Variation of the nutritional content of different genotypes of *Lotus corniculatus* L. under optimum and sub-optimum soil moisture conditions throughout the seasons of the year

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ABSTRACT

Objective: To evaluate the leaf nutrient content of four accessions and one variety of *Lotus corniculatus* L. trefoil throughout the seasons of the year and under optimum and sub-optimum soil moisture content, established under a shade mesh in northern Mexico.

Design/Methodology/Approach: During the 2021-2022 period, an experimental randomized block design, in a split-plot arrangement, with three replicates was established. The large plots had an optimum (26±1.5%) and sub-optimum (22±1.5%) soil moisture content. The small plots consisted of the trefoil accessions: 255301, 255305, 202700, 226792 origin code, and the Estanzuela Ganador variety.

Results: At optimum soil moisture content, the 202700 accessions recorded a K deficiency throughout the year. Meanwhile, regardless of the soil moisture content, the 255301 accessions recorded a Mg and a Mn deficiency only in summer. N, P, Fe, and Mn recorded deficient contents: N and P throughout the year and Fe and Mn only in winter and spring.

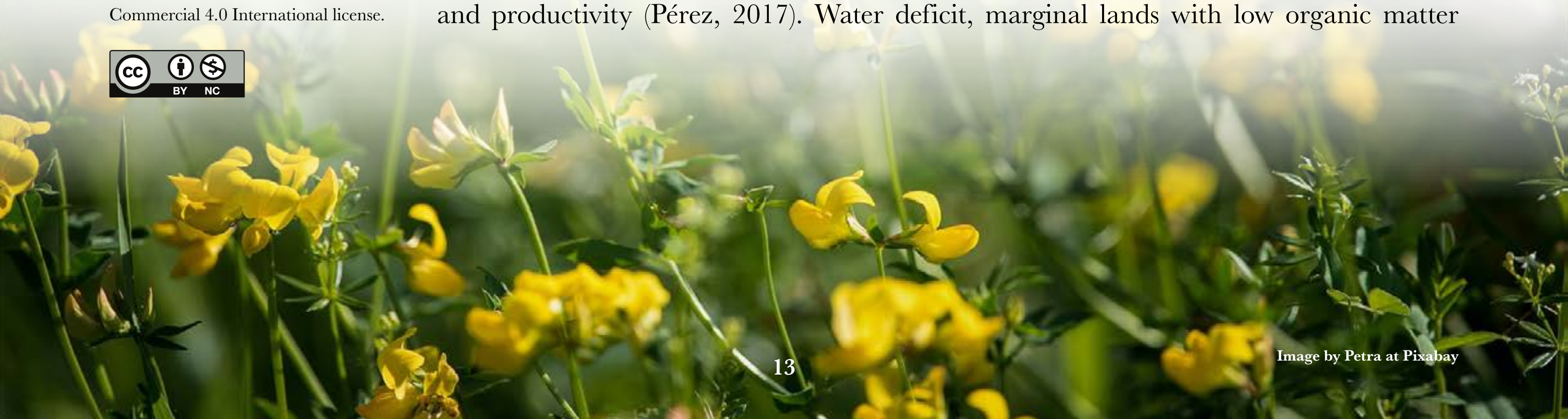
Study Limitations/Implications: These results could require a field validation, since the experiment was carried out in semi-controlled conditions, under shade mesh.

Findings/Conclusions: Developing an adequate annual nutrition program for *L. corniculatus* L. that improves its forage productivity requires several key factors, including the behavior of the nutritional status (dependent on the type of nutrient), the growth stage of the plant, the season of the year, and the soil moisture content.

Keywords: Plant nutrition, stress physiology, soil moisture, macro- and micro-nutrients, drought.

INTRODUCTION

Nutrition is fundamental for the growth of plants. Water plays a key role in the use of the different nutrients required by plants (Levinsh, 2023). Although plants require a minimum percentage of minerals, these are fundamental for their growth, development, and productivity (Pérez, 2017). Water deficit, marginal lands with low organic matter



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content, and extreme temperatures cause stress in plants (Bhattacharya, 2021). Some plant species have developed tolerance mechanisms to face biotic and abiotic limiting factors. This phenomenon promotes a better productive performance in marginal agricultural areas (Pedroza-Sandoval *et al.*, 2022).

