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Agronomic biofortification of potato through Zn foliar and soil fertilization

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Abstract

Zinc is an essential micronutrient in human health; however, its deficiency is considered a limiting factor for growth and development in children, especially in developing countries. Therefore, agronomic biofortification, the practice of applying fertilizers to crops to increase micronutrient concentrations in the harvested product, seeks to reduce the low intake of zinc in diets. Experimental plots in La Molina and Cañete, Peru were used to test agronomic biofortification in potato through soil and foliar fertilization. Three experiments were conducted, comprising 6 potato fields cultivated under furrow irrigation in the coastal region, characterized by an arid climate, low rainfall, and sparse natural vegetation. Experiment 1 included 3 fields with a complete block design and a factorial arrangement of 3 soil-applied Zn doses (0, 20, and 40 kg ha^{-1}) and 2 foliar Zn doses (0 and 5 kg ha^{-1}). Experiment 2 involved two fields with a complete block design, where foliar fertilization was applied at i) 0, 4, 8, and 16 kg ha^{-1} and ii) 5 and 10 kg ha^{-1}, fractionated into 4 and 8 applications. Experiment 3 was carried out using a complete block design with soil-applied Zn doses of 0, 10, 20, and 40 kg ha^{-1}. Zinc concentrations were increased in potato tuber flesh and skin employing fertilization methods. A more significant response to foliar fertilization than to soil fertilization was observed, soil being a limiting factor in fertilization efficiency. In general, foliar applications can increase Zn levels in pulp by 40%. Potato yield was not affected by soil applications, but foliar fertilization can affect yield.

1. Introduction

The levels of available micronutrients in soils represent important factors in determining micronutrient content in crops. Its deficiency limits nutrient uptake in plants and ultimately humans (Dhaliwal et al., 2022). In general, crops growing in poor soils have a lower concentration of micronutrients in their edible parts (Miller and Welch, 2013). Therefore, micronutrient application is necessary to correct soil deficiencies and improve crop yield.

Because of the deficiency of zinc in humans, some approaches are used to increase its content, in conjunction with other micronutrients needed in the daily diet. A recent global analysis estimates that half of all preschool-aged children are affected by deficiencies in at least one of three micronutrients (Zn, Fe, Vit. A) (Stevens et al., 2022). Some of them are food supplements, fortified foods, diet diversification and biofortification (Das and Green, 2016; Veena and Puthur, 2021). The first two require infrastructure, access to markets and health centers, as well as to have a greater purchasing power (Das and Green, 2016; Walker, 2014). Therefore, it is considered a disadvantage for many low-income families dependent only accessing small amounts of food with very limited availability of nutrients, making them vulnerable to growth and cognitive development problems (Ul-Allah, 2018).

A relatively new strategy is biofortification of staple crops, practiced in different parts of the world since the 1990s (Mishra et al., 2022), with the importance of supplying nutritious foods (quality) rather than adequate calories (quantity) (Freire et al., 2020). It involves the use of agronomic and plant breeding methods like the application of micronutrient-enriched fertilizers to elevate the concentrations of key minerals in the food crops that are part of the family diet (Bouis et al., 2011; Bouis and Saltzman, 2017; Garg et al., 2018; Praharaj et al., 2021; Stangoulis and Knez, 2022). Biofortification offers two key comparative advantages: long-term cost-effectiveness and the ability to reach disadvantaged rural populations (Bouis and Saltzman, 2017). Furthermore, the consumption of biofortified staple foods complements existing nutrition strategies, as it does not fully meet the total daily requirements for minerals and vitamins, unlike food supplements or industrially fortified foods. Consequently, biofortification can naturally contribute to the daily intake of micronutrients throughout the consumers' lifetime (Bouis, 2018).

