USE OF ACETYLSALICYLIC ACID AND AGRONOMIC PERFORMANCE OF POTATOES IN LIMA REGION

Abstract

Background: Salicylic acid participates in the regulation of the plant's response to a series of environmental stresses such as extreme temperatures, salinity, and oxidative condition of potato production, so it is necessary to determine a safe application dosage for potato in field conditions.

Methods: The purpose of the research was to evaluate the effect of increasing doses of acetylsalicylic acid on the agronomic traits of two potato cultivars in Lima, Peru. In a field experiment, an RCBD with 3 replications was used under a split-plot arrangement in which five doses (0.0, 0.2, 0.4, 0.6, and 0.8 mM) of acetylsalicylic acid plus control with Biol (biostimulant) were assigned to plots, and two potato cultivars ('Perricholi' and 'Única') were assigned to subplots. The average temperatures were between 15 and 21° C, the air relative humidity between 61 and 73% and the soil was loam textural class, free of salts.

Results: Statistical differences (p <0.05) were found between the two potato cultivars for most of the characters studied. The effects of doses of acetylsalicylic acid showed statistical differences for sprouting, vegetative vigor, and percentage of flowering; likewise, significant interactions (p <0.05) were shown between potato cultivars and dose of acetylsalicylic acid for the number of stems per plant and percentage of flowering, which would indicate a specific effect for the dose of acetylsalicylic acid in each potato cultivar.

Conclusion: No dose of the product significantly affected the potato yield in two potato cultivars however, the effect of the dosage of acetylsalicylic acid improved the performance of the crop in terms of sprouting in the field, vegetative vigor, a greater number of stems per plant as well as stimulation of flowering, with respect to control.

Keywords: Potato, agronomic performance, salicylic acid, field experiment

Introduction

Background

The potato (*Solanum tuberosum*) is the main food of the Andean people (BID, 2020; FAO 2013). This region is the center of origin of the potato and its wild relatives (de Haan and Rodríguez, 2016), so it is convenient to develop new agronomic management technologies with less impact on the ecosystem since the use of chemical pesticides in the potato is increasing in developing countries as farmers intensify production (FAO 2008a). Likewise, the virtues of the potato, in particular its great nutritional value and its ability to increase farmers' income, have not received the attention they deserve from governments, therefore it is necessary to invest in new technologies with the potential to reduce crop risks (FAO, 2008b), as climate change has a significant effect on the magnitude and frequency of the incidence of pests and diseases in potato (Quiroz et al. 2018).

Some signals from low molecular weight compounds, including jasmonic acid, ethylene, and salicylic acid, regulate the expression of defense-related genes. With the use of these regulators, the plant refines its expression of defense genes against aggressors that, in some cases, may be able to alter or expand defense signal pathways (Raymond and Farmer, 1998). Salicylic acid (SA) is a molecule related to the stress response in plants (Hayat and Ahmad, 2007) and therefore considered a candidate for exogenous applications as an activator of induced systemic resistance. In particular, acetylsalicylic acid (the active ingredient in aspirin) is a phenolic compound analogous to SA, which has been identified as a low-cost, non-phytotoxic product (Raskin, 1992). Typically caused by local infection, plants respond with a cascade of salicylic acid-dependent signaling that leads to systemic expression of broad-spectrum resistance and long-lasting resistance that is effective against fungi, bacteria, and viral infections.

In the words of Walters et al. (2013), induced resistance has the potential to revolutionize disease control in crops, but it remains an unconventional type of crop protection. The results of numerous investigations conducted over the last two decades have shown that SA plays an important role in various aspects of defense responses after a pathogen attack (Vlot et al., 2009). These include, among others, the activation of cell death, the expression of pathogen resistance proteins, as well as the induction of local and systemic resistance to diseases (Hayat and Ahmad, 2007).

