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Seed Mineral Content and Nutrient Harvest Index of Common Bean Variety "Azufrado Reyna"

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is an important legume that is highly consumed in Latin America and other countries as a part of feeding. It provides proteins, carbohydrates, vitamins, fiber and a wide range of minerals, which are essential in human nutrition. Determining the nutrient removal values on seeds is crucial to better adjust fertilization rates, increase nutrient use efficiency and nutritional quality. Therefore, a field experiment was conducted in northern Sinaloa in order to investigate the response of common bean variety Azufrado Reyna to different NPK fertilization rates on seed mineral content and nutrient harvest index values. The experiment was arranged as a randomized complete block design with three replicates. Based on the results, fertilization rates only influenced on the total mean protein content, phosphorus and iron. The total nutrient removal values were K>Ca>P>Mg and Fe>Zn>Mn>B>. While nutrient harvest index values indicated that N, P, Fe and Zn exhibited higher degree of mobility to grain compared to the rest of nutrients. These parameters could represent a good trait in terms of nutritional quality for common bean varieties, also, serve as reference values to precisely estimate nutrient removal from soil.

KEYWORDS: Extraction, Fertilizer, Crop, Nutrient.

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Contenido mineral de la semilla e índice de cosecha de nutrientes de la variedad de frijol común "Azufrado Reyna"

RESUMEN

El frijol es una leguminosa muy importante que constituye parte de la alimentación en Latinoamérica y otros países. Actualmente, es importante conocer los valores de remoción nutrimental en frijol para ajustar recomendaciones agronómicas, incrementar la eficiencia en el uso de nutrientes y conocer el contenido mineralógico de la semilla como parámetro fundamental de calidad. Se estableció un experimento con el propósito de investigar la respuesta del cultivo de a diferentes dosis de fertilización NPK en concentración mineral de semilla e índice de cosecha nutrimental. El experimento consistió en bloques completos al azar con tres repeticiones. De acuerdo a los resultados, las dosis de fertilización influyeron únicamente en la concentración total de proteína, fósforo y hierro. Los valores de remoción nutrimental total fueron K>Ca>P>Mg y Fe>Zn>Mn>B>. Mientras, que los valores del índice de cosecha nutrimental indicaron que el N, P, Fe y Zn exhibieron un grado de movilidad mayor hacia el grano en comparación con el resto de los nutrientes evaluados. Estos parámetros podrían representar un rasgo especifico en términos de calidad nutricional en variedades de frijol común, además de servir como valores de referencia para estimar más precisamente la remoción nutrimental del suelo.
PALABRAS CLAVE: Extracción, Fertilizante, Cultivo, Nutriente.

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important grain legumes cultivated worldwide and it is considered a nutrient demanding crop (Nakitto et al., 2015).

Mexico represents the origin center and diversification of this specie, and the actual genetic variability (physical characteristics, type of seed and chemical composition) has given diverse genetic forms (Hernández-López et al., 2013). Nutritional value of this legume is determined by its protein content (23%) minerals (iron, potassium, magnesium, zinc, calcium and phosphorus) as well as fiber, starch, folic acid and thiamine (Karim et al., 2020), which are strongly associated to human nutrition.

It is documented that common bean cultivars have shown variations in dry matter accumulation rates, nutrient export rates and harvest index values depending upon the cycle length, commercial group and plant growth type (Nascente & Carvalho, 2018; Leal et al. 2019; Silva & Moreira, 2022). In that aspect, the processes of remobilization (nutrient movement to the grain) and the nutrient harvest index values (quantity of nutrient uptake partitioned to grain) (Bender et al., 2015) are being used to assess sustainability in grain production systems (Esper-Neto et al. 2021), maintain soil fertility levels and adjust the rates and source of fertilizers (Tiecher et al., 2017). Other reports argue that underestimated nutrient replacement will impact cropping systems and decrease yield (Houx et al., 2014; Hansel et al., 2017). While, Gouveia et al. (2014), mention that mineral content in beans has varied as a function of genetic material, crop management practices and storage conditions.

