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Agronomic biofortificación in two native potato cultivars (*Solanum tuberosum* L.)

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Summary

During the 2012-2013 cropping season in the areas of Coacollo, Yana Muyu (La Paz, Bolivia) and El Paso (Cochabamba, Bolivia), plots were established in a randomized complete block design in stripe plot arrangement with four replications (experiments in series), in order to: a) determine the response of two potato native crops at Fe and Zn soil fertilization and b) determine the concentration of Fe and Zn in tubers. Two factors were considered: A) two cultivars and B) sixteen levels of Fe and Zn. The response variables were: yield ($t.ha^{-1}$) and the Fe and Zn content ($mg.100g^{-1}$ FW) in tubers. The Fe and Zn content were quantified with atomic absorption technique. Result showed that yield for Waych'a (25-35 $t.ha^{-1}$) and Pinta Boca (8-15 $t.ha^{-1}$) are affected by the environment. There was significance for Fe*locality interaction and for Zn. The optimum level for Fe in El Paso was 10 $kg.ha^{-1}$ of ferrous sulphate in order to achieve 15 $t.ha^{-1}$ and for Zn in the three localities was 9.71 $kg.ha^{-1}$ Zn sulphate to achieve 16 $t.ha^{-1}$. In Fe content in tubers was significant for cropping. Boca Pinta (0.60 mg.100 g^{-1}) was higher in 22% compared to Waych'a (0.49 mg.100 g^{-1}). There was no crop*location interaction. However, for Zn there were significances for location, crop and levels. Pinta Boca had higher Zn content (0.30 mg/100 g^{-1}) than Waych'a (0.26 mg.100 g^{-1}).

Additional Key words: Ploidy, Zn, Fe, nutrition, yield.

Biofortificación agronómica en dos cultivares nativos de papa (*Solanum tuberosum* L.)

Resumen

En la campaña agrícola 2012-2013 en las zonas de Coacollo, Yana Muyu (La Paz, Bolivia) y El Paso (Cochabamba, Bolivia), fueron establecidas parcelas en diseño de bloques completos al azar en arreglo de parcelas en franjas con cuatro repeticiones (experimentos en serie), con el objetivo de: a) determinar la respuesta de dos cultivares nativos de papa a niveles de fertilización de Fe y Zn al suelo y b) determinar el contenido de Fe y Zn en tubérculos. Se consideró dos factores: A) dos cultivares y B) dieciséis niveles de Fe y Zn. Las variables de respuesta fueron: El rendimiento ($t.ha^{-1}$) y el contenido de Fe y Zn (mg. 100 g^{-1} de PF) en tubérculos. El contenido de Fe y Zn fueron cuantificados con la técnica de absorción atómica. Los resultados mostraron que el rendimiento para Waych'a (25 a 35 $t.ha^{-1}$) y Pinta Boca (8 a 15 $t.ha^{-1}$) son afectados por el ambiente. Hubo significancia para la interacción Fe*localidad y para Zn. El nivel óptimo para Fe en El Paso fue de 10 $kg.ha^{-1}$ de sulfato ferroso para obtener 15 $t.ha^{-1}$ y para Zn en las tres localidades fue de 9.71 $kg.ha^{-1}$ de sulfato de Zn para lograr 16 $t.ha^{-1}$. En contenido de Fe en tubérculos hubo significancia para cultivar. Pinta Boca (0.6 mg. 100 g^{-1}) fue superior en 22% respecto de Waych'a (0.49 mg.100 g^{-1}). No hubo interacción

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cultivar*localidad. En cambio, para Zn hubo significancias para localidad, cultivar y niveles. Pinta Boca tuvo mayor contenido de Zn (0.30 mg.100 g^{-1}) que Waych'a (0.26 mg.100 g^{-1}).

Palabras clave adicionales: Ploidía, Zn, Fe, nutrición, rendimiento.

Introduction

The malnutrition caused by the lack of micronutrients, also called "hidden hunger", affects more than half of the world population, especially women in reproduction age and pre-school children in developing countries (Graham y Welch, 1999; Pfeiffer y McClafferty, 2007).

In Bolivia, recent studies found out that the high altitude production regions have persistent conditions of chronic malnutrition with 8 of 10 children (between 6 to 23 months) suffering anaemia, which is considered the problem of deficiency on macro and micro nutrients of most prevalence in the country (Mallea, 2010).

The principal consequence of ferrum (Fe) deficiency is anaemia ferropenic. However, there are also adverse effects on the psychomotor and cognitive development in children younger than two years old, their capability of learning, behaviour and body condition. They will also be more susceptibility to infections (especially respiratory tract). They will have a decrease on the speed of their physical development and increase on mortality rate (Grandy *et al*., 2010).

The deficiency of zinc (Zn) is frequent in developing countries (Brown *et al.*, 2001) in which there is a low ingestion of animal products in contrast with a high ingestion of fitates (Bhan *et al*., 2001). A study performed with school children from the rural area of a community in Bolivia, showed prevalence on the deficiency of zinc corresponding to 61% (lower cutting point of 80 μg.dL^{-1}).

Studies reveal that children with moderate deficiency of Zn show delay in their linear growth, as well as adverse effects in their neuro-behavioural and psychomotor development. There is also a decrease in appetite (due to a negative effect in the capacity of detection of food flavours) and immune response capacity (Grandy *et al.*, 2010).

The food sources of Fe and Zn are similar, reason for which, the deficiencies could be expected to appear simultaneously (Krebs y Hambidge, 1997). Besides diarrhoea, the prevalent disease responsible for 36% of death in children younger than five in Bolivia, also increases the disposal of zinc and ferrum (Grandy *et al.*, 2010).

