EFFECTS OF MINERAL NUTRITION ON IRON TOXICITY SEVERITY, LEAF CHOROPLYL LEVELS AND GRAINS SOLUBLES SUGAR OF FLOODY LOWLAND RATOON RICE (*Oryza* sp) Nerica L 14 UNDER RAINFED REGIME

ABSTRACT

Field fertilization trials were conducted on 15 cm high rice stubble in lowland rainfed rice to determine mineral nutrition (N-, P-, K-, Ca-, Mg-, and Zn-) effects on iron toxicity, agromorphological parameters, average contents of leaf chlorophyll and grains soluble sugar contents of Nerica L 14. Four complete block design of seven randomized treatments was used. Results indicate that iron toxicity occurred in unfertilized ratoon crops grown on undeveloped gleysol containing some iron concretions in upper layer. Characteristic symptoms covered about 6-25% of the field. Nitrogen fertilization totally corrected these symptoms, significantly increased ($p = 0.00$) chlorophyll a (0.21 $mg.g^{-1}$ FL), b (0.30 $mg.g^{-1}$ FL) and total (0.51 $mg.g^{-1}$ FL) average contents of the three primaries functional leaves, height (60.11 cm) and tillering (8.56 tillers/feet) average of ratoon rice. In addition, Zn applied to soil induced an increase in grains soluble sugar content (3.23 mg glucose/g DM). Conclusion: iron toxicity, mainly caused by antagonism between Fe^{2+} and K^+ as well as Fe^{2+} and Mg^{2+}, also remains a constraint to rice ratoon cultivation. Its effects on agromorphological and agrophysiological parameters can be corrected by mineral fertilization and nitrogen applies particularly.

Keywords: Mineral fertilization, grains, rice ratoon, soluble sugars, iron toxicity.

1. INTRODUCTION

In sub-Saharan Africa, rice represent the second most consumed cereal after maize, while its production does not cover consumption needs [1]. Particularly in West Africa, 60% of the population's needs are covered by local production estimated at 23 million tons in 2018 [2,3]. This production shortfall is under the triple influence of population growth, increased per capita consumption of rice and strong urbanization at the expense of arable land. In Côte d'Ivoire, rice is also the most consumed cereal [4], although production covers 50% of average needs [5]. In 2021, the country had to import 1,044,000 tons of paddy rice, whereas its production was 1,659,000 tons [5]. The shortfall production is linked to several causes, including low grain yields, reduced arable land, and soil infertility. The average production capacity without fertilizer inputs is low and does not exceed 1 tha^{-1}, with rainfed rice production accounting for 80% of rice farming systems [6]. Based on a yield ceiling of 6-8 tha^{-1}, 1,000,000 ha would need to be planted to make up for the deficit recorded since 2009.

However, heavy urbanization negatively impacts availability of the potential 200,000 ha of arable land [7]. Under these conditions, it is imperative to generate new production strategies to achieve rice self-sufficiency for Côte d'Ivoire. Thus, the cultivation of lowland rice ratoon appears to be an opportunity to intensify rice production in a context of scarcity of sown land due to excessive urbanization [8,4]. However, in lowland areas with acidic alluvial soils, iron toxicity remains a major constraint to rice production ([9,10]. Trials on iron toxicity expression of rice ratoon crops has not yet been initiated in Côte d'Ivoire. Mineral fertilization trials of lowland rice ratoon were therefore conducted to reduce iron toxicity, increase plant height and tillering, and improve chlorophyll content of the three primaries functional leaves and grains soluble sugar content.

2. MATERIEL AND METHODS

2.1. Study zone

Experiment was conducted in Dabou (05°21'484''N, 04°22'518''W and 80 m asl). The city, with a bimodal climate, is located in a savannah zone included in the humid forest of southern Côte d'Ivoire. The soils of Dabou are ferralitic, sandy to sandy-clay, highly denatured, on neogenous sand. In the lowlands, the upper layer is composed of a Neogene sedimentary cover by a sandy-clay texture with more than 40% clay in the savanna zone, and silty to very fine sand (10% clay) in the forest zone [11,12]. The climate is characterized by two rainy seasons. The most important, from April to July, is focused on June. Rainfall average is 511.7 mm. The second rainy season is located around the months of September and November, and is marked by 137.5 mm of rain. These two rainy seasons are interspersed with two dry seasons. The most important of these covers the period from December to March while the least extensive is in August. The temperature drops from 27.5 to 25.5 °C during the main rainy season. During the dry periods, the temperature rises from 26.7 to 27.8 °C from December to March, and is 25.5 °C in August.