The importance of wheat flour in the daily diet has driven numerous studies demonstrating that biofortification methods using zinc sulfate, in soil and foliar levels, cause significant change in crops, by increasing the content of this nutrient in the grain (Cakmak and Kutman, 2018; Prasad and Shivay, 2020; Ram et al., 2024; Suganya et al., 2020). Foliar applications of Zn have been shown to be more efficient than soil applications in agronomic biofortification (Chattha et al., 2017; Hidoto et al., 2017; Melash and Mengistu, 2020; White and Broadley, 2011). Similarly, crops show a better response to oxidative stress induced by water deficit (Sattar et al., 2022). Signorell et al. (2019) stated that foliar application of zinc in wheat can enhance dietary zinc absorption in humans. It should be considered that each crop requires different amounts of nutrients, and the application of Zn varies from one crop to another (Younas et al., 2022).

Potatoes, rice, bananas, cassava, sugarcane, corn (as kernels and starchy form), pineapple, sweet potato, wheat, tomato, and barley are the crops most consumed in Peru (Ortiz, 2010). Highlighting the potato for its affordability, nutrition, good source of vitamin C (ascorbic acid), protein and high carbohydrate energy source (Singh et al., 2021). In addition, the bioavailability of micronutrients in potatoes is potentially high because of the low concentrations of compounds that limit their absorption, i.e., phytates and oxalates (White et al., 2009).

Considering the successful application of zinc biofortification in wheat and the fact that potato is a staple food in the Peruvian diet, we hypothesized that agronomic zinc biofortification would increase Zn content and enhance the nutritional value of potato tubers, representing an accessible strategy for farmers. Therefore, the present study aimed to evaluate the increase in Zn content in tubers by applying zinc sulphate through foliar and soil fertilization in potato varieties 'UNICA' and 'Yungay'.

2. Materials and methods

2.1. Experimental tests

During the 2017–2018 seasons, six experimental trials were installed in the Department of Lima, Peru. Four of them at the Universidad Nacional Agraria La Molina (UNALM), in Lima, (Fields 1, 2 and 3). The other two were placed at the Regional Development Institute located in the city of Cañete (Field 4). Soil characteristics are presented in Table 1. Soils at the UNALM exhibited calcium carbonates, high zinc, and high phosphorus. On the contrary, soil in Cañete showed a minimum amount of calcium carbonates, low zinc content, and medium amounts of phosphorus content. In addition, Table 2 summarizes the three experiments (Experiment 1, 2, and 3).

According to the Holdridge Life Zone System (Holdridge, 1967), the region is classified as a Subtropical Very Dry Desert, which corresponds to an arid environment with scarce vegetation and minimal precipitation. The Fig. 1 shows the average, maximum, and minimum air temperatures, as well as relative humidity, during the crop growth period. Precipitation was 0 mm throughout the entire growing season.

The 'Yungay' variety, released in 1971, has an erect growth habit, oval-shaped tubers with yellow flesh, a late vegetative period (up to 180 days), and yields of up to 58 Mg ha^{-1}. It is adapted to altitudes up to 3,700 m a.s.l. and is the most widely cultivated potato variety. The 'UNICA' variety, released in 1998, has a low growth habit, elongated tubers with ivory-white flesh, a semi-early vegetative period (90 to 110 days), and yields of up to 40 Mg ha^{-1}. It is adapted to altitudes up to 3,800 m a.s.l. (Egusquiza, 2014; Gutiérrez-Rosales et al., 2007).

Table 1
Physicochemical characteristics of the fields used in the experiments

Characteristics	Method	Field 1	Field 2	Field 3	Field 4
Soil Texture Class	Bouyoucos	Loam Clay Sand	Loam Clay Sand	Loam Clay Sand	Loam Clay Sand
pH	1:1 soil-water	8.11	8.2	7.82	7.74
CE dS m^{-1}	1:1 soil:water	1.35	1.09	0.71	0.51
SOM %	Walkey & Black	2.17	1.84	1.29	0.75
$CaCO_3$ %	Gas-Volumetric	5.4	7.8	3.0	0.3
P extractable mg kg^{-1}	Olsen modified	30.1	16.9	16.2	11.4
K extractable mg kg^{-1}	Ammonium Acetate	262	250	129	174
CIC cmol$_c$kg^{-1}	Ammonium Acetate	14.08	10.88	13.92	12
Zn extractable mg kg^{-1}	Olsen modified	24.2	21.8	14.55	0.37
Fe extractable mg kg^{-1}	Olsen modified	44.6	11.8	13	43.8

