and heat tolerant clones recommended for distribution as they could express toxic levels of glycoalkaloids. These clones should not be recommended for variety release despite its good resistant to late blight and heat tolerance.

Table 3: TGA concentration (mg/100g FW) in late blight heat tolerant (LBHT) clones upper to 20mg/100 FW obtained in three locations.

	TGA concentration*		
	San Ramon	Huánuco	Huamachuco
CIP312889.018	23.27 ± 2.32	28.34 ± 5.11	18.50 ± 8.24
CIP312900.155	24.61 ± 3.54	33.38 ± 1.12	45.64 ± 4.38
CIP312888.039	25.85 ± 5.97	23.31 ± 4.75	13.38 ± 4.05
CIP312905.156	28.31 ± 11.62	13.14 ± 2.42	24.52 ± 7.15
CIP312889.082	28.93 ± 4.87	109.60 ± 17.90	72.40 ± 20.20
CIP312909.046	32.00 ± 7.34	16.97 ± 1.39	20.98 ± 3.11
CIP312903.013	41.30 ± 17.50	5.73 ± 2.05	7.28 ± 1.15
CIP312903.066	43.82 ± 5.86	47.11 ± 2.88	24.38 ± 2.93
CIP312898.077	45.10 ± 9.15	25.35 ± 5.20	14.93 ± 1.68
CIP312890.040	49.38 ± 15.32	107.02 ± 10.35	98.55 ± 6.73

*Data are the average ± standard deviation of three repetition for each sample

The mean TGA concentration of the late blight resistant clones was higher in San Ramon (16.96 mg / 100 g FW) than in Huánuco (14.39 mg / 100 g FW) and in Huamachuco (14.12 mg / 100 g FW). A study developed by Fogelman, E., et al 2019 evaluated and monitored the levels of secondary metabolites as glycoalkaloids under high-temperature conditions, and concluded that the heat treatment affected the level of secondary metabolites. Fogelman suggests that the response of health-related secondary metabolites to high temperatures must be monitored to enable future manipulation of new potatoes with improved adaptation to the environmental constraints prevailing in warm regions. San Ramon has a mean temperature (24°) higher than Huamachuco (18°) and Huanuco (15°) and this could have promoted the glycoalkaloid production in this location. However, it is important to mention that although the mean TGA for San Ramon location is higher than in Huanuco and San Ramon, the TGA concentration in each location depends of the clone. A statistical analysis of the TGA results together with agronomical values will be performed and reported separately by Manuel Gastelo.

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