The nutrition diagnosis of plants and soils —using critical, standard, or referential concentration indicators— enables the inference of the potential risks that may limit production, based on the deficit or the excess of nutrients (Kathpalia & Bhatla, 2018). Quintero-Azueta *et al.* (2021) reported that in several forage crops, the flowering stage is appropriate for the nutrition diagnosis of the plant.

Lotus corniculatus L. is a profitable pulse, with a nutritional quality similar to alfalfa (*Medicago sativa* L.) (18-22% raw protein); it has less cellulose and more non-structural carbohydrates (Álvarez-Vázquez *et al.*, 2018). Traditionally, the nutritional management of the common bird's-foot trefoil has been restricted to a P fertilization process, based on the idea that N can fix P in a soil in which pulses are grown (Barbazán *et al.*, 2008).

According to González-Espíndola (2024), the different seasons of the year impact the production of *L. corniculatus*. This situation is the consequence of extreme temperatures that modify several agronomic, such as the nutrients of the plants (Freire *et al.*, 2019; Márquez *et al.*, 2024). Therefore, the objective of this study was to determine the nutrient content of the leaves of different genetic plant materials of the common bird's-foot trefoil (*Lotus corniculatus* L.), under optimum and sub-optimal soil moisture content, throughout the seasons of the year. The hypothesis of the study was that the macro- and micro-nutrient requirements of the plant change, depending on the *L. corniculatus* L. genotype and the season of the year.

MATERIALS AND METHODS

The study was carried out in the experimental field of the Unidad Regional Universitaria de Zonas Áridas of the Universidad Autónoma Chapingo, in Bermejillo, Durango, Mexico (23° and 27° N and 106° and 102° W, at 1,130 m.a.s.l.). The area has a dry desert climate, with summer rains and cold winters. The region has a 258 mm annual mean precipitation and a 2,000 mm annual mean potential evaporation; the annual mean temperature is 21 °C, with 33.7 °C maximum and 7.5 °C minimum temperatures (Medina *et al.*, 2005).

Experimental design and management. An experimental randomized block design with divided plots and three replicates was used. The large plots had two moisture contents: optimum soil moisture content (OSMC: 26±1.5%) and sub-optimal soil moisture content (SSMC: 22±1.5%). Both were established based on the soil moisture drawdown curve determined by the pressure-membrane method proposed by Richards (1948), and through of this method the soil moisture constants were determined, corresponding to 26.5% field capacity (FC) and 17.5% permanent wilting point (PWP). The small plots used the *Lotus corniculatus* L. genetic plant materials of four accensions (255301, 255305, 202700, and 226792 identification codes) and the Estanzuela Ganador variety. The experimental unit consisted of a 20-kg plastic pot with a plant.

The pots were filled with 18 kg of a soil:compost:sand substrate (50:30:20 ratio). The substrate had a sandy-loam texture, with a 52:22:26 ratio of sand, clay, and slime, respectively. The experiment was carried out from March 2021 to May 2022, under shade-mesh conditions.

Irrigation was carried out every four days via gravimetry. The weight of the OSMC and SSMC pots was kept at 23.9 and 23.0 kg, respectively. An average of 0.6 L of water was added via irrigation to both moisture contents, reestablishing the upper limit of soil moisture of OSMC to 27.5% (slightly higher than FC) and of SSMC to 23.5%. Both percentages were allowed to decrease to 24.5% and 20.5%, respectively. A 3% margin (20.5-17.5) was considered as the usable moisture range, to prevent the plant from reaching PWP.

Measured variables. To determine the macro- and micro-elements, leaf samples from *Lotus corniculatus* L. were taken from the upper third of the plant. The samples were collected every 45 days (March 15, April 30, June 15, July 30, August 15, September 30, and November 15, 2021). From November 15, 2021 to March 15, 2022, the samples were collected every 90 days, because the low winter temperatures impacted plant growth. The Kjeldahl method (AOAC, 1984) and the Molybdenum blue method (Murphy and Riley, 1962) were used to determine N and P content, respectively.