2.2. Site description and chemical characteristics of 0 - 20 cm layer

Trials were conducted on a floodplain soil in a plot at an elevation of 29 m asl that lies between coordinates 5°19'54.3" N and 4°22'44.1" W. This field was used for an experimental operation from 2012 to 2014 and planted on soil from two years of fallow [8].

The 0 - 20 cm layer was acidic relative to the optimum values. The recorded pH_{water} of 5.19 was higher than pHKCl (4.07). Carbon contents of 5.14 $g.kg^{-1}$ were low. Similarly, organic matter content of 8.86 $g.kg^{-1}$ was low. This layer was particularly poor in K (0.18 $cmol.kg^{-1}$), Mg (0.5 $cmol.kg^{-1}$), Ca (1.14 $cmol.kg^{-1}$), and assimilable P (0.04 $g.kg^{-1}$), and very rich in the trace elements Zn (6.76 ppm) and Fe (174 ppm) compared to the threshold values with respect to chemical equilibrium, the Ca/Mg (11.40) and Mg/K (0.55) ratios were respectively greater than 10 and less than 2. Ca/K ratio values that were included in the standards range. K/CEC and K/Ca + Mg ratios were less than 2. Base saturation (13.29%) was below compared to the optimum values between 60 and 90%. Thus, in the study soil, potassium and magnesium are limiting factors for rice ratoon production and may lead to nutritional disorders [8].

2.3. Material

2.3.1. Rice variety

The plant material used was NERICA L14 rice stubble. Nerica are interspecific species resulting from hybridization between O. *sativa* and O. *glaberrima* [13]. NERICA L14 blooms between 63 and 73 days after germination. Its cycle length is between 95 and 110 days for potential grain yields fluctuating between 4 and 8 tha^{-1} [14]. These agronomic performances and the short duration of its production cycle favored its choice. In fact, in a bimodal climate zone, the cultivation of Nerica L 14 ratoons from a main crop planted at the beginning of the main rainy season can coincide with the short rainy season.

2.3.2. Fertilizers

Minerals tested were N, P, K, Ca, Mg and Zn. Calcium resulted from calcium carbonate ($CaCO_3$) dosed at 40% CaO. Potassium was spread as chloride salt (KCl, 50% K). The nitrogen source was urea [$CO(NH_2)_2$, 46% N]. Magnesium applied was in the form of magnesium sulfate powder ($MgSO_4.H_2O$, 17% Mg). Zinc sulfate ($ZnSO_4H_2O$, 36% Zn) was used as a zinc provider. Triple superphosphate [$Ca\ (H_2PO_4)_2$-H_2O, 38 - 46% P_2O_5] was used as a phosphorus source [8].

2.4. METHODS

2.4.1. Trials setting

2.4.1.1. Plot layout and weed control

A 1050 m^2 plot was marked out and then treated with 0.5 L glyphosate acid equivalent (360 gL-1). Three weeks later, the plot was cleaned manually. The plant debris was then removed and burnt off-site. The plot was subdivided into four blocks. The layout of the plot into blocks composed of microplots was done according to the work of [8].

2.4.1.2. Stubble production

The plots underwent a two-week planting period, followed by drainage 24 h before application of 200 kg.ha^{-1} NPK as a basal fertilizer, followed by transplanting at 20 cm row spacing of two 21-day-old plants from a nursery. The field was kept flooded from 10 days after transplanting (DAT) except during urea application (35 kg.ha^{-1}) at 21 days (tillering stage) and 45 days (panicle initiation stage). Manual weeding was also carried out twice before urea application, thus completing the management of the first crop. At maturity, mowing the rice with a sickle at 15 cm above ground level resulted in stubble as recommended by [8].