Table 2
Experimental design and treatments

Exp.	Location / Season	Variety	Design	Field	Soil Zn (kg ha⁻¹)	Foliar Zn (kg ha⁻¹)	Description / Frequency
1	La Molina (2017, 2018), Cañete (2018)	UNICA	RCBD (Factorial 3x2)	1,2,4	0, 20, 40	0, 5	Factorial combination (6 treatments). Foliar applied at 40, 47, 54, 61 DAP.
2a	La Molina (2017)	Yungay	RCBD	3	–	0, 4, 8, 16	Applied from 40 DAP (4 times). High doses (8, 16) split into 6 apps.
2b	Cañete (2017)	UNICA	RCBD	4	–	0, 5, 10	Applied from 42 DAP. Variable frequency (every 4–5 days or 8–10 days).
3	La Molina (2017)	Yungay	RCBD	3	0, 10, 20, 40	–	Soil application at planting only.

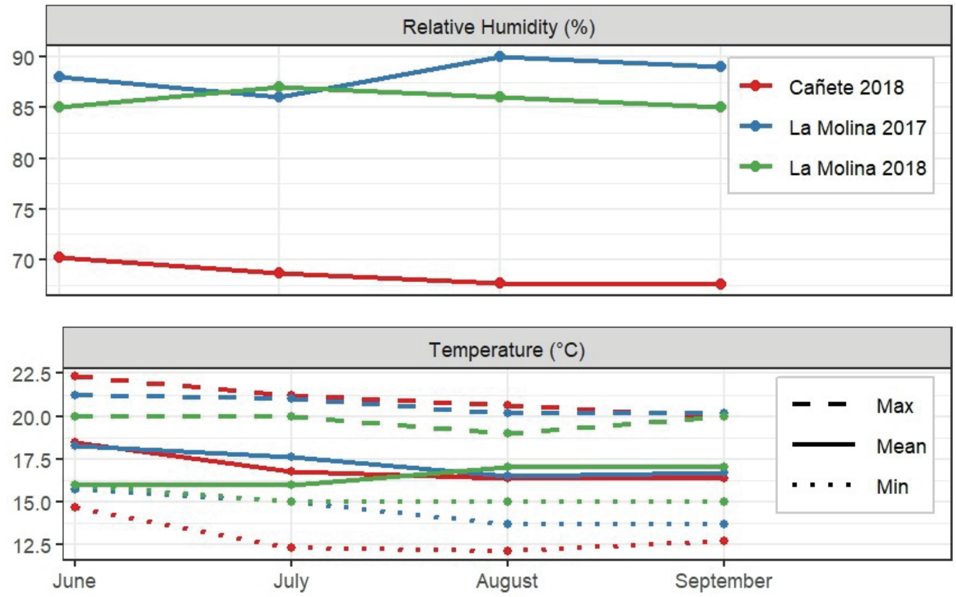

Fig. 1. Monthly variation of temperatures and relative humidity in Cañete and La Molina (2017–2018)

2.1.1. Experiment 1

In June 2017 (field 1) and June 2018 (field 2), two plots were installed in La Molina. In May of 2018, one plot was installed in Cañete (field 4). The trial consisted of 3 doses of soil Zn (0, 20, and 40 kg Zn ha^{-1}) and 2 doses of foliar Zn (0 and 5 kg Zn ha^{-1}), arranged in a randomized complete block design in a factorial arrangement (3x2) for a total of 6 treatments. Seed tubers were planted at a spacing of 30 cm and the distance between rows was 1 m. Zinc sulfate heptahydrate (21% Zn) was applied manually at planting and placed at the bottom of the furrow, halfway between adjacent seed tubers (15 cm), together with the other fertilizer, at the same depth as the seed tubers. Foliar fertilizations were done at 40, 47, 54 and 61 days after planting. The variety used here was 'UNICA', which is known for earliness with a harvest time of 130 days.