In general, the boy or girl that had suffered malnutrition will have problems at school performance and cognitive achievement (Ordinola, 2012). These circumstances have negative consequences in their future such us low salaries and low performance (Burgos *et al.*, 2007; Mallea, 2010; Ordinola, 2012).

There are three strategies of intervention that could contribute on the prevention and decrease of malnutrition due to lack of micronutrients: a) supplementation with pharmaceutical products, b) fortifications of food and c) improved basic crops. The biofortification could also be at agricultural level, genetic or biotechnological (Graham *et al*., 2001; PMA, 2010; Devaux, 2013).

The Agronomic Biofortification involves the application of chemical fertilizers to the soil and /or the plant to elevate the concentrations of micronutrients in vegetables (tubers). At the same time the characterization and identification of native cultivars with high concentrations of micro-nutrients (Devaux, 2012), it is a complementary approach that makes it possible to achieve it in a shorter time scheme (Cakman, 2008).

In recent years, the International Centres of Agricultural Research organized on the CGIAR (Consultation Group for the International Agricultural Research), together with numerous collaborators that belong to a multidisciplinary group, are looking at different breeding methods, for successful results in the increase of proteins, vitamins and minerals in the crops of maize, wheat, rice and frijol; with the support of the programme Challenge for Biofortificación Harvest Plus (Pfeiffer and McClafferty 2007, Bouis and Welch 2010).

In the case of potatoes, genetic and agronomic studies are required in order to find the genotypes with the higher contents of Fe y Zn, as well as the adequate doses of fertilization with Fe y Zn, in order to make it possible for this nutrients to be absorbed in higher proportion by the tubers and so for to be available in the needed quantities for human nutrition, particularly in areas where there is deficiency of this micronutrients (Devaux, 2013).

Previous studies, in agronomic biofortification, were performed by Val Verde *et al.* (2013) in Ecuador. They applied Fe and Zn to the soil and to the foliage in five cultivar of potatoes (some native others bred), their results showed that the concentrations of Fe and Zn in the tubers were high, though not of significance. Besides, in Perú, Burgos *et al*. (2007) characterised 37 native cultivars of potato for its content of Fe y Zn and found out that some of the cultivars reached the deliverance of 29 y 26 % of the recommended ingest (6 $mg.day^{-1}$ and 4.1 $mg.day^{-1}$) of Fe y Zn respectively. These results, for children 1 to 3 years old, with an average consumption of potato of 200 $g.day^{-1}$.

This present research had two objectives: a) determinations of the differentiated response of two native potato cultivars to different levels of fertilization of Fe and Zn in soil in three different towns in Bolivia and b) determine the content of Fe and Zn in the tubers for each level of fertilization applied in each of the two native potato cultivars.

Materials and Methods

Location

This study was done in the cropping season of 2012-2013 in three locations of Cochabamba and La Paz. One of the trial pots was established in the land belonging to PROINPA Foundation, known as El Paso, distant 15 km of Cochabamba city in the Quillacollo district, with the exact location of 17° 21’ of southern latitude and 66° 15’ of West longitude, at an altitude of 2620 meters over sea level. The second trial was established in the town of Coacollo, distant 92 km of La Paz city in the Ingavi district, located at 16°28’ southern latitude and 68°54’ of west longitude, at an altitude of 3845 meters over sea level. The third plot was established in the town of Yana Muyu, district Ingavi of La Paz, located at 16°38’ of southern latitude and 68°29’ of west longitude, to an altitude of 3960 meters over sea level.

Plant material

For this study, two native potato cultivars were tested: a) Pinta Boca (*Solanum stenotomum* - STN), which is a diploid cultivar (2n=2x=24), its tubers are purple colour and cream flesh with strings of antioxidants of purple colour, its shape is oblong and with round partially deep depressions on the tuber and b) Waych’a *(S.tuberosum* subsp. *andigena* - ADG), which is a tetraploid cultivar (2n = 4x = 48), with read-creamy tubers of white flesh, their shape is rounded and with deep depressions on the tuber (Cadima *et al*., 2004).

Experimental Design

The plots were established in the field in an experimental design of randomized complete blocks designed in stripe plot arrangement with four replications, and they were analysed in series of experiments (Martínez-Garza, 1988). Two factors were analysed: A) Two native cultivars of different Ploidy number, and

B) Sixteen levels of Fe and Zn as a result of the combination of four levels of Fe and four of Zn.

The fertilization was done immediately after seeding in manual form and continuous drip. Two types of fertilization were performed, one basic and another for research purposes. In the basic fertilization bovine manure was used together with Di-ammonic Phosphate (18-46-00) with doses of 5 t.ha^{-1}and 0.26 t.ha^{-1}respectively. In the research fertilization ferrous Sulphate (20 % Fe) and zinc sulphate (22 % Zn) were used in doses of: 0, 10, 20 y 40 kg.ha^{-1} of Fe and 0, 5, 10 and 15 kg.ha^{-1} of Zn. Urea (46-00-00) was also applied at the moment of rising the soil bed at a doses of 72.07 kg.ha^{-1}. This was as a complement to the basic fertilization done during the planting. The doses of fertilizers were measured with scales Ferton Professional ACS – B and the Acculab analytic AL 64.

To be able to define the correct levels of Fe and Zn a previous research done in Ecuador by Valverde *et al*. (2013) used the doses of 0 and 40 kg.ha^{-1} of Fe and 0 to 15 kg.ha^{-1} of Zn in the soil. On the present study intermediate levels were also used in amounts of 10 and 20 kg.ha^{-1} for Fe, and 5 to 10 kg.ha^{-1} for Zn, with the purpose of obtaining trends and to then determine the optimum level for each fertilizer. The optimum level was calculated for both the yield as well as the tuber nutrient contents.