2.4.1.3. Ratoon fertilizer treatments

One day after first crop (main crop) harvest, N-, P-, K-, Mg-, and Zn- fertilizers were applied at the rate of 100, 55, 150, 33, 15, and 10 kg.ha^{-1}, respectively, as recommended [15,16,8]**.** No fertilizer application was made in the control

2.4.1.4. Experimental design

The experimental design (Fig. 1) was a randomized complete block design of seven treatments with four replications. Each rectangular block was 29 m long and 6 m wide and was further subdivided into seven 5 m x 3 m microplots [4,8].

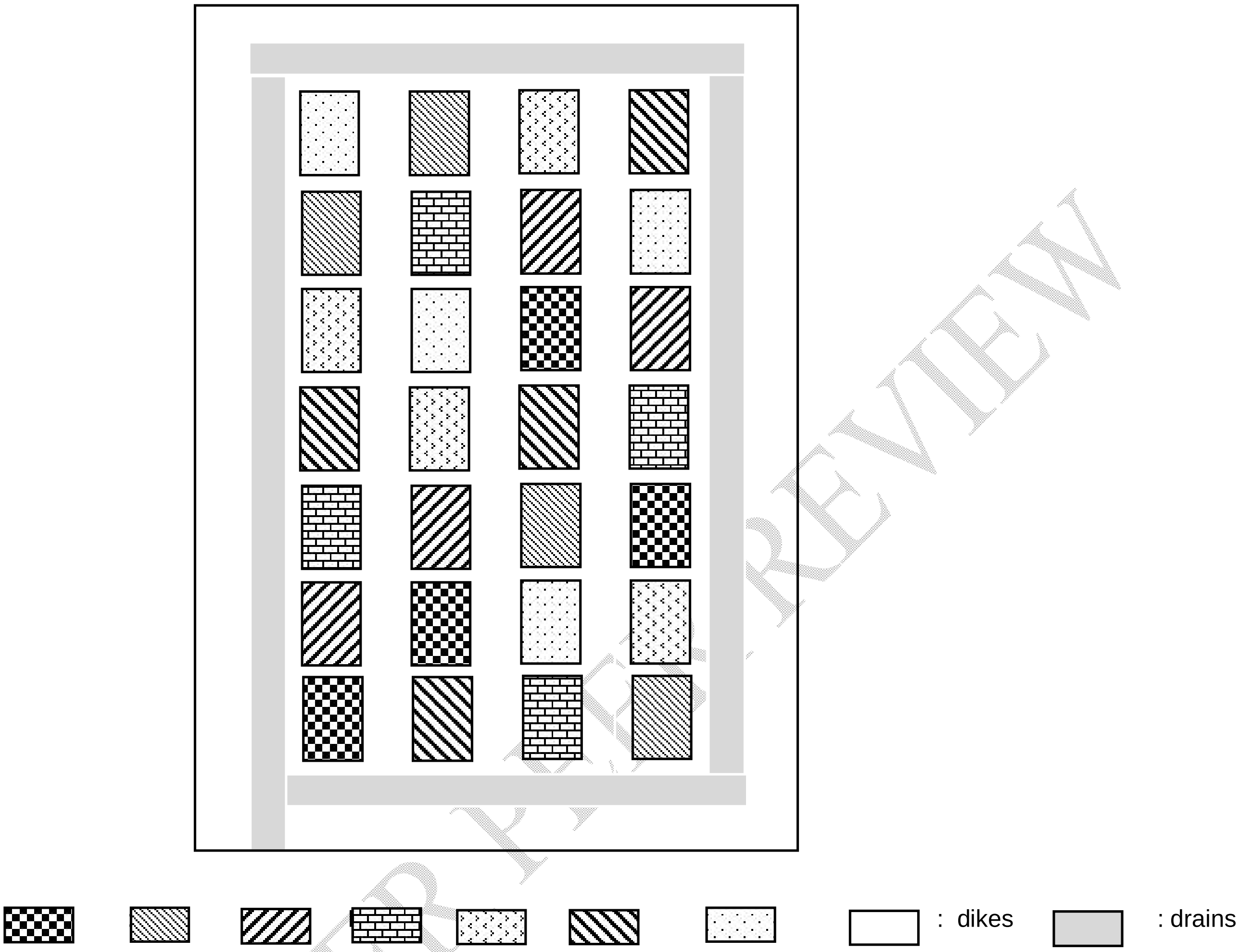


Fig. 1. Randomized complete block design
C: control.