SA stimulates resistance to viral replication, intercellular spread, and systemic movement in plants (Murphy et al., 2020). The efficiency in obtaining potato seedlings without virus infection by thermotherapy increased from 30.6% to 72.2% in seedlings treated with salicylic acid (Gonzalez and Huarte, 2011). The importance of SA in the viral multiplication of PVY and in symptoms was confirmed by Baebler et al. (2011) with the development of pronounced symptoms in a NahG-Desiree transgenic potato variety devoid of salicylic acid and the reversal of the effect after spraying with 2,6-dichloro isonicotinic acid, a compound analogous to salicylic acid. Halim et al. (2007) determined in transgenic NahG potato plants, incapable of accumulating salicylic acid, a drastic increase in the growth of pathogens and increased susceptibility of the transformed plants. These same plants previously treated with the salicylic acid analog 2,6 dichloro-isonicotinic acid allowed the growth of pathogens to a level similar to that of non-transgenic plants, indicating that salicylic acid is an important compound that is required for basal defense of the potato against late blight. *B. subtilis*, individually and in composition with SA, increased ascorbic acid content and decreased pathogen-induced proline accumulation and lipid peroxidation, for postharvest diseases (*Phytophthora infestans*, *Fusarium oxysporum*) development in stored potato tubers (Lastochkina et al. 2020).

Salicylic acid treatments significantly stimulated ethylene production in potato slices during 24 hours of aging. With 90 uM of salicylic acid, the stimulation was positively correlated with the applied concentrations (Liang et al. 1997). Salicylic acid showed a maximum stimulating effect on ethylene production at pH 6.4. This effect of salicylic acid is different from the general view that salicylic acid ultimately inhibits ethylene biosynthesis in plants. Zhang et al. (2013) consider that the enzyme salicylic acid 3-hydroxylase regulates the longevity of the leaf in Arabidopsis through the catabolism of salicylic acid.

According to González-Gallegos et al. (2015), the production of salicylic acid in potato plants was manifested by inoculation with *Bacillus* spp. and *Pseudomonas fluorescens*, which led to increased resistance to plant diseases. Numbers and size of lesions per plant was reduced by the application of SA against *Phytophthora infestans* in potatoes and plants treated with SA had higher peroxidase and polyphenol oxidase activity (Ghazanfar et al. 2020). Zhou et al. (2018) demonstrated that the gene StbZIP61 functions in concert with StNPR3L to regulate the temporal activation of SA biosynthesis, which contributes to SA-mediated immunity against *P. infestans* infection in potatoes.

Makarova et al. (2018) suggested that responses to heat stress and PVY infection in potatoes have some common underlying mechanisms, which may be integrated into a specific consolidated network that controls plant sensitivity to multiple stresses in a cultivar-specific manner, and that the SA pretreatment subverted the sensitive combined (heat and PVY) stress phenotype in potatoes implicating SA as a key component of such a regulatory network. Li et al. (2019) indicated that exogenous SA can play a positive regulatory role in alleviating Cd toxicity in potato plants.

Despite the high basal levels of salicylic acid that potatoes naturally have, this crop can respond to exogenous application of salicylic acid at a low concentration when sprayed on plants. However, plants grown in field conditions seem less sensitive at certain times (Navarre and Mayo, 2004). It is necessary to continue exploring the signaling pathways of this molecule in the context of resistance to diseases in crops (Delaney et al. 2007) and to evaluate the dose-effect of salicylic acid since, like any phytohormone, it requires a specificity for its action in the induction of defenses in plants (Van Loon, 2008). The weakness of salicylic acid is that its life inside the plant is very short, being immobilized in the cell walls, which is why routine application is necessary throughout the life of the crop in order to maintain high levels of resistance (USAID, 2006). The role of SA in potato and its involvement in SAR signaling have been an ongoing debate because, unlike tobacco and Arabidopsis, potato is considered to display high levels of endogenous SA (Aleksandersson et al. 2016). The objective of the present investigation was to determine response to exogenous application of acetylsalicylic acid -a pharmacological compound derived from salicylic acid- on the agronomic performance of two commercial potato cultivars under cultivation conditions in Lima, Peru.