Despite the importance of common bean in Mexico, there is very limited information regarding nutrient content and nutrient harvest index values for this crop. The existing reference values are outdated with cultivars grown in different soils and climate conditions of other countries. Therefore, the objectives of this study consisted of determining the amount of nutrients present in grains and estimate the nutrient harvest index of this crop.

1. Literature review

Studies on soybean grain composition have only estimated single nutrients such as (N) as the most important (Divito et al., 2016; La Menza et al., 2017), followed by phosphorus (P) or potassium (K) in some cases. Some others have studied how water stress and management practices during seed filling affected grain composition (Farmaha et al., 2016; Maciel de Oliveira et al., 2019). Nevertheless, very few studies have focused on the effects of different environments on soybean growth and nutrient grain content (Moreira & Moraes, 2016). Araméndiz-Tatis et al. (2016) found great differences in protein content, Ca, Mg and K in several black-eyed pea varieties (*Vigna anguiculata* L.) as compared to local landraces. They also mentioned that those varieties could be used in breeding programs to assure a higher nutritional value.

Espinoza-García et al. (2016) found high content of P, Na, Ca, Zn and Cu for several landraces of common beans grown in central Valley of Oaxaca, Mexico; while, the landraces grown in the north, attained high values of S, K, Fe, Zn and Mn under the same agronomic practices. They concluded that location and weather conditions influenced the seed nutrient concentration.

Works by Sarwar et al. (2016), Wan et al. (2011) have shown that P application enhanced micronutrient levels in plant tissues (leaves, stems, pods) which could also promoted higher quality parameters in seeds especially with Fe and Zn. Smith et al. (2019) found that water stress and P deficiency decreased the concentration of mineral nutrients such as K, P, S and Ca in seeds, but they observed that nitrogen concentration enhanced by 50% as well as the concentration of amino acids (alanine, arginine, asparagine, histidine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine) in response to water deficit conditions, with no differences between genotypes.

Machado-Silva et al. (2024) reported average nutrient removal values of 33 kg N, 11 kg P_2O_5, 17 kg K_2O, 1.5 kg Ca, 2.2 kg Mg and 2.3 kg S per ton of grain; while the average amounts of micronutrients were 14 g B, 3.7 g Cu, 70 g Fe, 13 g Mn and 34 g Zn for determinate and indeterminate common bean cultivars. They argued that the amounts of exported nutrients occurred because the cultivars tested yielded more than older cultivars and were also larger than those exported by the genotypes studied by Westermann et al. (2011). Nonetheless, Bulyaba et al. (2020) and Mukankusi et al. (2020) stated that variations in nutrient removal values are due to differences linked to genetic variability among the cultivars used. Silva and Moreira (2022) found a higher initial nutrient accumulation for early cultivar “TAA Gol” in the stage of V4 compared to other cultivars.

Khazaei and Vandenberg (2020) reported that seed mineral concentrations in low-tannin faba beans were rich in Ca, Mg, Fe, and Zn, which are usually lacking in the human diet. They also found higher protein content when compared to tannin-containing faba beans.

Finally, studies have focused on nutrient content in common bean as a way to enhance concentration of zin and iron in biofortified cultivars in Africa and Latin America (Haas et al., 2016; Andersson et al., 2017).

Regarding the nutrient harvest index, Westermann et al. (2011) found differences in the values depending upon the nutrient evaluated. They reported high harvest index values on N (1.0), P (1.09), K (0.59), Zn (0.83) and Cu (0.80) which were attributed to their mobility within the plant, except for Mn (0.29), evaluated on 16 bean genotypes and seven organic and conventional production systems. In that aspect, Bray (1954) proposed that nutrient mobility in soils is determined by the ionic state at which nutrients are taken up by plant roots and that partially influence the effectiveness of nutrient applications (Havlin et al., 2005).