2.4.2. Data collection

2.4.2.1. Determination of the soil morpho pedological characteristics

Submersion water surface

The water surface wave was observed in rainy season. The colorations of this one were noted.

Water table level and layer coloring

In dry season, a soil pit measuring 1 m x 0.6 m x 1.5 m [17] was dug and examined to determine the depth of the water table using a 1.5 m scale and to describe the color of the different layers using Munsell code [18]. To do this, a sample of each layer was taken and compared to the different colors of the Munsell code. The ratings corresponding to the observed colors have been listed below.

10YR/2/1: black,

10YR/2/2 : very dark brown,

10YR/3/2: very dark brownish gray,

10YR/3/4 : dark yellowish brown,

10YR/4/2 : dark greyish brown.

Structure, texture, porosity and organic matter of the layers

After draining the pit, the structure, texture [19], porosity and organic matter content of each layer were determined.

2.4.2.2. Description of Iron Toxicity leaf symptoms

In the ratoon, iron toxicity symptoms were investigated and described on the leaf blades of functional first three leaves on days 14, 21 and 28 after the main crop harvest. The signs observed were compared with a reference for leaf symptoms [20] of nutritional disorders in rice (**Fig. 2**).

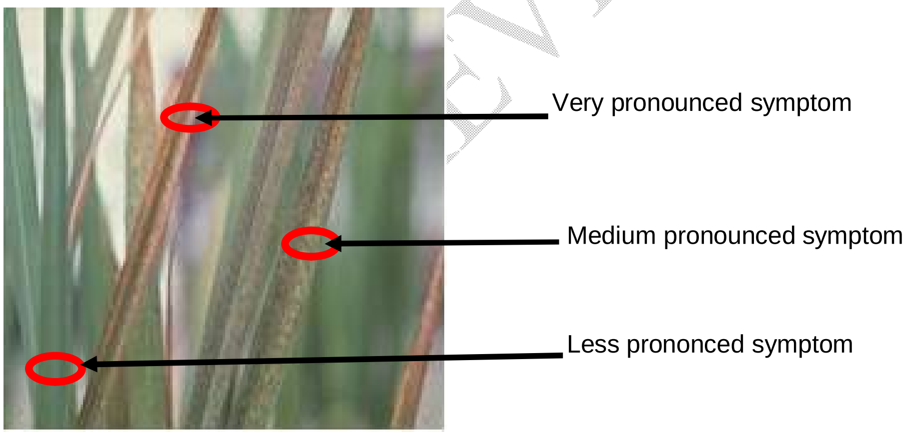


Fig. 2. Rice iron toxicity foliar symptom [20].

2.4.2.3. Evaluation of iron toxicity severity

The extent of the damage was assessed using a severity scale established by [20].

According to this scale, the following field coverage indices were applied:

- 0: no visible symptoms;
- 1: less marked visible symptoms covering less than 5% of the field;
- 3: marked symptoms covering less than 5% of the field;
- 5: marked symptoms covering 6 to 25% of the field;
- 7: marked symptoms covering more than 25% of the field;

- 9: very marked symptoms covering more than 25% of the field.

2.4.2.4. Height and number of tillers per plant at maturity

Averages of ratoon height [21] and number of tillers per plant [22,23] at maturity were determined.

2.4.2.5. Leaf chlorophyll contents

Chlorophyll extraction was performed from leaf blade of three first functional leaves taken fresh at the grain filling stage. A composite aliquot fraction of 0.25 g was used for extraction by 96° ethanol [24]. The determinations of chlorophylls a, b, and total were performed following the methods of [25,26].

2.4.2.6. Soluble sugar content of rice grains

An aliquot fraction of 0.1 g of rice grain powder, previously sun-dried and oven-dried at 70 °C for 24 h, was used to prepare an alcoholic extract of water-soluble sugars [27]. The determination of soluble sugars was performed according to [28,29].