Material and Methods

Description of the experimental site: The experiment was carried out under field conditions during the winter season in the district of Imperial, province of Cañete (Peru). The average temperatures were in the range between 15 and 21º C, the relative humidity between 61 and 73%, with an average of 2 hours of sunshine per day and 3 mm of evaporation per day. The soil was of a loamy textural class, pH 7.5, low content of organic matter, high content of phosphorus, and medium in potassium (Table 1). A fertilization formula of 100-80-120 and the equivalent of one ton of dry manure per hectare was applied, according to the soil analysis carried out.

Materials: Two potato cultivars, "Unica" (CIP 392797.22) and "Perricholi" (CIP 374080.5) provided by the International Potato Center (Lima), which are commercial varieties for direct consumption in Peru, were used as plant material. The seed tubers were disinfected with Benlate (Benomyl) and when they were properly sprouted, they were applied with the selected treatments and sown. On the other hand, the doses of acetylsalicylic acid were formulated using the commercial pharmacological product aspirin® (diluted previously in ethanol 90º) at concentrations of 0.2, 0.4, 0.6, and 0.8 mmol L^{-1} in filtered water (Table 2). An organic treatment with Biol (liquid commercial product) at a concentration of 1% was included, in addition to the absolute control without acetylsalicylic acid. Agricultural pesticides were also used to control the leaf miner fly (*Liriomy*za sp.), hyaline mite, and *Alternaria* sp., as part of the control of pests and diseases in the field.

Form of application of the treatments: Five applications were made during the vegetative period of the crop (105 days). The first application was in seed tuber immersion; the following applications of the treatments were sprayed on the foliage at the doses considered in table 2, with a fortnightly frequency from the sprouting in the field. The volume of water applied was dependent on the size of the foliage of the plants, maintaining the concentration of the treatments. The plots with the control treatment were applied with running water.

Characteristics evaluated: The following agronomic characteristics were evaluated: plant density per experimental unit 30 days after sowing, number of stems per plant at 45 days, vegetative vigor (phenotypic scale 1-9), plant height, percentage of flowering, percentage of plants with virus (the incidence data of plants with virus symptoms were transformed to square root), percentage of survival to harvest, the weight of foliage per plant, number of tubers per plant, weight of tubers $plant^{-1}$ and total weight of biomass (kg ha^{-1}).

Experimental Design and Statistical Analysis: A Random Complete Blocks Design with three replications was used, under an arrangement of split-plots, in which the acetylsalicylic acid treatments and the control were assigned to complete plots (six) and the cultivars (two) to subplots. The size of the basic experimental unit (subplot) was 60 plants per treatment (18.5 m2) and the total experimental area was 640 m^2. The data were statistically analyzed to determine the significance of the main effects and interactions at a 95% confidence level, using analysis of variance. The means of the treatments were compared using the Tukey test and the data were processed with the Infostat program, version 2019.

Results

In table 3, it can be shown that the treatments applied with acetylsalicylic acid and Biol affected the variability of agronomic characters such as plant density per experimental unit, vegetative vigor, and the percentage of flowering, and there were some significant interactions between the potato cultivars and the treatments applied with acetylsalicylic acid, in plant density, number of stems per plant, and flowering.

There were statistical differences for the potato genotypes in the characters studied, such as sprouting, number of stems per plant, plant height, percentage of flowering, the incidence of viruses, weight of foliage, weight of tubers, number of tubers, and plant survival to harvest (tables 3 and 4).

There were no significant differences for the acetylsalicylic acid and Biol treatments in terms of biomass yield per hectare, nor their components of number and weight of tubers per plant; there were also no statistical differences in the fresh weight of the foliage as a result of the inducing treatments (table 4).