2. Materials and Methods

2.1. Description of the experiment

A field experiment was conducted in northern Sinaloa, Mexico, during the winter season 2021-2022. The experimental site was located at Química Internacional Aplicada S.A de C.V (25°47’39”N 108°54’49”W) and 15 m above sea level. The soil of that region is classified as clay loam (50% clay, 30% silt y 20% sand), low organic matter content (< 1%), bulk density of 1.15 g cm^{-3} and volumetric water content of 0.155 cm^3 cm^{-3}.

Soil tillage techniques were realized following the guideline provided by Instituto Nacional de Investigaciones Agrícolas y Pecuarias (INIFAP). A composite surface 30 cm soil samples were collected before fertilization. The planting was realized on moistened soil and pest management was controlled during the season. Irrigation scheduling was managed by the water balance method (software IrriModel) (Sifuentes et al., 2012), which estimates the depletion levels in the root zone. Irrigation targets were set as a 50% depletion of plant available water (PAW), monitoring water content with Time Domain Reflectometry. Total N, P and K were pre-plant applied using Compo NovaTec® Classic 12-8-16 (+3+TE). Treatments consisted of different NPK rates T1: (45, 30, 60 $kg.ha^{-1}$), T2: (90, 60, 120 $kg.ha^{-1}$), T3: (150, 100, 200 $kg.ha^{-1}$) and control (100 $kg.ha^{-1}$ urea).

The experiment was carried out in a randomized complete block design with three replicates. The experimental unit had a dimension of 64 m^2.

2.2. Determination of nutrients in foliar tissues and grain

Destructive sampling of plants was performed to estimate nutrient uptake of crop at different stages of growth (third trifoliate, flowering, pod filling and physiological maturity). Plant samples were taken to the laboratory where all structures were separated (leaves, stems and pods). Vegetative and grain samples (taken at harvest) were oven-dried at 70 ºC until constant dry weights. Nitrogen was determined by wet digestion with Kjeldahl method and protein content was quantified with N concentration and a factor of 6.25 (Nelson & Sommers, 1973). Phosphorus was analyzed by wet digestion with nitric acid (HNO_3) + perchloric acid ($HClO_4$) + sulfuric acid (H_2SO_4). All the filtered material obtained by wet digestion was used to determine the concentration of K, Ca, Mg, Fe, Mn, Zn, Cu by spectrophotometry, following the guideline on the Official Mexican regulation (NOM-021-RECNAT-2000). Results of macro and micronutrients were reported on percentage and parts per million respectively.

The grain nutrient content ($kg.ha^{-1}$) was calculated as it follow:

Macronutrient removal ($kg.ha^{-1}$) = [yield ($kg.ha^{-1}$) * (% nutrient)/100)]

Micronutrient removal ($g.ha^{-1}$) = [yield ($kg.ha^{-1}$) * (% nutrient/1000)]

Nutrient harvest index (Nu-HI) was calculated by the ratio of grain nutrient content with respect to total nutrient uptake as outlined by Dass et al. (2010).

Nu-HI= [uptake of nutrient (fruit-pod) ($kg\ ha^{-1}$)/total nutrient uptake in biomass ($kg\ ha^{-1}$)] *100

2.3. Data analysis

Data of total grain nutrient content and the nutrient harvest index were subject to analysis of variance. Treatment mean differences were separated using Fishers' least significance difference (LSD) test at $P \leq 0.05$ (Minitab, 2017).

3. Results and Discussion

3.1. Grain nutrient removal

Grain nutrient removal is a very important parameter in terms of quality for human nutrition. As observed from data, protein content was significantly ($P \leq 0.05$) affected by the application of NPK rates. The greatest percentage was recorded in plants on T2.

The mean protein content (20.5%) was within the range as reported on previous works by Aramendiz-Tatis et al. (2016) who found 22%. Other studies have reported from 21 to 27% protein in seeds of cowpea beans (*Vigna unguiculata*) (Antova et al., 2014) (table 1). Smith et al.