The 16 levels of fertilization applied for Fe, and Zn respectively (T1: 0,0; T2: 0,5; T3:0,10; T4: 0,15; T5: 10,0; T6: 10,5; T7: 10,10; T8: 10,15; T9: 20,0; T10: 20,5; T11: 20,10; T12: 20,15; T13: 40,0; T14: 40,5; T15: 40,10; T16: 40,15) at planting, were the result of a factorial combination of the four levels for each element. The treatments T1, T3, T4, T9, T11, T12, T13, T15 and T16 were selected for the analysis of the content for Fe y Zn in the tubers.

The planting was done between the months of September and October of 2012, with 30 tubers of basic seed in lines 3 m long, 0,30 m between plants and 0,8 m between lines (7.2 m^2 per experimental unit). The cultural labour on the crop was the one traditionally performed for potato in the area. Irrigation was applied according to the crop requirements.

A specific herbicide (metribuzina) was applied pre-germination for the control of weeds. When 80% of the seed had emerged a systemic fungicide (metalaxyl) was applied, seven days after, a contact fungicide (mancozeb) was applied, in order to prevent diseases such as late blight (*Phytophthora infestans*), due to an approaching rainy season.

Tested variables

Tuber yield (t.ha^{-1}). At harvest the yield of tubers was tested, for all the treatments in kg/experimental unit, afterwards calculated in t ha^{-1}.

Analysis of the content of *Fe and Zn in tubers.* Once harvested, tuber samples of the same size approximately of 500 g., were put and labelled in fresh bags of paper that measured 21.59 cm x 35.56 cm. Afterwards they were sent to the Institute on Technology of Food (ITA) located in the city of Sucre, where they were analysed by Spectroscopic of Atomic Absorption (Herrero y Vigil, 2003).

Statistical analysis

The yield data and the content of Fe and Zn in tubers was analysed under a model of experimentation in series (Martínez-Garza, 1988). Once the variable satisfied to approximate of the supposed values of normal distribution and homogeneity of varianzas, they were analysed according to the proposed statistical model (Martínez-Garza, 1988).

With the background of the mentioned analysis model, the analysis of variance was done in order to probe the hypothesis regarding the fix effects and comparison of average values of yield and content of Fe and Zn in tuber. Indicators of one degree of freedom were used for the determination of the difference between cultivars, levels of fertilization and locations. The analysis of variance also allowed to estimate the components of variance for the randomised effects; with the use of the PROC MIXED and GLM, features of the statistical programme SAS version 9.2 (SAS, 2004).

Since there were differences between the levels of fertilization (quantitative factor), indicators of one degree of freedom were employed for the determination of the significance of the tendencies (linear, square and cubic) of yield and content in the tubers as a result of the levels of application of the fertilizers. The indicators were employed with the use of the PROC MIXED y GLM, features of the statistical program SAS version 9.2 (SAS, 2004). Afterwards the optimum levels of Fe and Zn were determined with the derivate of the respective functions.

Results and Discussion

Yield Analisys

The yield factor presented highly significant differences for cultivar*location ($p<0.01$) (Table 1). This means that the differences in yield between cultivars were not the same in both locations.

Table 1. Varianza analysis for yield (t.ha^{-1}) in two native potato cultivars tested in three locations of Bolivia. Year 2013.

FV	Gl	CM
Loc	2	154.699
Blq (Loc)	7	85.495
Cult	1	187.704
Fe	3	30.068
Zn	3	49.482**
Cult*Loc	2	884.600**
Fe*Loc	6	28.289*
Zn*Loc	6	15.185
Fe*Cult	3	1.546
Zn*Cult	3	21.227
Fe*Zn	9	13.537
Fe*Zn*Loc	18	16.746
Fe*Zn*Cult	9	7.605
Fe*Cult*Loc	6	1.961
Zn*Cult*Loc	6	10.074
Fe*Zn*Cult*Loc	18	5.747
Blq*Cult (loc)	7	40.622

**: Highly significant to p< 0,01 of probability, *: Significant to 0,05 of probability, Loc: locality, Blq: block, Cult: cultivar.

Figure 1 shows that in the locations of El Paso (Cochabamba) and Yana Muyu (La Paz) there were no significant differences ($p<0.05$) between cultivars. However, in Coacollo (La Paz) there were significant differences between cultivars, being Waych'a the one with highest yield.

The cultivar Pinta Boca (STN), was on the average yield range of 8 to 15 t.ha^{-1} for the three locations. In contrast, the cultivar Waych'a (ADG) had an average yield of 25 a 35 t.ha^{-1} for the three locations (Figure 1).

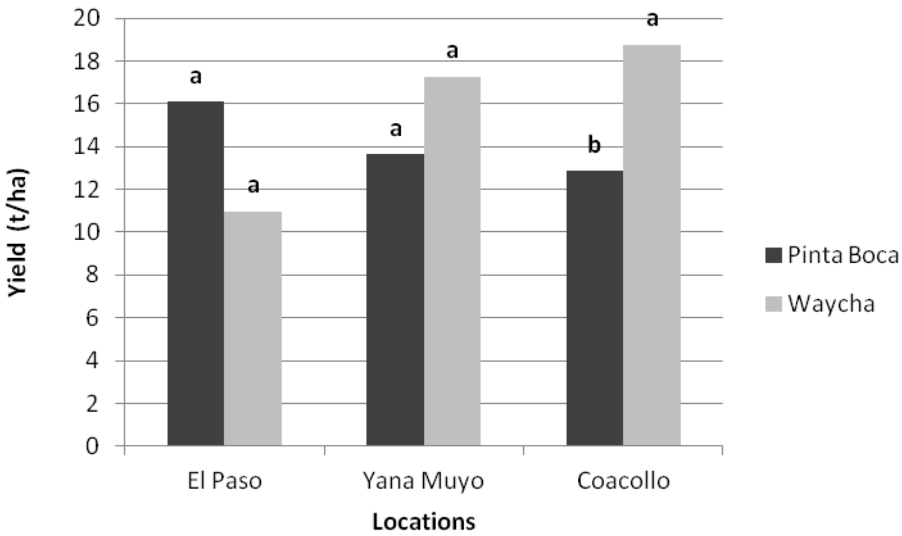

Figure 1. Response to yield (t.ha^{-1}) by cultivar in three tested locations. Year 2013.