For plant density per experimental unit, it is noted in table 5 that Biol and acetylsalicylic acid treatments at a dose of 0.4 mmol significantly increased sprouting in both cultivars compared to the control. In the case of the number of stems per plant at 30 days, significant differences were observed between doses and in the interaction of doses and cultivars.

Likewise, in the number of stems per plant, the treatments with Biol or with the dosage of acetylsalicylic acid between 0.2 to 0.4 mmol had a significant effect in the Perricholi variety for this character in relation to the control without application, not so in the cv. Unica where no significant differences were evidenced to stimulate sprouting per plant (table 5).

The vegetative vigor showed an interaction effect between potato cultivars and dosage as observed in table 5; 0.4 mmol dose of acetylsalicylic acid had a better effect on vigor for the Perricholi cultivar than in the control treatment without application for cv. Única.

Flowering was stimulated by the effect of the application of acetylsalicylic acid in cv. Perricholi at a dose of 0.4 mM, as can be seen in table 6 where this treatment statistically surpassed the control and the application of Biol; cv. Unica did not show flowering.

It can be seen in table 6, that the acetylsalicylic acid and Biol treatments did not significantly affect the biomass per hectare (fresh weight of tubers and foliage), number and weight of tubers per plant in both cultivars under field conditions.

Discussion

The results show that the effects of the application of acetylsalicylic acid in the evaluated potato cultivars were mainly on characters related to the physiology of the plant such as sprouting, plant density, vigor, and flowering. This may be related to the fact that the potato responds to the exogenous application of salicylic acid at a relatively low concentration (Navarre & Mayo, 2004). The doses used in the established application frequencies did not significantly affect the weights of tubers, foliage, and total biomass, so they can be considered safe for field application. It is considered that the plant uses part of the energy in the systemic resistance and could have production loss problems due to overdosing; likewise, an overdose of salicylic acid can stress the plant, causing a reduction in yield, as also considered by USAID (2006).

Regarding the aspects related to the production of tubers and potato biomass per hectare, no differences were found for the dosage used of acetylsalicylic acid in the present investigation, although some authors affirm that salicylic acid can improve the production of photosynthates in potatoes under conditions of temperature stress and protect potato plants from damage caused by phytoplasmas, thus improving the photosynthetic assimilation of the tuber (Sanchez Rojo et al., 2011), thermo-tolerance *in vitro* (López Delgado et al., 2007), tolerance to heat and PVX (López Delgado et al., 2004), induced resistance to *P. infestans* (Ghazanfar et al. 2020), preventing postharvest diseases development in stored potato tubers (Lastochkina et al. 2020), or tuber rot caused by *Erwinia* sp. (Lopez-López et al., 1995).

Fakhimi et al. (2020) showed that exogenous application of salicylic acid increased the expression level of genes and lead to enhancement of plant tolerance to salinity stress; the efficacy of salt tolerance of salicylic acid was proven to improve potato yield under salt stress, according to Faried et al. (2017), recommending using a dose of salicylic acid at 0.5 mmol L^{-1} in potato under saline soil, a similar concentration of salicylic acid as we found in the present research to improve agronomic characters.

On the other hand, unlike what was found by Gonzáles & Huarte (2011) and Murphy et al., (2020), no significant differences were shown in terms of the incidence of viruses in potato cultivars due to the effect of acetylsalicylic acid, but this could be due to various factors, among which we can highlight the potato genotypes used that could have differences in terms of the degree of susceptibility to virosis, as well as the incidence of the virus under field conditions.

Conclusions

The dose of 0.4 mmol (72 mg L^{-1}) was the most appropriate to apply acetylsalicylic acid during potato cultivation to improve sprouting under field conditions, plant density per unit area, vegetative vigor, and flowering, although it is necessary to test these doses in other environmental conditions since plants grown in field conditions seem to be less sensitive to exogenous applications of salicylic acid or its derivatives at certain times. At the mentioned dose, both the yield and the biomass produced by the crop were not significantly affected.