2.1.2. Experiment 2

During the 2017–2018 seasons, two plots of Zn fertilization through foliar applications were installed:

a) For 'Yungay' variety, in La Molina (field 3), doses of 0, 4, 8 and 16 kg Zn ha^{-1} were applied from the 40th day after planting (DAP), at intervals of 8 days, up to 4 times. For doses 8 and 16, the applications were split into 6 times to avoid stress to the plant due to high concentrations of the fertilizer.
b) For 'UNICA' variety, in Cañete (field 4), two doses (5 and 10 kg Zn ha^{-1}) and two fractionating (for applications 4 and 8) plus one control were applied. The application started at 42 DAP, treatments for dose 4 were done every 8 to 10 days, while dose 8 treatments took place every 4 to 5 days.

2.1.3. Experiment 3

In 2017, a plot was installed in La Molina (field 3), using four soil application treatments of 0, 10, 20 and 40 kg Zn ha^{-1}. In this case, the variety used was 'Yungay'.

2.2. Field work and data collection

The size of the plot used was 30 m^2. The plots of 'UNICA', in La Molina, were fertilized with a dose of N-P-K: 200–70–115 kg ha^{-1} using urea, diammonium phosphate, and potassium chloride. In Cañete, the dose of NPK was: 130 – 70 – 230 kg ha^{-1}, using ammonium nitrate, potassium sulfate, potassium chloride and diammonium phosphate. The application of N was applied at planting and hilling (30 DAP), both in equal parts.

In 'Yungay', guano (bird poop) was applied during the land preparation, at a rate of 4 Mg ha^{-1}. The harvest was semi-mechanized at 130 DAP for 'UNICA' and 150 for 'Yungay'. All tubers from 3 representative plants per plot were taken for nutritional content measurements.

2.3. Determination of Zn content

In all experiments, leaves and tubers were washed with distilled water, peeled, and separated into peel and flesh. They were placed in an oven at 60°C for 48 hours. The dry matter was obtained and 1 g of it was taken for digestion at 180°C with 10 ml of a 5:1 solution of HNO_3 and $HClO_4$. The samples were analyzed using an atomic absorption spectrophotometer. Method validation was performed by evaluating linearity, limit of detection (LOD), and limit of quantification (LOQ). Linearity was assessed using a calibration curve with five concentration levels, yielding a correlation coefficient (r) of 0.9997. The LOD and LOQ were calculated based on the standard deviation of the response of 20 analytical blanks (σ = 0.010) and the slope of calibration curve (m = 0.3087), following ICH guidelines (LOD = 3.3 σ/m; LOQ = 10 σ/m). The LOD and LOQ obtained were 0.107 mg L^{-1} and 0.324 mg L^{-1}, respectively, demonstrating the method's sensitivity for the analyzed samples.

2.4. Statistical analysis

Effects of foliar and soil fertilization of Zn on yield and, concentrations of Zn in leaves, peel and flesh were analyzed using an ANOVA model, after verifying normality and homogeneity. Significant differences between treatment means were analyzed using Tukey's test (α = 0.05). Statistical analysis was performed using R software.

3. Results and discussion

In Table 3, the results for the 'UNICA' variety are presented. In experiment 1, no significant differences were found in Zn concentrations in the plant tissues for fields 1 and 2, when soil applications were used. However, in field 4 there was an increase which was proportional to the increased doses of soil Zn. The variability in initial soil conditions presented in Table 1, particularly differences in $CaCO_3$ and initial Zn levels, was critical for delineating the limitations of soil applications. The availability and absorption of Zn is reduced when the pH of the soil and the concentration of $CaCO_3$ in calcareous soils increase (Havlin et al., 2016). This phenomenon is supported by recent studies indicating that calcium-rich materials promote the precipitation of zinc into insolulbe carbonate forms, rendering it unavailable for root uptake. Adiccionally, the alkalinization caused by calcium amendments restricts the quantity of bioactive zinc species in the soil (Skuta et al., 2025). Therefore, the lack of response in field 1 and 2 (La Molina) compared to field 4 (Cañete) is not a contradiction, but a reliable indicator the high soil pH and carbonates mask the effect of soil Zn fertilizer. At the same time, excessive P fertilization can limit Zn absorption by plants (Zhang et al., 2017). Consequently, the experimental fields at La Molina, characterized by a higher content of $CaCO_3$, high pH, and high contents of P and Zn, did not show a positive response to soil fertilization of Zn. On the contrary, foliar fertilization did have a significant positive response in the three fields evaluated regardless os soil characteristics. Phattarakul el al. (2012) found that rice cultivation in calcareous soil had an increase in Zn in grains when foliar application of Zn was used. In addition, the diffusion of Zn in the soil is lower in a calcareous soil, thus foliar application is more effective (Zhao et al., 2014). Kromann et al. (2017) reported a higher content of zinc in Andean potato varieties in response to foliar applications compared to soil applications in different production areas.