It was established that there were significative differences ($p<0.05$) for the interaction Fe*location (Table 1). This means that differences were observed in yield for at least some of the levels of Fe in at least one of the locations. Significative differences were not detected ($p<0.05$) for the interaction Fe*cultivar*location (Table 1), for which it can be said that the differences will be the same in both cultivars.

On the other hand, high significant differences were observed for Zn ($p<0.01$) (Table 1). This means that at least one level of Zn caused differences in the yield. Since there were no significative differences ($p=0.149$) in the interactions of Zn*cultivar and Zn*location, it can be tell that the differences in yield were the same for both cultivars in the three locations.

Analysis of the optimal level of Fe in soil

Being Fe a quantitative factor in its different levels, indicators of one degree of freedom were employed to be able to determine the significance of the trends (linear, square and cubic) of the yield as a function of the application of four levels of Fe in each location. This was done for each location, due to the fact that the interaction Fe*location was present.

Table 2 shows that yield follows a cubic trend highly significant ($p<0.01$) that grows with the increase of the level of Fe (kg.ha^{-1}) in the location of El Paso (Cochabamba).

Table 2. Indicators of one degree of freedom for the linear trends, square and cubic of yield (t.ha^{-1}) in relations to four levels of fertilization of Fe and four levels of Zn in two native potato cultivars tested in three locations. Year 2013.

FV	Gl	CM
Linear_Coacollo x Fe	1	18.865
Linear_ElPaso x Fe	1	46.078*
Linear_YanaMuyu x Fe	1	1.105
Cuad_Coacollo x Fe	1	20.705
Cuad_ElPaso x Fe	1	54.843*
Cuad_YanaMuyu x Fe	1	7.926
Cub_Coacollo x Fe	1	9.594
Cub_ElPaso x Fe	1	91.037**
Cub_YanaMuyu x Fe	1	1.284
Linear_ Zn	1	55.667*
Cuad_Zn	1	75.154*
Cub_Zn	1	17.632

**=Highly significative to $p< 0.01$ of probability, *=significative to 0.05 of probability, Cuad=square trend, Cub=cubic trend.

The relation yield/levels of fertilization with Fe in El Paso (Cochabamba) is a cubic function (Figure 2), with an optimum level of fertilization of 10 kg.ha^{-1}of Fe to achieve a maximum yield of 15.28 t.ha^{-1}.

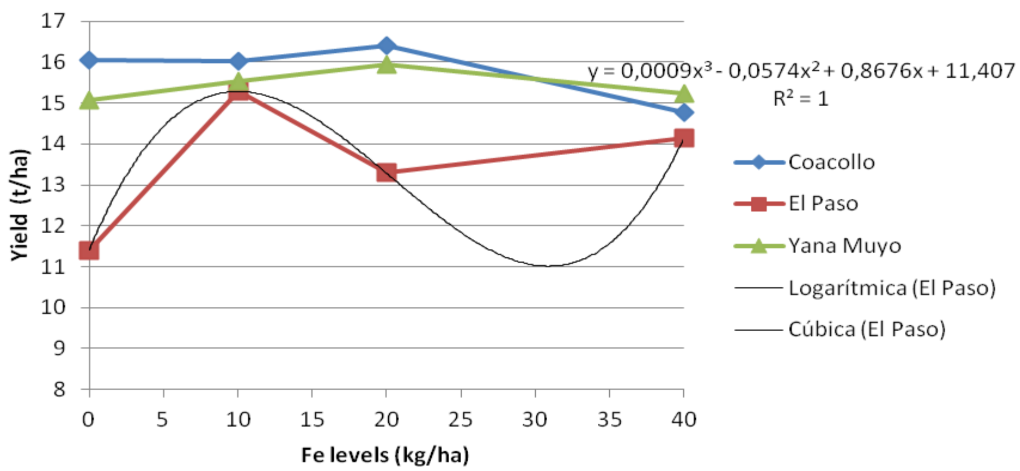

Figure 2. Trends of regression for yield (t.ha^{-1}) in relation to four levels of Fe (kg.ha^{-1}) applied in two native potato cultivars in three locations. Year 2013.

On the other hand, in the other two locations neither of the trends was significant. This is showing that there was no effect of the fertilization for the levels of Fe applied. These results are in agreement with the findings of Valverde *et al*. (2013), whom found that there were no significative differences in yield when a dose of 40 kg.ha^{-1} was applied for Fe. The lack of fertilization with Fe could be due to three principal reasons: a) The presence of diseases, b) Fe is not available at the time when the plant requires it, which affects the production as it is reported by Patiño (2000) and Venegas (2010), also Barrera (1998); Westfall and Bauder (2011) mentioned that the application of $FeSO_4$ to the soil is relatively inefficient, due to its fast conversion to non-available sources c) that there was toxicity with Fe, as it is reported by Beltran and Guerra (2013) and Vélez (2013) who tested the effect of the foliage and edaphic fertilization with Fe in a tetraploid variety of potato [I-Gabriela x (*S. phureja* x *S. Pausissectum*)] under greenhouse conditions. In that experiment he used ten treatments coming from the combination of five doses of edaphic fertilization (0. 38, 76, 114 y 152 kg.ha^{-1} de Fe) and two doses of foliar fertilization (0 and 117.2 ppm). The results expressed that there were no significative differences in the yield for neither of the doses applied in the foliage. However, there were significant differences for the doses applied to the foliage, being that the absence of ferrum (0 ppm) reach higher yield that the doses of 117.2 ppm. The author concluded that the reduction in yield was due to fitotoxicity.