2.4.3. Statistical analysis

The mean values of the study data were generated. The data on height, number of tillers per plant, chlorophyll and soluble sugar content were separated into homogeneous groups by analysis of variance using the Student-Newman-Keuls test at the α threshold of 0.05 by SAS (Statistical Analysis System) for Windows version 9.1.

3. RESULTS AND DISCUSSION

3.1. Pedomorphological characteristics of the site

The pedomorphological characteristics of the study soil are shown in Figures 3, 4 and 5.

The soil of the study plot is a brown hydromorphic soil (gleyic cambisol), not very developed, with ferrous concretions. It is characterised by the presence of a layer of submerged water over which an amorphous layer of pale color with a hydrocarbon sheen is observed (**Fig. 3**). According to **Fig. 4**, the water table is at a depth of 75 cm. The A horizon was deep. The A0 layer (0 - 5 cm) has a very dark brownish grey color (10YR3/2). Those of A11 at 5 - 10 cm and A12 (10 - 25 cm) are very dark grey-brown (10YR3/2) and very dark brown (10YR2/2) respectively. At A21 (25 - 50 cm) the observed color is very dark brown (10YR2/2). From 50 cm to 75 cm, the A22 layer was black (10YR2/1). From the surface to a depth of 150 cm, the soil is poorly porous, generally rich in organic matter, with a predominant color of 10YR, with some iron concretions at depths of 10 - 25 cm. The size of the roots increases from millimeters at the surface to decimeters at depth. Their orientation is generally vertical throughout the soil profile. The texture was silty-clay with a polyhedral to lumpy structure between 0 and 10 cm, sandy-clay at depths of 10 - 25 cm, silty-clay from 25 to 50 cm, sandy-clay after 50 cm. Above 10 cm the structure was polyhedral sub-angular (**Fig. 5**).

SW: Submerged water

PL : pale layer with hydrocarbon reflection

Fig. 3. Lowland soil floodwater surface characteristics

Layer A subdivided into A0, A1 and A2

0 - 5 cm: A0, shade: very dark grey-brown (10YR3/2)

5 - 10 cm: A11, shade: very dark grey-brown (10YR3/2)

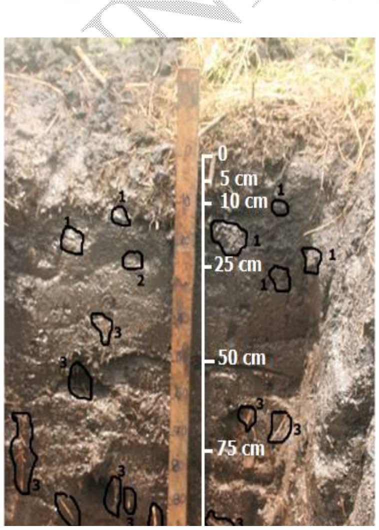
10 - 25 cm: A12, shade: very dark brown (10YR2/2)

25 - 50 cm: A21, shade: very dark brown (10YR2/2)

50 - 75 cm: A22, shade: black (10YR2/1)

Fig. 4. Shades of 0 - 75 cm layers and water table of the lowland soil

np: water table



A0, 0 - 5 cm: rich in organic matter, clay-loam texture, polyhedral to lumpy structure, many small millimeter roots with vertical orientation, not very porous

A11, 5 - 10 cm: rich in organic matter, clay-loam texture, polyhedral to lumpy structure, many small roots and a few decimetric roots with vertical orientation, not very porous

A12, 10 - 25 cm: rich in organic matter, sandy-clay texture, sub-angular polyhedral structure, a few vertically oriented decimetric roots, a few ferruginous concretions, little porous.

A21, 25 - 50 cm: rich in organic matter, clayey-silty texture, sub-angular polyhedral structure, vertically oriented decimetric roots, little porous.
A22, 50 - 75 cm: rich in organic matter, clayey-sandy texture, sub-angular polyhedral structure, many vertically oriented decimetric roots and large horizontally oriented roots, little porous.
A23, 75 - 105 cm: rich in organic matter, clayey-silty-sandy texture, sub-angular polyhedral structure, very numerous vertically oriented decimetric roots and very large horizontally oriented roots, not very porous.