The dry digestion method (Isaac & Kerber, 1971) was used to determine the Cu, Mn, Zn, Fe, Ca, Mg, K, and Na micro-nutrients, with a PerkinElmer AAnalyst 200 Atomic Absorption Spectrometer. On the one hand, the Ca, Mg, K, and Na macro-nutrients were established preparing a 1 mL + 9 mL triple distilled water dilution of the sample; on the other hand, 10 mL of the extract were used to determine the Fe, Cu, Zn, and Mn micro-nutrients.

The following formulas were used to determine the macro- and micro-nutrient percentages:

$$\text{Ca, Mg, K, Na}\,(\%) = \frac{ppm\ CC \times Dm \times Vd}{10\ 000} \tag{1}$$

$$\text{Fe, Cu, Zn, Mn}\,(\%) = ppm\ CC \times D_m \times V_d \tag{2}$$

Where: *ppm CC*=parts per million in the calibration curve; D_m=dilution of the mass (gauge volume/g of the sample); V_d=Volume dilution (gauge/aliquot, in case of dilutions).

Statistical Analysis. An analysis of variance (ANOVA) and Tukey's Multiple Comparison Test ($P \leq 0.05$) were used to process the database in the Statistical Analysis System version 9.0 software (SAS Institute, 2011). The statistical analysis was based on the corresponding model of the divided plot experimental design, where the treatments were the different genotypes within the moisture content. The Minitab v.18.0 statistical software was used to perform the Normality test (Johnson technique); this analysis was carried out before the ANOVA and the Tukey's Test.

RESULTS AND DISCUSSION

The Normality test recorded a positive value (P=0.956). Based on the results of the F-test, significant differences were found only between the optimum irrigation genotypes, throughout the seasons of the year (Table 1).

Based on the Normality test, the Ca, Mg, and Na values were P=0.325, 0.946, and 0.506, respectively. According to the analysis of variance, the F-probability identified a significant effect between the *L. corniculatus* genotypes, within each soil moisture content: Mg, Ca, and Na recorded no differences in sub-optimal soil moisture content, while Na recorded differences in the optimum soil moisture content (Table 2).

Behavior of the macro-elements between genotypes. The 202700 accession was the most sensitive and recorded the lowest K values in OSMC, throughout all the seasons of the year (Table 3); these results indicate that the plant consumes high amounts of K, which results in a low K content during the whole growth cycle (Sardans & Peñuelas, 2021).

Table 1. Analysis of variance of P content (%) of the different *Lotus corniculatus* L. genotypes, throughout the seasons of the year, with optimum and sub-optimal soil moisture content.

	Spring 2022		Summer 2021		Autumn 2021		Winter 2021-2022	
	OSMC	SSMC	OSMC	SSMC	OSMC	SSMC	OSMC	SSMC
Fc	62.9	1.57	8.39	2.49	6.85	0.82	5.58	1.78
Prob.	0.018*	0.295	0.008**	0.138	0.014*	0.55	0.024*	0.238

OSMC=optimum soil moisture content (26±1.5%); SSMC=sub-optimal soil moisture content (22±1.5%).

Table 2. Analysis of variance of the macro- and micro-nutrient content of the different *Lotus corniculatus* L. genotypes, with optimum and sub-optimal soil moisture content (summer 2021).

	Calcium (%)		Magnesium ($mg\ kg^{-1}$)		Sodium ($mg\ kg^{-1}$)	
	OSMC	SSMC	OSMC	SSMC	OSMC	SSMC
Fc	5.54	4.6	2.72	10.69	0.59	2.69
Prob.	0.023*	0.38*	0.01*	0.004**	0.681	0.000**

OSMC=optimum soil moisture content (26±1.5%); SSMC=sub-optimal soil moisture content (22±1.5%).

Table 3. Effect of the K content variation in *Lotus curniculatus* L. in different soil moisture contents and seasons.

Accessions	Spring (2022)		Summer (2021)		Autumn (2021)		Winter (2021-2022)	
	OSMC	SSMC	OSMC	SSMC	OSMC	SSMC	OSMC	SSMC
255301	5.0^{a}	3.9^{a}	5.8^{a}	4.0^{a}	5.7^{a}	3.6^{a}	5.3^{ab}	4.3^{a}
255305	4.3^{ab}	4.4^{a}	4.5^{ab}	4.4^{a}	3.9^{ab}	3.7^{a}	3.9^{ab}	3.7^{a}
202700	3.0^{b}	3.5^{a}	2.9^{b}	3.5^{a}	3.6^{b}	3.6^{a}	2.9^{b}	3.4^{a}
226792	4.7^{a}	4.11^{a}	4.7^{ab}	4.5^{a}	5.6^{a}	4.4^{a}	5.6^{a}	4.5^{a}
Estanzuela Ganador	4.3^{ab}	3.7^{a}	4.3^{ab}	3.8^{a}	4.9^{ab}	3.7^{a}	4.1^{ab}	3.5^{a}

Tukey's Test (P≤0.05). Figures with the same letters within each column are statistically equal. OSMC=optimum soil moisture content (26±1.5%); SSMC=sub-optimal soil moisture content (22±1.5%).