In our case the reason for the lack of response in Coacollo and Yana Muyu could be due to the presence of disease or to the lack of availability of Fe since there was no evidence of toxicity in the plants at none of the locations. However, late blight infections were reported in both locations. Besides, as reported by Barrera (1998) and Westfall and Bauder (2011) the application of $FeSO_4$ to the soil was relatively inefficient, so for the Fe should be applied in quelates form (at the beginning of the vegetative period of the crop), but its use at field scale fertilization is limited by its high Price.

Analysis of the optimum level of Zn in the soil

Once it was determined that there were highly significative differences ($p<0.01$) for yield for at least one of the levels of fertilization with Zn, and being Zn a quantitative factor in its different levels, indicators of one degree of freedom were employed to be able to determine the significance of the trends (linear, square and cubic) of yield as well as the equation of the four levels of Zn (Table 2). It was observed that this equation follows a square trend ($p<0.05$) (Figure 3).

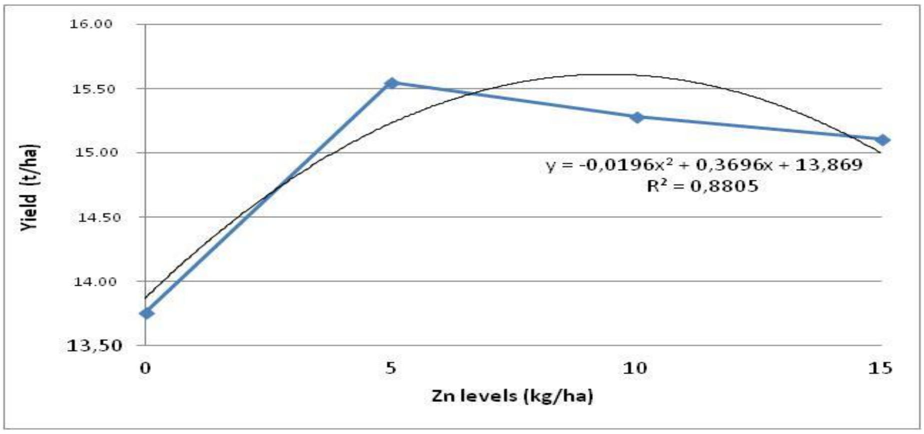

Figure 3. Tendency of regression for yield (t.ha^{-1}) in relation of four levels of Zn (kg.ha^{-1}) applied in two native potato cultivars in three locations. Year 2013.

The optimum level of fertilization is 9.71 kg.ha^{-1}of Zn to be able to obtain the yield of 15.65 t.ha^{-1} (Figure 3). As it is reported by Barrera (1998), the recommended doses of micronutrients for the potato crop at field level, is normally 10 kg.ha^{-1}, mixed with N-P-K. Besides, Westfall y Bauder (2011) recommended a range of application of 5.5 a 11.0 kg.ha^{-1} of Zn or of 16.5 a 33.0 kg.ha^{-1} of $ZnSO_4$ (36 %) for two or three potato cropping campaigns. The range also depends on the availability of irrigation (lower levels for soils naturally irrigated) and the level of Zn in the soil. Our results are inside the recommended range by the above authors.

Regarding the yield response to fertilization, our results are different from those reported by Valverde *et al*. (2013), whom applied doses of 20 kg.ha^{-1} for Zn, but they did not find significative differences (p<0.05) on yield. This means that the fertilization with Zn in doses of 20 kg.ha^{-}, did not influence the yield significantly in that particular experiment. This could be due to the fact that the soil where the experiment was performed presented high content of Fe; being Fe a biological antagonist of Zn (Piaggesi, 2004) it will decrease its availability at the time the plant requires it, interfering in this way with the production of yield as reported by Patiño (2000) y Venegas (2010).

Analysis of the content of Fe in tubers

The analysis of variance for the content of Fe in tubers (Table 3), showed significative differences (p<0.05) for cultivars. This implies that the content of Fe in tubers is different in both cultivars.

Table 3. Analysis of variance for the content of Fe in tubers of two native potato cultivars tested in three locations. Year 2013.

FV	Gl	CM
Loc	2	0.019
Var	1	0.485*
Fe	2	0.009
Zn	2	0.020
Var*Loc	2	0.021
Fe*Loc	4	0.011
Zn*Loc	4	0.019
Fe*Cult	2	0.026
Zn*Cult	2	0.008
Fe*Zn	4	0.010
Fe*Zn*Loc	8	0.004
Fe*Zn*Cult	4	0.003
Fe*Cult*Loc	4	0.033
Zn*Cult*Loc	4	0.008
Fe*Zn*Cult*Loc	8	0.013
Blq*Cult (Loc)	6	0.043
Blq*Fe*Zn (Loc)	47	0.018

**: Highly significant to p< 0.01 of probability, *: significant to 0.05 of probability, Loc: location, Cult: cultivar, Blq: block.