Fig. 5. Textures, structures, organic matter richness, presence of roots according to the depth of the lowland soil.
1: ferruginous concretion, 2: horizontally oriented root, 3: vertically oriented root

The thin, pale-colored layer observed on the surface of the floodwater is the result of the excessive presence of soluble iron in the soil solution. As the 0-20 cm layer of the study site is characterized by standard iron contents [4], these excessive quantities could be due to the leaching of ferric iron from the upper and lower slopes and their accumulation in the lowland, and also to the ferruginous concretions found in the A12 layer, which are indurated elements rich in iron hydroxide [30]. The release of iron hydroxides and their reduction due to the waterlogging conditions of the environment leads to an accumulation of iron ions in the soil solution [31, 10], which is manifested by the amorphous layer of pale color with a hydrocarbon reflection. A layer is thick and has an overall color of 10YR which has changed very little from 10YR/3/2 between 0 and 10 cm to 10YR/2/1 beyond 50 cm depth. The overall 10YR hue is a characteristic of lowland soils. This small variation in the grey-black hue characterizes a poorly developed soil with a high content of poorly mineralized organic matter and medium to low levels of iron in the form of oxide and hydroxide [32,17]. These variations in the hue of the shallow layer confirm previous work by [4] who showed that in this layer the C/N ratio was low compared to the optimum values. The textural characteristics revealed that the texture changed very little with depth from sandy-clay at the surface to sandy-clay from 20 cm depth. Thus, the fine clay-silt-sand texture was dominant in this soil. This evolution of texture suggests a transgression of coarser layers over finer ones linked to colluvium. Similarly, the structure changed little with depth. It was polyhedral to lumpy in the first 20 cm and sub-angular polyhedral beyond 20 cm.

3.2. Leaf symptoms of iron toxicity in untreated rice ratoon

Foliar symptoms of iron toxicity, observed in the control (untreated), were characterised by appearance of brown to purple spots on the tips and leaf margins of young leaves. These spots became increasingly visible as the leaves aged. In the adult stage of the leaves, these increasing spots turned rusty on an increasingly yellow colored leaf blade (**Fig. 6**).

The typical leaf symptoms of iron toxicity observed on untreated ratoon in the study lowland site could be the consequence of exchangeable cation poverty [4], excessive levels of iron in the soil solution and its excessive tissue accumulation by rice ratoon [10, 33]. Indeed, in main crop, iron toxicity in rice is generally associated with excessive amounts of reduced iron (Fe^{2+}) in the soil solution, acidic pH, low CEC, exchangeable Potassium and Zn values [34, 35].

Figure 6. Evolution of leaf symptoms of iron toxicity in rice ratoon.

tbv: brown to purple spot, tb: brown spot, trlch: rust spot in a chlorotic leaf blade, a: F0 first leaf or panicle leaf, b: F1 second leaf, c: F2 third leaf.

3.3. Fertilizer treatments effects on iron toxicity coverage index

The highest average coverage index for iron toxicity was that of the control (C), while the lowest was observed following nitrogen fertilization (N). Between these two extremes, K and Mg caused average coverage indices (Figure 7). The values were respectively 5 (marked symptoms covering 6 to 25% of the field), 0 (not visible symptoms) and 3 (marked symptoms covering less than 5% of the field).

Only the ratoons fertilised with N were spared from iron toxicity, in contrast to the control which was severely affected by iron toxicity. In addition, the other ratoons treated with P, Ca and Zn showed little disease, reducing the severity of iron toxicity. The observation on nitrogen fertilisation is probably a consequence of the effect of reduced Fe uptake [36] relative to nitrogen [37], stimulation of metabolism, chlorophyll synthesis by increased magnesium uptake [4] and nitrogen assimilation. High iron concentrations cause antagonism with P, K, Ca, Mg and Zn. This antagonism, which is more important for K and Mg, would have limited their uptake in contrast to P, Ca and Zn. Thus, their higher uptake relative to Fe^{2+} would have reduced the severity of iron toxicity in the ratoon [38]. Thus, iron toxicity in the rice ratoon may be mainly related to antagonism between Fe^{2+} and K^{+}, and between Fe^{2+} and $Mg2^{+}$.