The percentage of increase in leaves, peel and flesh using foliar application were 138.5, 20.5 and 34.4% (La Molina 2017); 116.6, 30.1 and 39.2% (La Molina 2018); and 704.2, 12.7 and 45.8% (Cañete 2018), respectively, compared to the control. Zinc values in leaves are known for most crops and they range from 30 to 100 mg kg^{-1} with critical levels of toxicity between 300 to 500 mg kg^{-1} (Chaney, 1993; Noulas et al., 2018), which can cause a decrease in yield. The potato crop holds concentrations in the range of 21 to 70 mg kg^{-1} and reaches toxicity levels from 150 mg kg^{-1} (Walworth and Muniz, 1993). For foliar applications in potato, concentrations from 792 mg Zn kg^{-1} have been reported without the visual observation of toxicity symptoms. An explanation for high concentrations is that part of the applied zinc is retained on the surface of the leaves or in apoplastic space of cells (White et al., 2012).

The increase in the concentration of Zn in tubers was lower than that observed in the leaves. This can be explained by a greater difficulty, or restriction of the absorption of zinc within the cells or phloem because translocation is limited in the phloem from the leaves towards the tubers (Longnecker and Robson, 1993; White et al., 2012). Higher concentrations of Zn are observed in the peel than in the flesh of the potato tuber (Subramanian et al., 2011), getting concentrations of 49.4 and 24.4 mg kg^{-1}, respectively.

Yield is not affected by the availability of zinc in soil, because the Zn value in the soil is possibly well above the critical level (which can range from 0.5 to 1 mg kg^{-1}). Although foliar application shows lower yields in two of the three fields evaluated, these results are like those observed in White et al. (2012) where lower yield was found in foliar application treatments (> 5.4 g Zn 1.84 m^{-2}). However, Amoros et al, (2020) reported that Zn has a weak direct negative effect on tuber yield.

For the variety 'Yungay' (Table 4), the same trend of experiment 1 was observed for experiments 2 and 3, in soil and foliar applications, respectively. That is, there was no significant response for soil fertilization, but there was for foliar fertilization in biofortification for potatoes. The increases from foliar application for leaves, peel, and flesh were 258, 21, and 27%, respectively. These increases were lower than those found in 'UNICA' cultivar. Depending on the cultivars, the Zn concentration may differ in the parts of the potato crop (Mengist et al., 2021).

In general, it can be concluded that applying fertilizer to the soil during planting, despite the high concentrations used,