The cultivar Pinta Boca (STN) was superior on the content of Fe in 22% in comparison to the cultivar Waych'a (ADG), with contents of 0.6 y 0.49 mg/100g in fresh weight (PF), respectively (Figure 4). There was no interaction cultivar*location, therefore is evident that there is no interaction genotype*environment for the content of Fe in tubers in neither of the cultivars. This means, that difference in Fe content in tubers between cultivars is the same in all locations.

cultivars

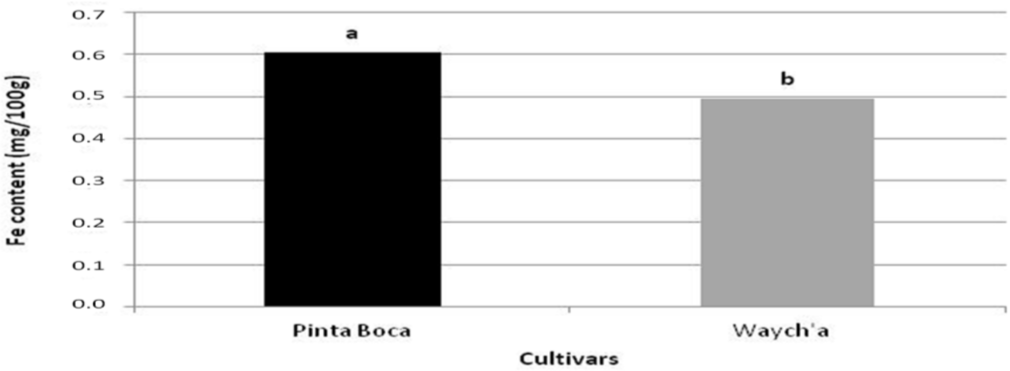

Figure 4. Fe content in tubers based in fresh weight, and for cultivar. Year 2013.

Other studies also found highly significative differences ($p<0.01$) between cultivars in relation to the Fe concentration. Burgos *et al*. (2007) for example, tested 37 native potato cultivars [*S. goniocalyx* (GON),*S. phureja* (PHU),*S. tuberosum* (TBR), *S. chaucha* (CHA), GON x STN, *S. andigena* (ADG) and *S. Stenotomum* (STN)] in two locations, he found that STN and ADG had concentrations between 0.45-0.74 and 0.43-0.73 mg.100 g^{-1} of Fe in PF, respectively; though the differences between this two species were not statistically significant. Besides, the specie PHU was superior to all the rest of the species regarding content of Fe in tubers.

Differences on the concentration of Fe was also found in germplasm tested by CIP between 2004 and 2007. The tested species were: GON, PHU, ADG y STN together with 315 advanced clones. STN and ADG had concentrations between 0.46 – 0.62 y 0.28 – 0.69 mg.100 g^{-1} of Fe in PF, respectively (CIP, 2007).

In this study we have determined that Pinta Boca (STN) has a medium content of Fe ranged between 0.60 mg.100 g^{-1} of Fe, that is a value inside the range found by Burgos *et al*. (2007) for this specie. Tisdale and Nelson (1991) reported in this respect that these cultivars have a marked effect on the response to an applied fertilizer.

On the other hand we have not observe the same effects due to the fertilization with Fe in the content of the tubers in any of three locations ($p=0.621$) and in none of the varieties. This means that in these locations the content of Fe cannot be increased under neither of the treatment of fertilization in none of the varieties. So forth, the only option would be the availability of cultivars with high content of Fe like the bred cultivar Chota Ñawi with 1,05 mg.100 g^{-1} of PF (Gabriel *et al.*, 2014a), Wila Surimana (STN) with 1.35 mg.100 g^{-1} PF, and Chilltu (STN) with 1.18 mg.100 g^{-1} (Gabriel *et al*., 2014b). It would be recommended to use these cultivars in a programme for crossing with more popular cultivars such as Waych'a, Sani Imilla, Imilla Negra, Malcacho, Desirée, etc., in order to obtain in the near future new bred cultivars with high content of Fe.

Valverde *et al.* (2013) found significative differences in the concentration of Fe in the tubers between cultivars but not in the levels of fertilization with Fe (the same result as ours).

On this regard, Graham *et al*. (2001), reported that the absorption of Fe is regulated to avoid the excessive accumulation of Fe in the cells. For this reason, trying to achieve that the crops absorb significantly more Fe than the required for satisfying the metabolic demands, is difficult and it must be done carefully.

If there is no response, to fertilization, it must be understood that the nutrient was deficient, and in reality, could be possible that it might have not been absorbed by the plant (Barker y Pilbeam, 2007). Many interactions could improve or suppress the absorption of other nutrients. In the case of maize for example, the organic manure that satisfied the requirements of the crop, decreased the antagonism of Mn and the Fe over the Zn. Besides, high concentrations of K decreased the effect of P over the Zn (Villarroel, 1979). Also the pH is an important factor that influences the availability of Fe, like is illustrated by the results obtained by López (2007), who found that in acid soils together with the lowest doses of organic fertilization (5 $t.ha^{-1}$), the potato tubers presented higher concentration of Fe. We infer that the non significative response to fertilization with Fe in this study could have been influenced by these factors.

Analysis of the cultivars output to the recommended ingest of Fe

The cultivar Pinta Boca (0.60 $mg.100\ g^{-1}$ of Fe) tested in this study, will give an average output of 10.3% and 2% of the daily recommended ingest of Fe for children between 1 to 3 years old (5.8 $mg.día^{-1}$) and women in reproductive age (29.4 $mg.día^{-1}$), respectively; and the cultivar Waych'a (0.49 $mg.100\ g^{-1}$ of Fe) will give an average output of 8.4% and 1.7% for children and women respectively; taking into account an average intake of potatoes of 100 $g.day^{-1}$. On the other hand the native cultivar with higher content of Fe: Wila Surimana (1.35 mg $mg.100\ g^{-1}$ of Fe), will have an output of 23.3 % for children and 4.6 % for women, respectively (Gabriel *et al.*, 2014b). The minimum content proposed by CIP in order for a potato cultivar to help decreasing the deficiency of Fe is approximately 0.95 $mg.100\ g^{-1}$ of Fe in PF. So forth, the cultivar Wila Surimana would be able to help in the deficiency of Fe in children between 1 to 3 years old and women in reproductive age, in Bolivia. Besides, it would be a valuable source of genes for its Ferrum content in tuber, since bred genotypes with a content of 1,2 $mg.100\ g^{-1}$ of Fe in PF are already considered biofortified (Ortiz, 2010).