The Ca, Mg, and Na macro-elements only recorded effects during summer. The 255301 accession recorded de lowest Ca and Mg values, regardless of the soil moisture content. These results indicate that these elements are the most required during summer season (Catzistathis *et al.*, 2010). The high temperatures recorded during this season resulted in a higher photosynthetic activity. Regarding SSMC, the Estanzuela Ganador variety recorded high Na values. The plant did not record negative effects, which suggests a potential salinity tolerance (Orosco *et al.*, 2018) (Table 4).

Temporal behavior of *L. corniculatus* L. regarding the macro- and micro-nutrient average

Nitrogen content. The average N concentration of the plants recorded was 1% in OSMC and 1.1% in SSMC, with a 0.79-1.19% seasonal range and a significant difference ($P \leq 0.05$) between seasons. The highest (1.4%) and lower (0.79%) N content for both soil moistures were recorded in winter and summer, respectively (Figure 1A). Based on the 2% critical level for alfalfa recommended by Barbazán *et al.* (2008), the *L. corniculatus* L. recorded a N deficiency throughout the year.

Phosphorous content. The average P concentration reached 0.1%, regardless of the soil moisture content. No statistical difference ($P \leq 0.05$) was found, and the range recorded was 0.08-0.11%. Figure 1B shows that the P content is lower than the critical values (0.24%) proposed by Pinkerton *et al.* (1997). The P deficiency symptoms are similar to those of N: a growth delay of all the plant organs (mainly leaves and stems). These phenomenon increases the impact on foraging crops, because these organs are the main source of fresh and dry biomass productivity (Marín, 2011).

Potassium content. The plants recorded an average K concentration of 4.4% (OSMC) and 4.0% (SSMC), with a 3.86-4.72% range between seasons (Figure 1C); however, there were no differences ($P \leq 0.05$) between seasons. In addition, these values are higher than the 1.4% critical value reported by Barbazán *et al.* (2008). Just like in the case of N and P, K travels from the old to the new organs of the plant. When a plant has a K deficiency, the leaves turn slightly yellow and then develop necrotic spots (Marín, 2011).

Based on these results, the N, P, and K concentrations are expected to change according to the season, showing deficiency levels as the metabolic activity of the plant increases

Table 4. Effect of the Ca, Mg, and Na content variation in *Lotus curniculatus* L., with different soil moisture contents (summer 2021).

Accession	Calcium		Magnesium		Sodium	
	OSMC	**SSMC**	**OSMC**	**SSMC**	**OSMC**	**SSMC**
255301	3.4^{ab}	2.5^{a}	1.04^{abc}	0.77^{c}	0.34^{a}	0.25^{bc}
255305	3.5^{a}	2.8^{a}	1.14^{ab}	0.89^{abc}	0.32^{a}	0.22^{c}
202700	3.1^{ab}	3.6^{a}	1.16^{a}	1.12^{a}	0.39^{a}	0.27^{bc}
226792	2.5^{ab}	2.6^{a}	0.78^{c}	0.84^{bc}	0.37^{a}	0.40^{b}
Estanzuela Ganador	2.4^{b}	3.1^{a}	0.85^{bc}	1.07^{ab}	0.44^{a}	0.64^{a}

Tukey's Test ($P \leq 0.05$). Figures with the same letters within each column are statistically equal. OSMC= optimum soil moisture content (26±1.5%); SSMC=sub-optimal soil moisture content (22±1.5%).