(2019) found 213 mg.g^{-1} in irrigated bean production and 226 mg.g^{-1} in drought conditions. They mentioned that the value was higher than protein supplied by amino acids. On the other hand, Leleji et al. (1972) who reported that crude protein in common bean was highly influenced by the environment. Bulyaba et al. (2020) found differences in seed crude protein content among varieties and they relate them to seed color. Strauta et al. (2013) reported differences in grain crude protein content between brown and white-colored *Phaseolus coccineus* L. also related to seed color.

The range of P found (4.5 g.kg^{-1} of seed) was similar to that reported by Aramendiz-Tatis et al. (2016), Mesquita et al. (2007) and Frota et al. (2008). While, Araujo (2007) found 3.0 and 5.4 mg.g^{-1}. Espinoza-García et al. (2016) found high values of P content in several landraces of common bean (4.9 g.kg^{-1} of seed) grown at different locations of Oaxaca. They reported that those values were higher from those found by Martinez-Meyer et al. (2013) and Prolla et al. (2010) who obtained 4.7 and 3.6 g.kg^{-1} of seed respectively.

Potassium had a mean concentration of 18 g.kg^{-1}, which was higher than that found by Avanza et al. (2013), Aramendiz-Tatis et al. (2016) and Mesquita et al. (2007) on some species. While, Jiang et al. (2019) found that the average N, P and K content in grain were 45.7 g.kg, 5.0 g.kg, and 10.1 g.kg on soybean.

Ca concentration (6.7 g.kg^{-1}) was higher compared to values exhibited by Frota et al. (2008) who reported 1.4 g.kg^{-1} of seed. Espinoza-García et al. (2016) found from 1 to 1.5 g.kg^{-1} of seed; Prolla et al. (2010) reported 4 g.kg^{-1} of seed, while Martinez-Meyer et al. (2013) found 1.41 g.kg^{-1} of seed. As indicated by Ribeiro et al. (2012), the differences in Ca content could be attributed to genetic variation (influence the capacity to form new roots), being the roots the determinants in uptake and accumulation in seeds. Possibly, the accumulation of Ca in this study was closely related to soil fertility where the experiment was conducted, due to its optimum concentration to meet plant requirement.

Magnesium content was around 4 g.kg^{-1} of seed, value that was above from that reported by Avanza et al. (2013) in cultivars of beans. Thus, total macronutrient contents were in the following order: K› Ca› P› Mg (table 1). In that aspect, Esper-Neto et al. (2021) found the following removal values, N=45.6 $kg.ha^{-1}$ of grain, P=4.95 $kg.ha^{-1}$, K=20.6 $kg.ha^{-1}$, Ca=1.76 $kg.ha^{-1}$, Mg=2.37 $kg.ha^{-1}$ for soybean varieties grown in Brazil.

Total concentrations o micronutrients were as follow: Fe> Zn> Mn> B> Cu> (table 1).

Fe content significantly differed ($P \leq 0.05$) in response to NPK fertilization rates. The highest value was found on T2 (0.43 $g.kg^{-1}$ of seed). The mean concentration was 0.378 $g.kg^{-1}$ of seed respectively. These values were higher than those reported by Espinoza-García et al. (2016), Prolla et al. (2010), Martínez-Meyer et al. (2013) and Gouveia et al. (2014) who found 37, 74, 33, 61 $mg.kg^{-1}$.

Bulyaba et al. (2020) reported that the range of Fe varied with location (61 to 150 mg kg^{-1}) and varieties (83 to 141 mg kg^{-1}) and was higher than the average Fe concentrations (55 mg kg^{-1}) found by Bänziger and Long (2000). Khazaei et al. (2017) reported that Fe concentration of Faba bean seeds ranged from 45 to 55 ppm, which was similar to that observed for other crops, but less than that reported for lentil (53-93 ppm) grown in Western Canada.

Zn content had a mean concentration of 0.32 $g.kg^{-1}$ of seed, a higher value than that found by Astudillo and Blair (2008) in 40 cultivars of common bean. They also suggested that application of P to soil can increase Fe concentration but at the same time it can decrease Zn concentration in bean seeds. Studies by Moreira et al. (2018) argue that low Zn concentrations in grains are related to poor supply by the soil, since part of total content (30 to 60%) is found as plant-unavailable forms, trapped in organic matter or adsorbed on mineral colloids. Petry et al. (2015) reported that location and management can alter iron and zinc seed concentration.

