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VARIATION IN MACRO-ELEMENTS AND PROTEIN CONTENTS OF ROSELLE (*HIBISCUS SABDARIFFA* L.) FROM NIGER

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ABSTRACT

Roselle is an important part of the human diet in many countries, particularly in the Sahel zone of West Africa. The leaves of Roselle are consumed as a green vegetable and in sauce. Nevertheless, little attention has been paid to their nutrient composition at different stages of plant growth. Therefore, an experiment was carried out under rainfall conditions from July to September 2006 at the experimental station of the Agrhymet Regional Centre in Niamey (Niger). The contents of Ca, K, Mg, P and protein in leaves of three ecotypes (A3, A7 and A9) were determined at stages I, II and III, corresponding, respectively to vegetative, flowering and mature stages. The experimental design was a randomized complete block with four replicates and one variable (ecotype). For ecotype A7, the protein content of the leaves decreased significantly ($p<0.05$) between stage I and II, than remained relatively constant up to stage III. In contrast for A3 and A9, protein content in leaves decreased significantly ($p<0.05$) between stage II and III. From stage I to III, the decrease of protein content in leaves was 41% for ecotype A3, 50% for A7 and 66% for A9. For all ecotypes, the P content of the leaves decreased progressively during plant growth. For A7 and A9, the reduction in P content from stage I to II and from II to III was about the same, namely 30%. However, for ecotype A3, the decrease was greater, respectively, 37% and 46%. The Mg content of the leaves was relatively constant for all three ecotypes at a given stage of plant development. However, the Mg content of the leaves decreased significantly ($p<0.05$) from flowering to maturity: 28% for A3, 23% for A9 and 14% for A7. For the three ecotypes, the Ca content of the leaves remained relatively constant from stage I to II, than increased markedly from stage II to III, to about 150% for A7, 85% for A3 and 50% for A9. From stage I to II, the leaves' K content decreased significantly for all three ecotypes, about 67% for A9, 62% for A3 and 44% for A7. The data indicated that Roselle can contribute useful amounts of essential nutrients such as Ca K, Mg, N and P mainly at vegetative stage in the diets of people who inhabit the western Sahel.

Keys words: Roselle, macronutrient, leaves, stage, Niger

INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) is an important part of human diet in many countries, particularly in the Sahel zone of West Africa. The calyces are utilized in producing drinks, jellies, sauces, chutneys, wines, preserves [1]. The calyces drink is a readily available and inexpensive source of vitamin C in addition to various medical values [2]. The seeds of Roselle are subjected to a solid-state fermentation process to produce a meat substitute condiment known in Niger as dawadawa-botso or Mari Mi, respectively in Hausa and Djerma language. The leaves are consumed as green vegetable (spinach) [3]. In Niger, they are also used as an ingredient in sauces and, therefore, serve as a nutrient complement in cereals such as sorghum or millet. Moreover, Roselle is regarded as one of the most popular folk medicinal plants.

The leaves of *Hibiscus sabdariffa* contain nutrients such as phosphorus, calcium, magnesium, and potassium [4]. The role these nutrients play in human nutrition has long been appreciated [5]. Calcium is major mineral constituent of the skeleton. Calcium is vital for a large number of functions, including prostaglandin and leucotriene synthesis and a signaling molecule in metabolism. Potassium is a factor in tissue elasticity, healing injuries, liver function, normal bowel activity and regular heart rhythm. It regulates nerve and muscle action and is needed for intercellular fluid homeostasis. A lack of potassium can result in liver ailments, pimpling of the skin, slow healing of sores, and muscle weakness. Phosphorus is necessary for the innumerable biochemical reactions in which phosphates serve as substrates or products such as ATP and as an integral part of phospholipids, phosphoproteins and phosphosugars. Magnesium, like P is widely distributed in soft tissues and in the skeleton, and is involved in a numerous metabolic processes [6].

However, the database of Roselle nutrient and chemical composition is incomplete and fragmentary [7]. The objective of this study was to investigate the macro-elements (Ca, Mg and P) and Protein contents in leaves of three Roselle ecotypes from Niger at different stages of plant growth.

MATERIALS AND METHODS

Experimental details

Field experiment was carried out from July to September 2006 at the experimental station of the Agrhymet Regional Centre in Niamey (latitude 13° 29’ N, longitude 2° 10’ E, altitude 222 m), Niger. Total rainfall during the experiment was 395 mm. During the growing season, the daily temperature varied from 20.3 to 27.4°C for the minimum and 28.8 to 37.6°C for the maximum and the air humidity, from 29.4 to 69.5% and 74.7 to 97%. The top 30 cm of the soil site had a pH in H_2O of 7.4.The soil was sandy with low moisture storage capacity and contained 0.20 % of C, 0.162% of total N and 0.0479% of P [8]. In order to improve the water storage capacity of the soil moisture, 10 tons/ha of organic matter was applied. In addition, 100 kg $N.ha^{-1}$ of composite fertilizer NPK (15-15-15) was incorporated into the soil using a plow.

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Experimental design

The experiment was laid out in a randomized complete block design with four replicates. The distance between two consecutive blocks was 2 m. Plot size was 10 m x 6 m with 1 m margin round each plot. All plots consisted of 6 rows of plants with 1 m apart between rows and 1 m between plants within rows. The four central rows were intended for plant sampling.

Three Roselle ecotypes (A3, A7 and A9) collected in the south sahelian zone of Niger were field tested [15]. Ten seeds per hole were hand sown on July 14, 2006. After 22 days, the crop was thinned to two plants. The plots were weeded manually with a hoe, at five times in order to assure good conditions for plant growth.

Plant sampling and analysis

Plants were sampled at three stages of growth: vegetative (stage I), flowering (stage II) and mature (stage III) corresponding respectively to 25, 75 and 115 days after sowing (DAS).