Analysis of the content of Zn in tubers

The variance analysis for the content of Zn in tubers (Table 4), showed significant differences ($p<0.05$) for location, Cultivar and levels of Zn. This means that there are differences in the content of Zn in tubers for at least one of the locations, one of the cultivars and one of the levels of fertilization with Zn.

cultivars

Table 4. Analysis of variance for the Zn content in tubers of two native potato cultivars tested in three locations. Year 2013.

FV	Gl	CM
Loc	2	0.308**
Var	1	0.072**
Fe	2	0.0002
Zn	2	0.080**
Var*Loc	2	0.003
Fe*Loc	4	0.012
Zn*Loc	4	0.005
Fe*Var	2	0.005
Zn*Var	2	0.008
Fe*Zn	4	0.008
Fe*Zn*Loc	8	0.005
Fe*Zn*Var	4	0.002
Fe*Var*Loc	4	0.010
Zn*Var*Loc	4	0.006
Fe*Zn*Var*Loc	8	0.002
Blq*Var (loc)	6	0.009
Blq*Fe*Zn (loc)	47	0.005

**: Highly significant to $p < 0.01$ of probability, *: Significant to 0.05 of probability, Loc: location, Cult: cultivar, Blq: block.

The comparison of averages between locations (Figure 5), showed that the major content of Zn in PF was in El Paso (0.35 mg.100 g^{-1}) followed by Yana Muyu (0.28 mg.100 g^{-1}) and finally Coacollo (0.20 mg.100 g^{-1}). This could be due to the existence of a major content of Fe, P, organic matter and K in the location of El Paso, where the organic matter could have acted to decrease the antagonism of the Fe over the Zn and the high levels of K in the soil could have inhibit the effect of P over Zn, favoring its availability. Villarroel (1979) found in maize that the antagonistic relationships between P y Zn decrease in presence of high levels of K. He also found antagonism between Fe and Zn and between Zn and Manganese (Mn); since the relationships Zn/ Fe+Mn< 0,16 caused deficiencies of Zn. However, the antagonism between Fe and Zn decreases under high content of Fe, Zn and Mn in organic matter.

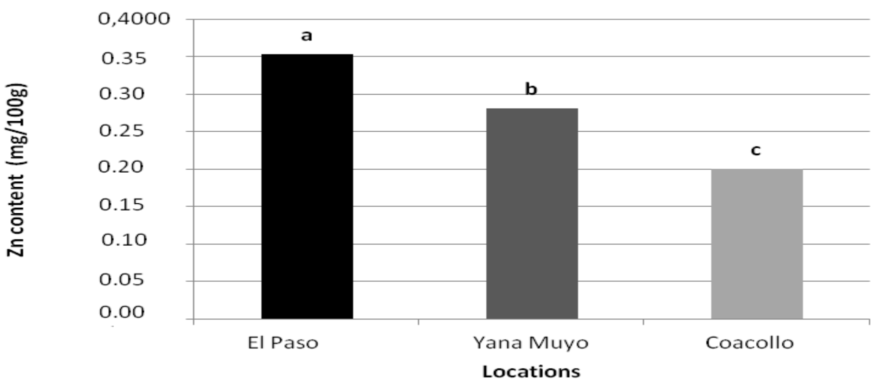

Figure 5. Zn content in tubers based in fresh weight, and for locations. Year 2013.

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Burgos *et al*. (2007), found differences highly significant ($p<0.01$) in the concentration of Zn in tubers in the testing of 37 native cultivars of potato between two high land locations (3800 y 3700 meters over sea level), in acid soils (pH 3.8 y 5.2), of high capacity of cationic exchange (20 y 26 meq/100 g), with high content of organic matter (5,1 y 6,4%) and enough content of Fe. In the same say, White *et al*. (2009), mentioned that the mineral concentrations in tuber vary significantly with the geographic location. This was truer for Zn tan for Fe, in our study.

In our Research there was no interaction between cultivar*location (Table 4) which indicates that the differences between cultivars were the same in the three locations.

Figure 6 presents the response of the Zn content in tubers in relations to the tested cultivars. In the same way as the factors content of Fe in the tuber, the native cultivar Pinta Boca (STN) presented a major concentration ($p<0.01$) of Zn in tuber (0.30 mg.100 g^{-1}) than the cultivar Waych'a (0.26 mg.100 g^{-1}).

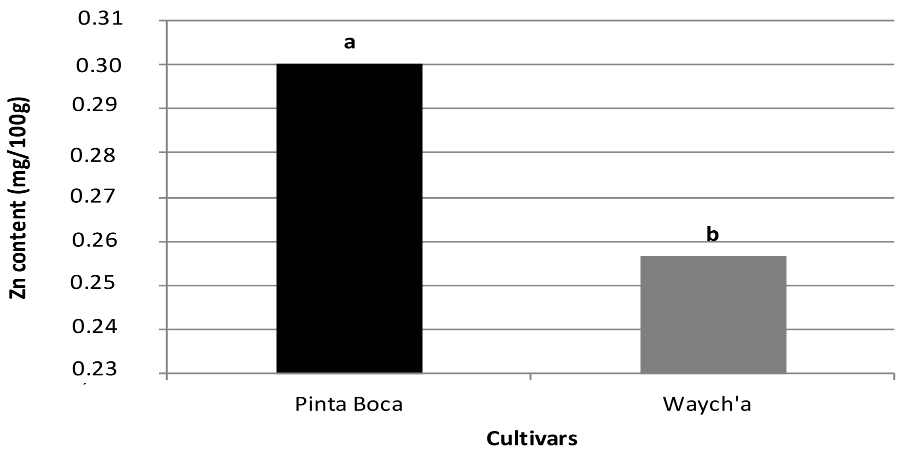

Figure 6. Zn content in tubers based in fresh weight, and for cultivars. Year 2013.