Table 3
Yield and concentration of Zn of potato in experiment 1

Treatment	Yield Mg ha^{-1}	Zn leaves	Zn peel	Zn flesh
		mg Zn kg^{-1} DW		
		Year 2017 – La Molina (Field 1)		
Soil	NS	NS	NS	NS
Foliar	NS	***	**	***
S*F	NS	NS	NS	NS
Soil				
0 kg Zn	28.9	123.2	44.3	22.2
20 kg Zn	29.5	115.9	46.4	22.2
40 kg Zn	31.1	114.3	45.5	21.9
Foliar				
0 kg Zn	30.6	69.6 b	41.4 b	18.9 b
5 kg Zn	29.0	166.0 a	49.4 a	25.4 a
		Year 2018 – La Molina (Field 2)		
Soil	NS	NS	NS	NS
Foliar	***	*	*	***
S*F	NS	NS	NS	NS
Soil				
0 kg Zn	22.3	118.9	32.9	19.8
20 kg Zn	23.3	146.2	37.6	20.5
40 kg Zn	22.9	95.4	31.1	20.1
Foliar				
0 kg Zn	24.8 a	75.9 b	29.6 b	17.1 b
5 kg Zn	20.8 b	164.4 a	38.5 a	23.8 a
		Year 2018 – Cañete (Field 4)		
Soil	NS	NS	NS	***
Foliar	***	***	NS	***
S*F	NS	NS	NS	NS
Soil				
0 kg Zn	25.3	363.2	39.9	15.8 a
20 kg Zn	24.6	352.4	43.1	17.4 b
40 kg Zn	24.2	358.9	45.7	19.0 c
Foliar				
0 kg Zn	27.1 a	79.3 a	40.3	14.2 a
5 kg Zn	22.3 b	637.7 b	45.4	20.7 b

Note: NS = non-significant; Treatments with the different letter are significantly different

Table 4
Yield and concentration of Zn in potato variety 'Yungay' for experiments 2a and 3

Experiment 2a – Foliar application					Experiment 3 – Soil application				
Zn dose	Yield	Zn leaves	Zn peel	Zn flesh	Zn dose	Yield	Zn leaves	Zn peel	Zn flesh
kg Zn ha^{-1}	Mg ha^{-1}	mg Zn kg^{-1} DW			kg Zn ha^{-1}	Mg ha^{-1}	mg Zn kg^{-1} DW		
0	18.3	99.6 b	41.7	21.0 b	0	33.4	114.3	40.7	24.5
4	16.2	340.1 a	58.7	24.3 ab	10	32.3	108.7	44	24
8	20.6	374.8 a	54.7	28.8 a	20	25.6	102.6	37.7	24.8
16	17.6	354.9 a	38.3	27.5 ab	40	32.9	103.9	39.7	22.8
	NS	**	NS	**		NS	NS	NS	NS

Note: NS = non-significant; Values in the same column followed by the different letter are significantly different according to Tukey's test (P < 0.05)

did not generate response in absorption by the plant, although this is a traditional fertilization practice in potato cultivation (Westermann, 2005).

In Experiment 2b, for the 'UNICA' cultivar, yield was affected by foliar zinc application, with the highest dose resulting in the lowest yield (Fig. 2). While the control treatment yielded an average of 29.04 Mg ha^{-1}, treatments with 5 kg Zn ha^{-1} and 10 kg Zn ha^{-1} showed average yields of 24.29 and 20.89 Mg ha^{-1}, respectively. No significant differences were observed between the 4- and 8-split applications. When performing foliar applications, the number of field entries for application must be considered, as other potato studies (Gaj et al., 2020; Srivastava et al., 2023) reported 1 or 2 applications with no associated yield reduction. However, no significant differences were found in the yield of the 'Yungay' variety, suggesting this cultivar might be less susceptible to yield reduction from foliar application.

Other aspects must be considered when performing foliar fertilization. Climatic conditions can determine the success or failure of the foliar application; for example, it should not be applied during high temperatures to avoid leaf burn (Fageria et al., 2009). The water used to dissolve the fertilizer is another important factor, as it may contain salts that increase the osmotic potential and cause leaf damage (Fernández et al., 2021). In our experiment, we used irrigation water instead of distilled water to simulate conditions closer to those of a farmer.

In Table 5, foliar applications increased zinc content in leaves, peel, and flesh of potatoes in Cañete. On average, fertilized treatments increased Zn concentration by 59, 88, and 637% in the pulp, peel, and leaves, respectively, compared to the control. No differences were observed between doses and fractionation.