Smith et al. (2022) reported that iron and zinc concentration in the seed were not affected by varying locations, soil properties or varieties. Works by Deckij-Kachinski et al. (2020) found that foliar Zn application increased the export rate of Zn, P, Ca, Mg, S, Mn, Cu and Fe in cultivar “IPR Campos Gerais”; while soil Zn application resulted in higher export rate of P, K and Mn in cultivar “BRS Esteio”. However, the nutrient export rate was N > K > P > Ca ≈ S > Mg and Fe > Mn > Cu > Zn for treatments without Zn application.

Overall, research evidence suggests that amino acid deficiency in Azufrado beans is a non-nutritional factor due to low use of iron and zinc. Others mention that seed nutrient quality is regulated by the source-sink dynamics and the manipulation of transport processes represents a possible strategy to increase nutrient allocation within the seed (Bennett et al., 2011; Pottier et al., 2014; García & Grusak, 2015; Tan et al., 2017). While, the specific nutrient mobility is influenced by the functions that nutrients play in plant metabolism and determines their mobility or redistribution within the plant. Finally, Smith et al. (2019) determined that rain-fed bean production did not impact the concentration of nutrients within the seed. However, it decreased the nutrient concentration in leaves. Works by Below et al. (2010) showed that environmental conditions, cropping rotations and fertilization rates had influenced grain nutrient concentrations as a crucial component of nutrient removal.

Table 1. Seed nutrient content of common bean (*Phaseolus vulgaris* L.).

Fertilizer	Protein	P	K	Ca	Mg
rate kg ha^{-1}	(%)		g kg^{-1} of seed		
control	18.6 b	4.5 ab	17.5	6.4	3.7
45-30-60	20.1 ab	4.9 ab	18.7	6.8	3.9
90-60-120	22.3 a	5.3 a	18.9	7.0	4.2
150-100-200	21.1 ab	4.1 b	17.7	6.7	4.2
OSL	0.05	0.03	0.6	0.6	0.6
Overall Mean	20.5	4.5	18	6.7	4
	Fe	**Mn**	**Cu**	**Zn**	**B**
		g kg^{-1} of seed			
control	0.30 b	0.13	0.087	0.30	0.10
45-30-60	0.41 ab	0.13	0.097	0.34	0.10
90-60-120	0.43 a	0.17	0.097	0.34	0.11
150-100-200	0.37 ab	0.14	0.092	0.30	0.12
OSL	0.005	0.8	0.7	0.4	0.4
Overall Mean	0.37	0.14	0.093	0.32	0.11

Values with different letters are significantly different. Fisher's mean separation test ($P \leq 0.05$), (OSL Pr> F).

3.2. Nutrient Harvest Index (Nu-HI)

The values found in this study showed that all nutrients exhibited different mobility. Approximately 75% of total P uptake was partitioned to grain, greater than N (37%), K ~ Ca (20%). These values indicated that P and N were mobilized to grains in a higher extend as compared to K and Mg which were partitioned in similar amounts (21%).

As expected, Ca-HI (17%) exhibited the lowest value, since this nutrient is immobile within the plant and less likely to be distributed to grains (Mascarenhas et al., 2013).

Of the measured micronutrients, Fe, Cu and Zn had the highest HI and Mn (10%) had the lowest. Some of the HI values are very similar to those of Karlen et al. (1988) in maize, who found the following values N (~60%), P (~80%), K (~25%), Ca (3%), and Mg (59%); this fact explains the behavior of nutrients within plants.