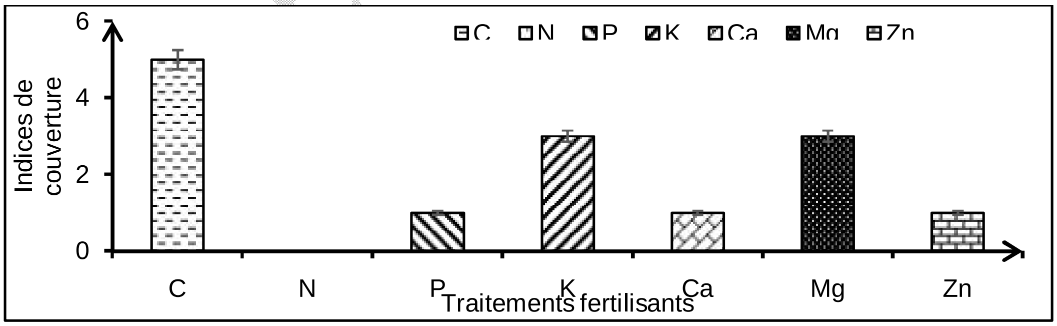


Fig. 7. Iron toxicity coverage indices in rice ratoon.

In the histogram, values of bands with different letters are statistically different at the 5% level. C: control.

3.4. Fertilizer treatments effects on mature rice ratoon average height and number of tillers

The average heights and numbers of tillers of mature rice ratoon plants according to the fertilizer treatments applied to the ratoon were recorded in Table 1. Differences between values were significant (p = 0.04) at the 5% level. Nitrogen induced higher mean height (60.11 cm) and mean number of tillers per ratoon (8.56 tillers/plant). In contrast, the unfertilized control, and those treated with Zn for height (49.81 and 52.20 cm) or with Ca, Mg and Zn for tillers average number (5.26; 5.41 and 5.82 tillers/plant) had lower values.

Height and tillering of ratoon plants were significantly stimulated by N supply. Similar observations were made in rice [39]. This mineral element is considered to be the one most likely to limit plant growth [40], because its availability is often low while the demand is high, especially for photosynthesis [41]. It is an essential component of nucleic acids, amino acids, enzymatic and structural proteins, and chlorophylls, molecules essential for plant growth and development. Nitrogen nutrition can influence the distribution of photoassimilates between aerial and root organs of herbaceous plants [42]. The large proportions allocated to aerial organs could explain high height growth and tillering, as previously observed in wheat [51]. This effect of nitrogen on height demonstrates its efficacy in the lowland trial [43].

Table 1. Average height and number of tillers per plant at maturity of lowland rice ratoon according to fertilizer treatments

Traitements	Average height at maturity (cm)	Average number of tillers (tillers/plant)
C	49,81b	4,38b
N	60,11a	8,56a
P	55,87ab	6,36ab
K	57,44ab	6,07ab
Ca	60,69a	5,82b
Mg	56,03ab	5,41b
Zn	52,20b	5,29b
P > F	0,04	0,04
VC	9,53	25,13
Mean	56,02	5,99

In each column, the means assigned to different letters in the same column are significantly different at the 5% level (Student-Newman-Keuls test). C: control.

3.5. Fertilizer treatments effects on leaf chlorophyll levels in rice ratoon and on grains soluble sugar content

The mean chlorophyll contents of ratoon rice first three functional leaves, determined according to the fertilizer treatments, were recorded in Table 2. Differences between the mean values were significant (P = 0.00) at the 5% level. The highest levels of chl a, chl b and chl t were only as a result of nitrogen fertilisation, respectively 0.21 - 0.30 and 0.51 mg.g-1 FL. The lowest values were recorded following magnesium fertilizer application and in the untreated control.

The soluble sugar content of ratoon grains as a function of the fertilizer treatments was also evaluated. The values are shown in Figure 8. The differences between the means were significant. The highest soluble sugar content of the grains (3.23 mg glucose. g-1 DM) was due to Zn fertilisation. The lowest value was found in the untreated control (2.21 mg glucose. g^{-1} DM).