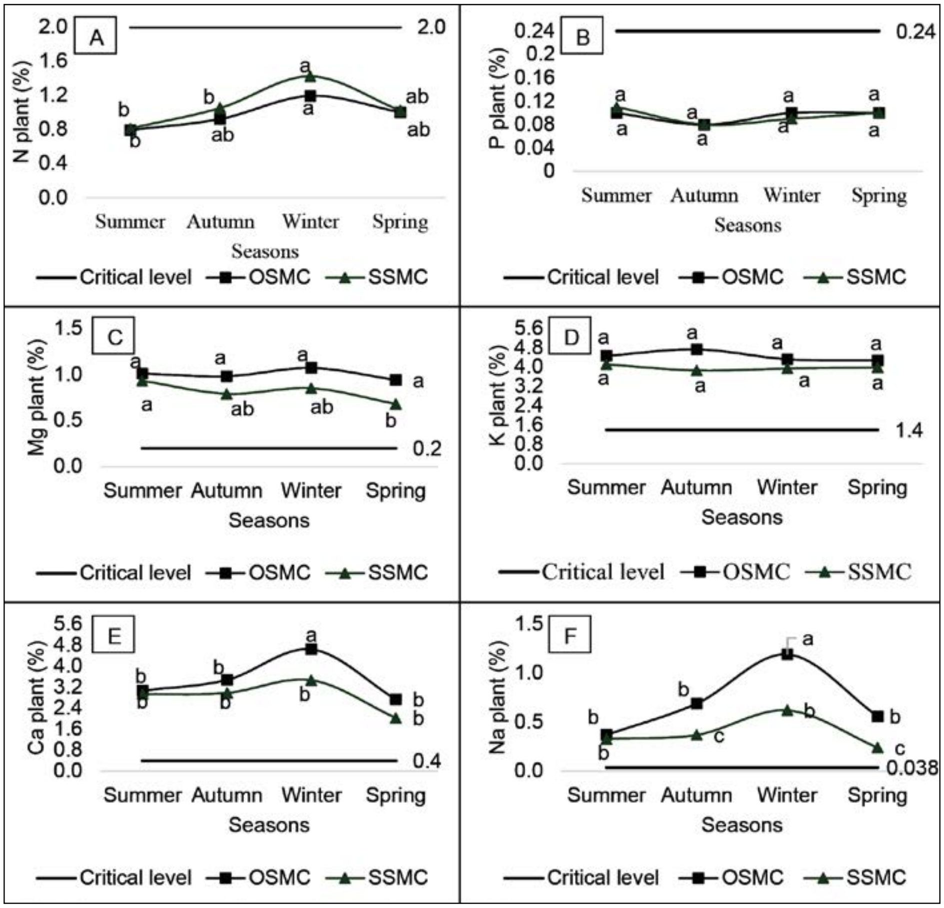

Figure 1. Seasonal behavior in the macro-nutrient content of the leaves of *L. corniculatus* L., with optimum soil moisture content (OSMC) and sub-optimal soil moisture content (SSMC). A=N concentration (%); B=P concentration (%); C=Mg concentration (%); D=K concentration (%); E=Ca concentration (%), and F=Na concentration (%).Tukey's Test (P≤0.05). Figures with the same letters on the same line of each graph are statistically equal. OSMC: optimum soil moisture content (26±1.5%). SSMC: sub-optimal soil moisture content (22±1.5%).

during spring and summer. The nutrients of a plant change with the seasons; this change is an indicator of how the plant and its organs absorb, use, redistribute, and extract nutrients (Galindo-Reyes *et al*., 2011).

Calcium content. Ca concentration showed significant differences (P≤0.05) between seasons. Winter recorded the highest concentration (4.64%). The Ca average reached 3.5% (OSMC) and 2.9% (SSMC) and the range between seasons was 2.9-3.5% (Figure 1D). These results surpassed the critical values (0.4%) reported by Thor (2019) for different plant species.

Magnesium content. The average Mg concentration recorded was 1% (OSMC) and 0.8% (SSMC), with a seasonal range of 0.68-1.07% (Figure 1E). Winter recorded the highest Mg concentrations (1%); however, the values decreased as the plant grew old. Nevertheless, the Mg values were always higher than the critical values (0.2%) of most plant species (Ishfaq *et al*., 2022).