It was also found that the differences in the concentration of Zn in a trail plot in which CIP tested four species of native potatoes (GON, PHU, ADG Y STN) and 315 clones between the years 2004 y 2007 (CIP, 2007). STN and ADG had concentration of Zn between 0.33 to 0.57 mg.100 g^{-1} g y 0.20 a 0.51 mg.100 g^{-1} in PF, respectively (CIP, 2007).

Besides, Burgos *et al*. (2007) found significative differences ($p<0.01$) in the content of Zn when he tested 37 cultivars of native potato (GON, PHU, TBR, CHA, GON x STN, ADG y STN) in two locations, they had concentrations between 0.24 – 0.53 y 0.26 – 0.46 mg.100 g^{-1} of Zn in PF, respectively. The concentration obtained for Pinta Boca (STN) in our study (0.30 mg.100 g^{-1} de Zn en PF) it is between the range that was reported by Burgos et al. (2007) and the CIP (2007), for the specie STN.

Apparently native diploid cultivars like STN (Pinta Boca) absorb better the Fe and Zn than the tetraploid cultivars like the ADG (Waych'a). This is probably related to the fact that the specie STN is the most ancient and is the one that has had more time of co-evolution (Grun, 1990), reason for which has genes for adaptation and rusticity that are very valuable.

Analysis of the outcome of cultivars to the recommended ingest of Zn

cultivars

The cultivar Pinta Boca (STN diploid) would have an outcome in average of 3.6% and 3% to the recommended daily ingest of Zn in Bolivia for 1 to 3 years old children (8.3 mg.día^{-1}) and women under reproductive age (9.8 mg.día^{-1}), respectively; taking into account a an average consumption of potato of 100 g.day^{-1}. The cultivar Waych'a (ADG tetraploid); on the other hand, would have an outcome of 31% y 27% for children and women, respectively. The bred cultivar Chota Ñawi could give an outcome of 0.61 mg/100g de Zn (Gabriel *et al.*, 2014a) which is 7.3% and 6.2% of the requirements of children and women respectively. The minimum proposed by CIP for a potato cultivar to be able to decrease the deficiency of zinc is approximately 0.83 mg.100 g^{-1} of Zn in PF (Ortiz, 2010). Therefore, the cultivar Chota Ñawi would be the best recommended, even though is not yet widely cultivated.

Optimum level of Zn for the best content of Zn in tubers

In this study, once established that significative differences were there ($p<0.05$) for the content of Zn in tubers (Table 4), it was established that the regression for the content of Zn in relation with the four levels of Zn would have a linear trend (Figure 7), for which the level of 15 kg.ha^{-1} allowed to obtain the major content of Zn in tuber (0.31 mg.100 g^{-1}).

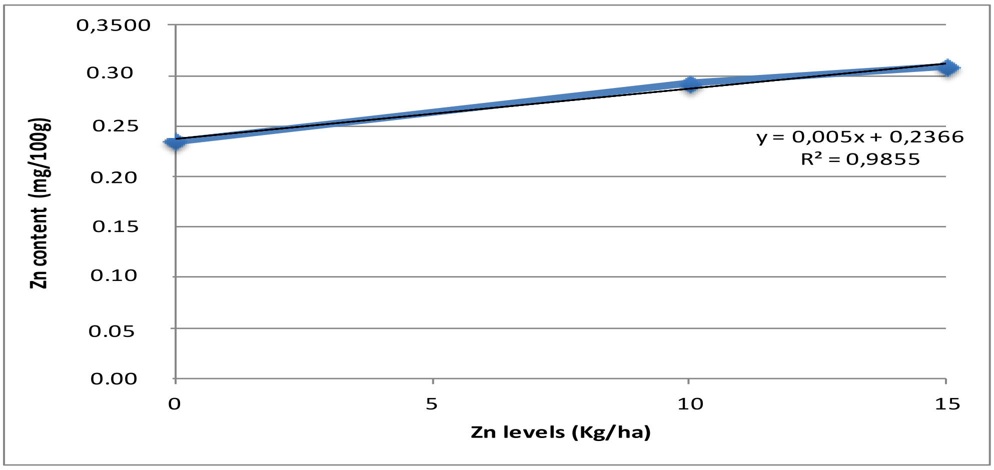

Figure 7. Trend of the regression for the Zn content in tubers based in PF in relation to four levels of fertilization with Zn (kg.ha^{-1}). Year 2013.

The response obtained on the content of Zn in tuber in relation to the level of fertilization with Zn was linear (Figure 7), meaning that, to the increase of the doses of Zn in soil, the content of Zn in tubers also increases. In a study developed by Vélez (2013) about edaphic and foliar fertilization and about fertilization with Fe and Zn in potatoes under greenhouse condition, a linear significative trend was found for the concentration of Zn in potato flesh, as an effect of soil fertilization. Besides, Shaver y Westfall (2008) analyzed the different sources of fertilization with Zn in maize, and found that only one source $ZnSO_4$ (36.5 %) showed a linear positive trend with the increase of Zn concentration and the absorption with the increase of doses application of 6.6; 11 and 22 kg.ha^{-1} de Zn; however there were no significative differences between levels.

As a conclusion it could be said that the yield of Waych'a and Pinta Boca is affected by the environment. The optimum level for Fe in El Paso was of 10 kg.ha^{-1} of $FeSO_4$ to achieve 15 t.ha^{-1} and for Zn in the three locations was 9.71 kg.ha^{-1} of $ZnSO_4$

to achieve 16 $t.ha^{-1}$. The content of Fe and Zn in tubers was greater in the cultivar Pinta Boca than in Waych'a.

Conflict of interests

The authors declare there is no conflict of interest in the publication of the results of this research.

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