Abbreviations

CIP: International Potato Center; CV: Coefficient of Variation; dF: degrees of freedom, mM: mMol; BID: International Development Bank; RCBD: Randomized Complete Block Design; PVY: potato virus Y.

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Tables and figures

Table 1 Soil Analysis in the experimental area

Parameter	EC	Texture	pH	$CaCO_3$	OM	P	K	CIC	PSI	Fe
Units	dS/m			%	%	ppm	ppm		%	ppm
	1.85	Fr.A	7.67	2.14	0.85	8.99	200	11.27	3.16	34.10

Table 2 Application treatments with acetylsalicylic acid

Code	Treatment	[Concentration][1]
0	Control	No app
1	Biol[2]	1%
2	Acetylsalicylic acid	0.2 mmol
3	Acetylsalicylic acid	0.4 mmol
4	Acetylsalicylic acid	0.6 mmol
5	Acetylsalicylic acid	0.8 mmol

[1] Taking the commercial formulation of the product and the molecular weight of acetylsalicylic acid.
[2] A commercial dose of the Biol was used.

Table 3 ANOVA for agronomic traits in 2 potato cultivars under the effect of acetylsalicylic acid

Sources	dF	Mean Squares				
		PD	NSP	VV	HP	PV
Blocks	2	ns	ns	*	ns	ns
Cultivars	1	*	*	ns	*	*
Treatments	5	*	ns	*	ns	ns
Error a	10					
Cultivar/Treatment	5	*	*	ns	ns	ns
Error b	12					
Total	35					
Means		87,90	2,63	7,06	47,42	2,24
R^2		0,87	0,83	0,86	0,93	0,88
CV, %		17,13	13,05	4,02	9,65	29,7
Units		n	n	Scale 1-9	cm.	$\sqrt{(\%+1)}$

ns = non significant mean squares, * = significant mean squares (P <0.05). PD = density of plants per experimental unit at 15 days, NS = number of stems/plant at 30 days, VV = vegetative vigor (phenotypic scale 1-9), HP = plant height at 60 days in cm., PV = percentage of plants with viruses (square root transformed data). R^2 = coefficient of determination, CV % = coefficient of variation

Table 4 ANOVA for productive traits in 2 potato cultivars under the effect of acetylsalicylic acid

Sources	dF	Mean Squares					
		PF	WFP	NTP	WTP	PSl	BIO
Blocks	2	ns	*	*	ns	ns	*
Cultivars	1	*	*	*	*	*	*
Treatments	5	*	ns	ns	ns	ns	ns
Error a	10						
Cultivar/Treatment	5	*	ns	ns	ns	ns	ns
Error b	12						
Total	35						
Means		19,04	0,31	5,4	0,48	93,17	29 601
R2		0,99	0,78	0,71	0,77	0,87	0,77
CV, %		16,48	20,74	26,23	19,97	3,92	17,68
Units		%	kg	n	kg	%	kg

ns, non-significant mean squares, *, significant mean squares (*P* <0.05). PF = percentage of flowering, PS= percentage of plants surviving to harvest, WFP = fresh weight of foliage per plant, NTP = number of tubers per plant, WTP =weight of tubers/plant, BIO = total weight of biomass (kg ha^{-1}).