Kumar et al. (2015) found N-HI values of 30.2%, P-HI 35.3%, K-HI 26.7%, B-HI 20.4% on Okra plants (*Abelmoschus esculentus* L.) at V_{12} with application of 50%P and 80% water regime. They also reported values of N-HI 43.6%, P-Hi 46.6%, K-HI 37.8%, Fe-HI 35.7%, Zn-HI 42.7%, Cu-HI 44.7%, Mn-HI 31% and Mo-HI 39.6% on garden pea (*Pisum sativum* L.). Dass et al. (2010) found P-HI range values from 0.67 with compost to 0.74 on control treatments, N-HI ranged from 0.63 with recommended NPK to 0.67 on the control and K-HI values from 0.31 with compost to 0.34 on the control rice cultivar.

Similarly, Westermann et al. (2011) reported that some common bean varieties such as pinto Othello, Bill Z, CO46348, Mexican red NW-63 and UI 239 had higher Nu-HI that should be deployed in sustainable and reduced-input planting systems.

Rotundo et al. (2014) and Tamagno et al. (2020) have reported high N allocations from stover to grain in modern soybean genotypes indicating the ability of the genotypes to maintain grain N content. The existing N-HI values in the literature range from 52 to 72 for soybean growing in Brazil (Moreira et al., 2016; Maciel de Oliveira et al., 2019), which represent small values compared to the N-HI measured in other places.

3.3. Nutrient removal as a function of yield

Overall, the removal values obtained for all treatments showed a linear increase as a function of yield. The range of total nitrogen removal values were of 100 and 113 kg.ha-1 for a yield between 2700 and 3200 kg.ha-1 (Figure 2A). These concentrations impact the seed protein content, which represent a good quality parameter. Removal of PO4- was in a range of 11 and 19 kg.ha-1 (Figure 2 B), potassium removal was in a range of 43 and 57 kg.ha-1 (Figure 2 C), while, calcium removal remained constant throughout the season and was in a range of 15 kg.ha-1 for the same yield (Figure 2 D). Ribeiro et al. (2011) reported that common bean genotypes with higher yield, also removed higher P on seeds.

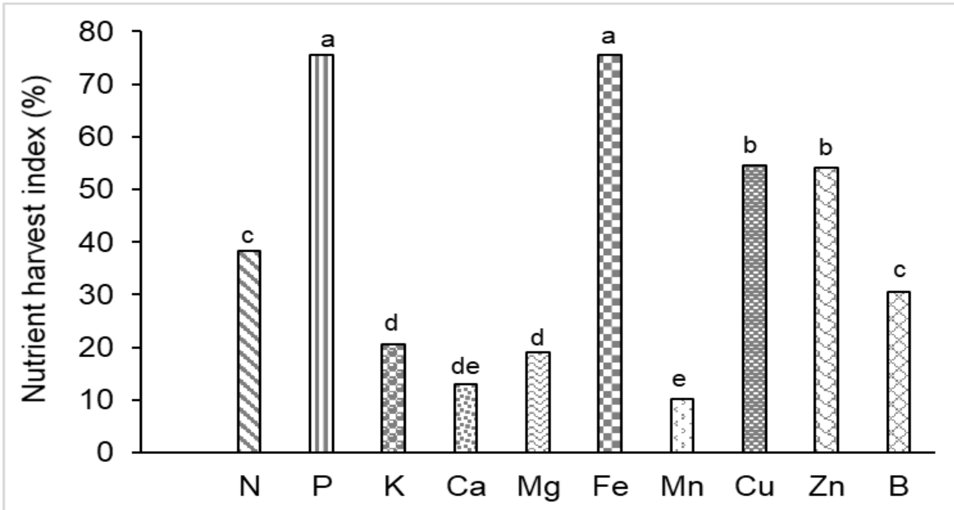

Figure 1. Nutrient harvest index in grains of common bean.

Bulyaba et al. (2020) found a positive correlation of yield with grain P content on different common bean varieties. They also mentioned that root morphology of those varieties could exploit more soil volume for P acquisition (Shen et al., 2011) and tended to accumulate it into the grains. The same authors observed varietal differences in grain K between varieties (Taurus, MY06326, Montcalm and Eclipse).