In the experiments, the Zn concentration in the potato crop generates a trend of leaves > peel > pulp. In different cultivars of Andean potatoes, the level of Zn concentration in the tuber varies between 8 to 20 mg kg^{-1} (Burgos et al., 2007), turning out to be lower, compared to some applications in experiment 1, 2 and 3. According to Subramanian et al. (2011), three minerals of dietary importance, such as iron, calcium, and zinc, have markedly higher concentrations in the potato tuber peel. For instance, the potato tuber peel contained 17% of the total zinc in the tuber. Although the contributing dry mass content is lower than that of the potato tuber flesh, the contribution of zinc is considerable. In short, the reduction in potential dietary zinc intake from this crop will occur, for example, when peeled tubers are consumed (White and Broadley, 2011).

On average across all experiments, the increase in Zn in the potato flesh is approximately 40%. This would mean that if an adult consumes 200 g of fresh potatoes (20% dry matter), it could represent an additional 2% towards the adequate daily zinc requirement (15 mg day^{-1}).

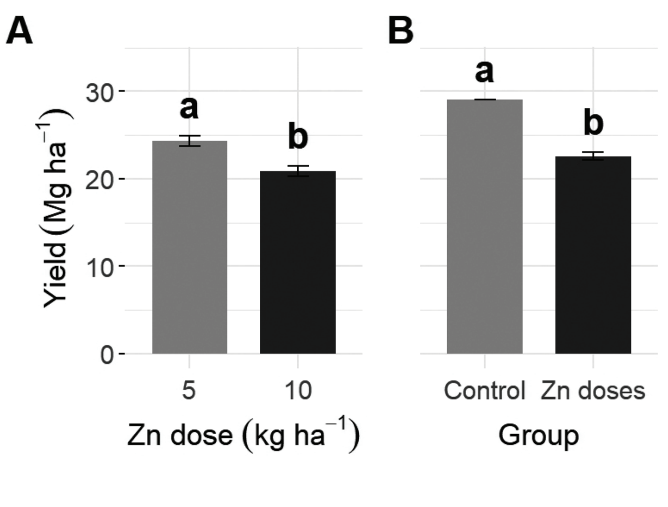

Fig. 2. Yield of potato variety 'UNICA' influenced by (A) Zn dose and (B) comparison between control and Zn-treated groups. Different lowercase letters indicate significant differences (P < 0.05) according to Tukey's test

Table 5
Zn concentration (mg kg^{-1} DW) in flesh, peel and leaves potato var. 'Única' in Cańete under foliar application for experiment 2b

Dose kg Zn ha^{-1}	Zn flesh Fractionation			Zn peel Fractionation			Zn leaves Fractionation		
	4	8	M	4	8	M	4	8	M
5	18.9	21.0	20.0	39.7	30.0	34.8	643.4	637.1	640.2
10	19.6	20.7	20.2	31.5	46.4	38.9	661.5	636.7	649.1
M	19.3	20.8	20.1 a	35.6	38.9	36.9 a	652.4	636.9	644.7 a
C	12.6 b			19.6 b			87.4 b		

Note: C = control, M = mean. The comparison is between the control group and the average fertilized treatments. Treatments with the different letters are significantly different

4. Conclusions

The present study, conducted in two locations in Lima, showed that foliar fertilization consistently increases Zn concentrations in potato tubers of the varieties 'Yungay' and 'UNICA'. In contrast, responses to soil fertilization were inconsistent and strongly influenced by soil chemical properties. Specifically, high soil pH, elevated $CaCO_3$ content, and high P concentrations were limiting factors that reduced Zn availability and uptake. Consequently, soil Zn application is not recommended in calcareous soils where these chemical constraints hinder effective biofortification. Regarding yield, soil fertilization did not affect tuber production. Overall, foliar Zn biofortification increased Zn concentrations in potato tubers and proved to be the most effective strategy under soil chemical conditions that limit Zn availability.

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Conflict of interest

The authors declare no conflict of interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. This research did not involve human or animal subjects.

Author Contributions

Pedro Gutierrez-Vilchez – Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Melissa Quispe-Poma** – Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. **Marilú Acuńa** – Conceptualization, Investigation, Methodology, Supervision. **Rolando Egúsquiza** – Conceptualization, Funding acquisition, Methodology, Supervision. **Ruby Vega-Ravello** – Conceptualization, Data curation, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. All authors read and approved the final manuscript.

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