Table 5 Effect of acetylsalicylic acid dose on agronomic traits of two potato cultivars

Cultivar	Dose	**PD**	**NSP**	**VV**	**HP**	**PV**
Perricholi	0.0	56,0^{b}	1,75^{b}	7,67^{a}	53,23^{ab}	2,90^{abc}
	0.2	105,3^{ab}	3,09^{a}	7,27^{ab}	59,27^{a}	3,87^{a}
	0.4	109,3^{a}	2,97^{a}	7,20^{ab}	59,61^{a}	2,97^{abc}
	0.6	71,0^{ab}	2,14^{ab}	7,07^{ab}	63,53^{a}	2,93^{ab}
	0.8	88,3^{ab}	2,69^{ab}	6,90^{ab}	60,37^{a}	3,67^{abc}
	Biol	84,3^{ab}	2,51^{ab}	6,87^{ab}	60,16^{a}	2,83^{abc}
Unica	0.0	103,3^{ab}	2,93^{a}	7,23^{ab}	39,31^{bc}	1,30^{c}
	0.2	98,7^{ab}	2,85^{ab}	7,23^{ab}	34,55^{c}	1,00^{c}
	0.4	107,7^{a}	2,82^{ab}	7,20^{ab}	41,06^{bc}	1,00^{c}
	0.6	106,7^{ab}	2,95^{a}	7,17^{ab}	43,58^{bc}	1,60^{bc}
	0.8	67,3^{ab}	2,67^{ab}	7,07^{ab}	42,20^{bc}	1,00^{c}
	Biol	112,0^{a}	2,99^{a}	6,57^{b}	40,27^{bc}	1,60^{bc}
Std.Error		9,15	0,2	0,17	2,77	0,38

Means with the same letter in columns are not significant (*P*<0.05)

Table 6 Effect of acetylsalicylic acid dose on production-related parameters of two potato cultivars

Cultivar	Dose	PF	WFP	NTP	WTP	PS	BIO
Perricholi	0.0	$38{,}07^{b}$	$0{,}25^{a}$	$3{,}72^{a}$	$0{,}35^{a}$	$91{,}2^{abc}$	$21666{,}7^{a}$
	0.2	$40{,}65^{ab}$	$0{,}39^{a}$	$5{,}73^{a}$	$0{,}52^{a}$	$86{,}8^{c}$	$31508{,}8^{a}$
	0.4	$50{,}97^{a}$	$0{,}39^{a}$	$5{,}43^{a}$	$0{,}48^{a}$	$88{,}6^{abc}$	$30877{,}2^{a}$
	0.6	$41{,}06^{ab}$	$0{,}40^{a}$	$5{,}01^{a}$	$0{,}43^{a}$	$91{,}2^{abc}$	$30140{,}4^{a}$
	0.8	$48{,}64^{ab}$	$0{,}29^{a}$	$4{,}69^{a}$	$0{,}35^{a}$	$87{,}7^{bc}$	$22438{,}6^{a}$
	Biol	$38{,}99^{b}$	$0{,}43^{a}$	$5{,}92^{a}$	$0{,}49^{a}$	$89{,}5^{abc}$	$32701{,}8^{a}$
Unica	0.0	nd	$0{,}29^{a}$	$6{,}46^{a}$	$0{,}56^{a}$	$99{,}1^{abc}$	$33964{,}9^{a}$
	0.2	nd	$0{,}30^{a}$	$5{,}13^{a}$	$0{,}58^{a}$	$100{,}0^{a}$	$35263{,}1^{a}$
	0.4	nd	$0{,}29^{a}$	$6{,}19^{a}$	$0{,}54^{a}$	$99{,}1^{ab}$	$32754{,}4^{a}$
	0.6	nd	$0{,}31^{a}$	$6{,}64^{a}$	$0{,}61^{a}$	$97{,}4^{abc}$	$35754{,}4^{a}$
	0.8	nd	$0{,}26^{a}$	$6{,}52^{a}$	$0{,}53^{a}$	$100{,}0^{a}$	$31543{,}9^{a}$
	Biol	nd	$0{,}28^{a}$	$6{,}37^{a}$	$0{,}55^{a}$	$100{,}0^{a}$	$33228{,}1^{a}$
Std.Error		2,05	0,04	0,86	0,06	2,13	3163,32

Means with the same letter in columns are not significant (*P*<0.05). nd = cv. Unica did not show flowering.