The relationship of micronutrient removal values also followed a linear increase as a function of yield. The range of total iron (Fe) removal values were of 43 and 8 $g.ha^{-1}$ for a yield between 2580 and 3200 $kg.ha^{-1}$ (Figure 3A). In that aspect, Hirschi (2009) exhibited that the absence of significant differences in grain Fe levels among locations even when soils had sufficient Fe concentration at planting may be due to the irreversible binding of Fe to soil particles immediately after application, which limits the uptake by plants and its accumulation in grain. Studies by Aciksoz et al. (2011) reported that application of Fe fertilizers to soils do not enhance seed Fe concentrations. Ramolemana, (2013) also reported no significant impact of soil type or location on Fe concentration of *Morama bean (Tylosema esculentum (Burch.) E. Schreib.)*

seeds. Removal of Zn was in a range of 77 and 114 $g.ha^{-1}$ (Figure 3 B), while, Mn removal values were in a range of 44 to 68 $g.ha^{-1}$ (Figure 3 C) for a yield between 2580 and 3600 $kg.ha^{-1}$.

Bulyaba et al. (2020) exhibited that grain Zn content on common bean was not affected by sites despite variations in soil pH (8.0 to 6.0). In that sense, Gül et al. (2008) mention that pH can reduce Zn solubility and impair root absorption. Marschner, (2012) reported that soil Zn concentration decreased by 30- to 45 fold for each unit increase in soil pH due to strong adsorption to soil constituents and reduce the desorption of the adsorbed Zn.

Similar studies by Zou et al. (2012) and Phattarakul et al. (2012) mention that grain Zn concentrations on wheat and rice did not enhance with the application of Zn fertilization. Finally, Moraghan and Grafton (2001) reported a negative correlation between seed weight and Mn concentration.