Table 2. Average chlorophyll content (mg.g^{-1} FL) of the first three functional leaves of lowland rice ratoon according to fertilizer treatments

Treatments	Chl a	Chl b	Chl t
C	0,11c	0,20c	0,31c
N	0,21a	0,30a	0,51a
P	0,13bc	0,26b	0,39b
K	0,13bc	0,25b	0,38b
Ca	0,17b	0,24bc	0,41b
Mg	0,11c	0,20c	0,31c
Zn	0,15bc	0,22bc	0,37bc
P > F	0,00	0,00	0,00
VC	23	15	15
Mean	0,14	0,23	0,38

In each column, the means assigned to different letters in the same column are significantly different at the 5% level (Student-Newman-Keuls test). C: control.

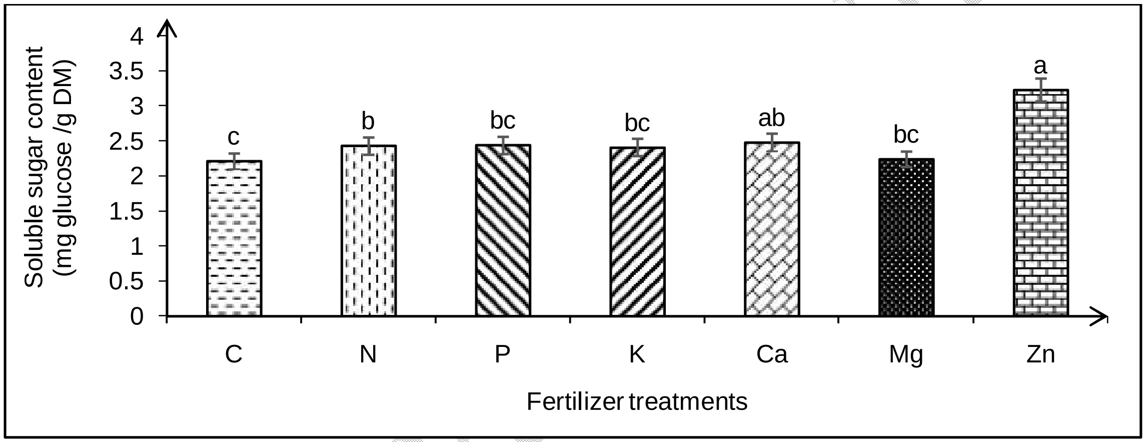


Fig. 8. Soluble sugar content (mg glucose per 1 g DM) of rice grains from lowland ratoon according to fertilizer treatments

In the histogram, values of bands with different letters are statistically different at the 5% level. C: control.

Chlorophyll levels (a, b and total) are increased after nitrogen application to the ratoon. This increase in chlorophyll is associated with a good uptake and assimilation of Mg [4], probably N [44] and Fe [45] to activate the synthesis of chlorophyll pigments in the rice ratoon. Indeed, these minerals are essential for the mechanisms of chlorophyll biosynthesis. Similar data have also been reported in durum wheat by [46], in peach by [47] and in black tea by [48]**.**

The soluble sugar content of the grains of zinc-treated ratoons was the highest, while that of leaf chlorophylls was low. According to [4], Zn application has an effect on Ca and K uptake by rice ratoons grown in a lowland area with low exchangeable cations. This application would also have suppressed iron uptake [49]. The high concentrations of soluble sugars in rice grains due to zinc fertilisation would be due to its involvement in photosynthesis rather than chlorophyll production. The assimilates strongly produced under the effect of its action would be progressively remobilised in the grains, as suggested by [50,51].

4. CONCLUSION

In lowland rainfed rice grown on an acidic sandy-clay soil with low exchangeable cation content and high Fe content, 25% of rice ratoon is damaged by iron toxicity due to antagonism between Fe^{2+}, K^+ and Mg^{2+}. The foliar symptoms of this nutrient deficiency are corrected by nitrogen fertilisation and greatly reduced by the application of P, Ca and Zn. The effect of nitrogen is accompanied by maximum tillering and height and an increase in leaf chlorophylls. In addition, Zn increases the soluble sugar content of grains.

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