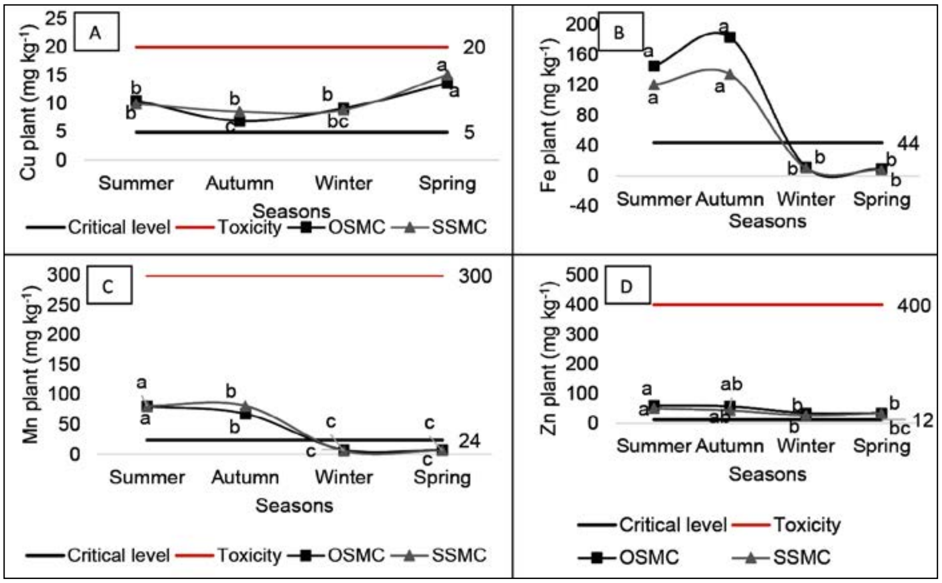

Figure 2. Seasonal behavior of the micro-nutrient content of *L. corniculatus* L., with optimum soil moisture content (OSMC) and sub-optimal soil moisture content (SSMC). A=Cu concentration (mg Kg^{-1}); B=Fe content (mg Kg^{-1}); C=Mn concentration (mg Kg^{-1}); D=Zn concentration (mg Kg^{-1}). Tukey's Test ($P \leq 0.05$). Figures with the same letters of each graph are statistically equal. OSMC: optimum soil moisture content (26±1.5%). SSMC: sub-optimal soil moisture content (22±1.5%).

Sodium content. The average Na concentration was 0.7% (OSMC) and 0.39% (SSMC), with a 0.24-1.19% range. The highest Na concentrations were recorded during winter, but only in the case of OSMC. The values decreased in spring (Figure 1F); however, they were always higher than the critical levels (0.038) established by Miloševi and Miloševi (2012).

Micro-nutrient content. A Cu deficiency was recorded throughout the year; however, Fe and Mn deficiency was recorded only in winter and spring. A similar response trend was reported by Ishfaq *et al.* (2022) in their study about alfalfa: 5, 44, 24, and 12 mg kg^{-1} for Cu, Fe, Mn, and Zn, respectively. At physiological level, the micro-nutrients impact the redox processes, which are important for the appropriate development of photosynthesis and the detoxification of free radicals in the oxygen (Marín, 2011).

Based on the results of the seasonal behavior of the micro-nutrients of the common bird's-foot trefoil (*L. corniculatus* L.), a higher seasonal influence was observed, depending on the type of nutrient, the moisture condition, and chemical characteristics of the soil. The behavior is modified by the relationships established between the genotypes and the plant species, strongly influenced by environmental conditions (particularly extreme temperatures and precipitation periods) (Prause and Fernández, 2012).

The nutritional state of a plant is partly related to the soil moisture content. However, its relation to the season of the year is even more important. The N and P macro-nutrient content is related to spring and summer, while the micro-nutrient content is related to winter. This information must be taken into account to improve the nutritional state of a plant using fertilization.

CONCLUSIONS

The nutritional state of *Lotus corniculatus* L. changed depending on the genotype, the type of nutrient, season, and, in a lower degree, the soil moisture content. The 202700 accession recorded K deficiency all year long in an optimum soil moisture content; meanwhile, the 255301 accession recorded Mg deficiency only during summer, regardless of the soil moisture content. N, P, Fe, and Mn recorded the highest deficiencies. N and P recorded deficiencies throughout the year, while Fe and Mn only in spring and summer. A plant and soil nutrition diagnosis is fundamental to develop an appropriate plant nutrition program.

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