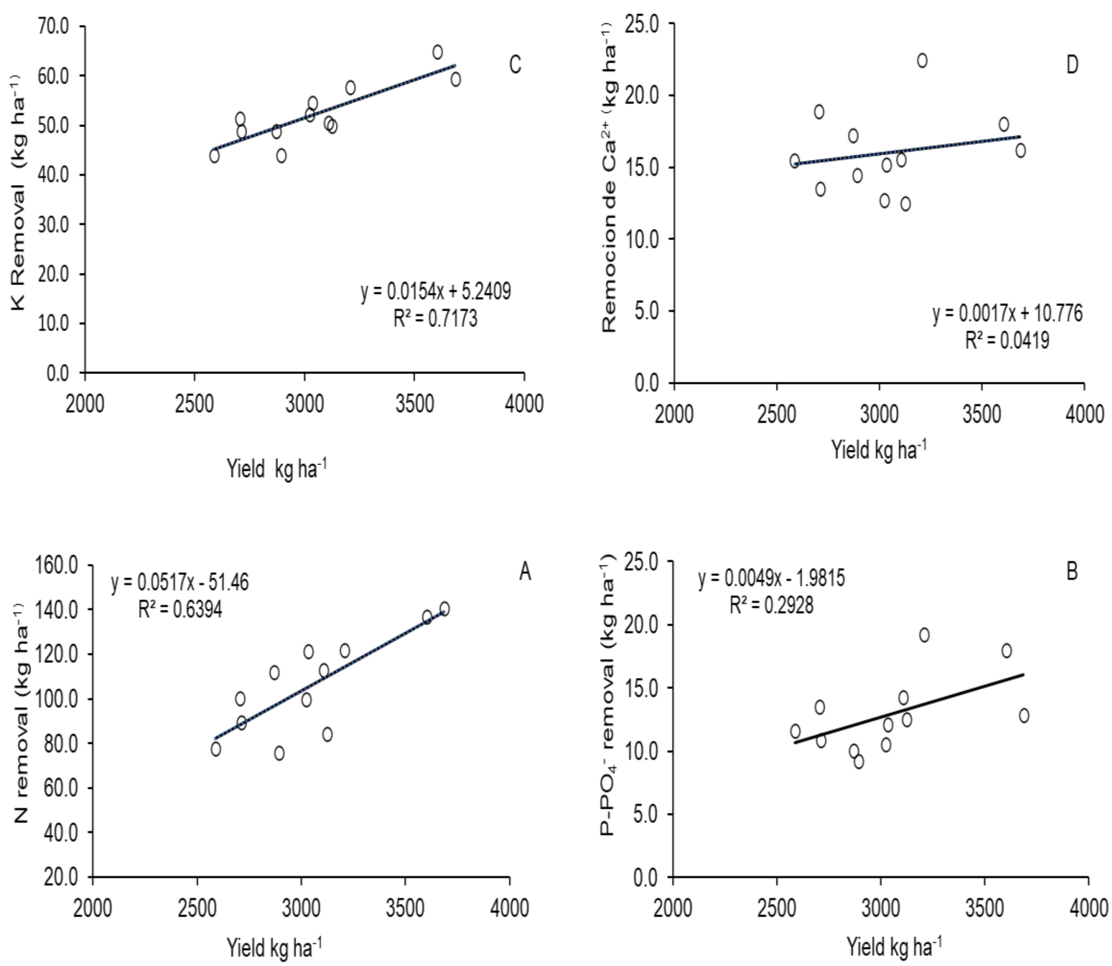

Figure 2. Relationship between yield and macronutrient removal values.

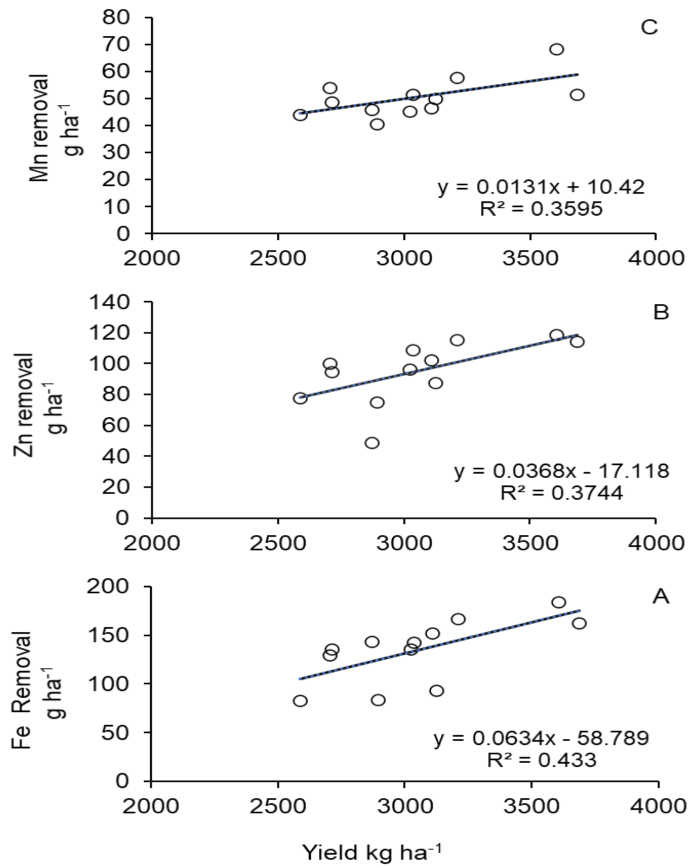

Figure 3. Relationship between yield and micronutrient removal values.

Conclusions

The mean protein concentration was 20.5 that was within the normal range, whereas the rest of concentration in seeds was in the following order: K› Ca› P› Mg and Fe› Zn› Mn› B› Cu.

The nutrient harvest index values indicated that N, P, Fe and Zn exhibited higher degree of mobility to grain compared to the rest of nutrients.

These parameters could represent a good trait in terms of nutritional quality for this variety. They can also serve as reference values to precisely estimate nutrient removal from soil and compare them with other varieties.

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Conflicto de interés

Los autores de este manuscrito declaran no tener ningún conflicto de interés.

Declaración ética

Los autores declaran que el proceso de investigación que dio lugar al presente manuscrito se desarrolló siguiendo criterios éticos, por lo que fueron empleadas en forma racional y profesional las herramientas tecnológicas asociadas a la generación del conocimiento.

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