For a given stage, five plants were randomly selected within central rows of each plot. The plants were then separated into different organs (leaves, stems, calyces and seeds), which were sun-dried.

The leaves of three replicates were oven-dried (65ºC overnight) and finely milled using the multi-beads shocker (Yasui Kikai, model MB500E). Thereafter total N, total P, Ca, K and Mg were determined at the Analytical Services Laboratory of ICRISAT Niamey (Sadoré, Niger) according to procedures of analysis [10].

The total N content was measured by colorimetric analysis by the salicylate/nitroprusside method. By this method, the ammonium reacts with salicylate in the presence of hypochlorite (oxidant) and nitroprusside (catalyst) to form an emerald green colour measured calorimetrically on an auto-analyzer Technicon AAII (Californie, USA) at 660 nm. The reaction takes place in a buffered alkaline medium at a pH of 12.8 - 13.0.

The total N content in leaves was converted to protein content as follows:
Protein content = total N content * 6.25.

The quantitative determination of Ca, K and Mg in the extract was done by atomic absorption spectrometry using the Atomic Absorption Spectrometer, model Perkin-Elmer AAnalyst 400 (Shelton, USA). The samples were nebulized into an air-acetylene flame where they were vaporized. The compounds were atomized. Thereafter, the atoms thus formed were measured by atomic absorption at a wavelength of 422.7 nm for the Ca, 766.49 nm for the K, and 285.2 nm for the Mg.
Phosphorus was determined by the molybdate-blue method using ascorbic acid as the reductant. Colour development was measured at 882 nm on a visible spectrometer (Turner model SP-850, Dublin, Ireland).

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Data analysis
The analysis of variance (ANOVA) was carried out to determine the differences among the three growth stages. Significantly-different means values were compared using the Student Newman Keuls test at 0.05 probability level.

RESULTS

Protein content The protein content of the leaves of Roselle decreased for all ecotypes during plant growth (Fig. 1). From the vegetative stage to maturity, the decrease was 41% for ecotype A3, 50% for A7 and 66% for A9. However, within plant growth, this decrease occurred at different growth stages according to ecotypes. Therefore, for A3 and A9, no significant difference in protein content in leaves was observed between vegetative stage and flowering and among ecotypes. For these ecotypes, protein content in leaves decreased significantly ($p<0.05$) between the flowering stage and maturity. For ecotype A7, the protein content of the leaves decreased significantly ($p<0.05$) between the vegetative stage and flowering, and remained relatively constant up to maturity (Fig. 1).

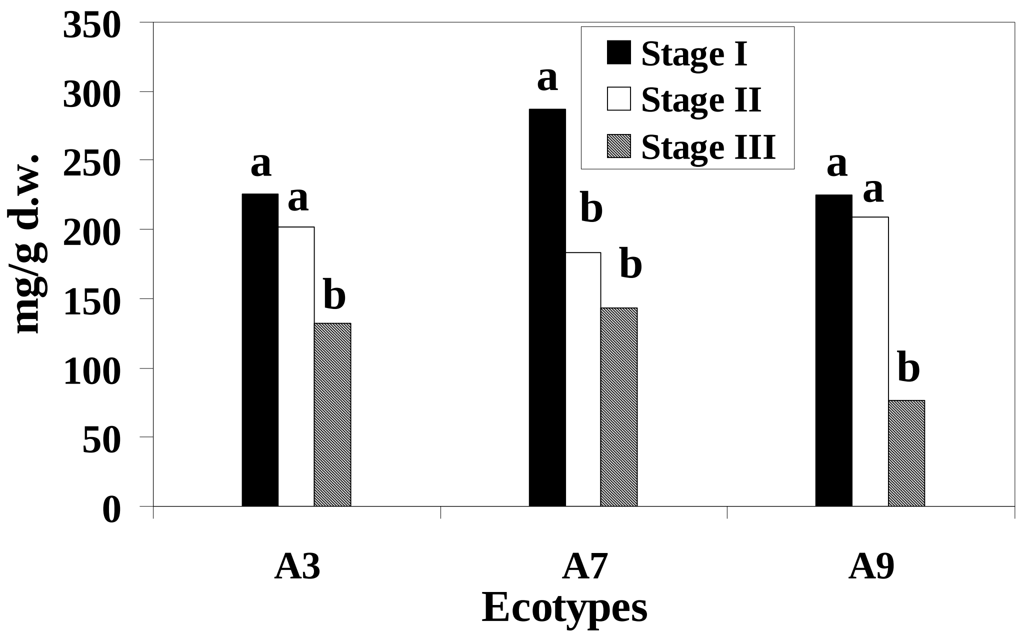

Figure 1: Protein content of leaves at different growth stages of Roselle
(Stages I, II, and III corresponded, respectively to vegetative stage (25 DAS), flowering stage (75 DAS) and maturity stage (115 DAS). Data of the same ecotype with the same letter(s) are not statistically significant at P=0.05 probability level).

At maturity, the lowest protein content in leaves was observed for A9 (76 mg/g d.w.). Ecotypes A3 and A9 had similar and higher protein content in leaves, respectively 133 mg/g d.w. and 144 mg/g d.w.

Phosphorus content For all ecotypes, the P content of the leaves decreased progressively and significantly ($p<0.05$) during plant growth (Fig. 2). For A7 and A9, the reduction in P content during each of the two periods of plant growth (from vegetative stage to flowering and from flowering to maturity) was about the same, namely 30%. However, for ecotype A3, the decrease was greater: 37% from vegetative stage to flowering, and 46% from flowering to maturity. At maturity, the P content of the leaves varied from 2010 µg /g d.w. for A3 to 3080 µg /g d.w. for A7.

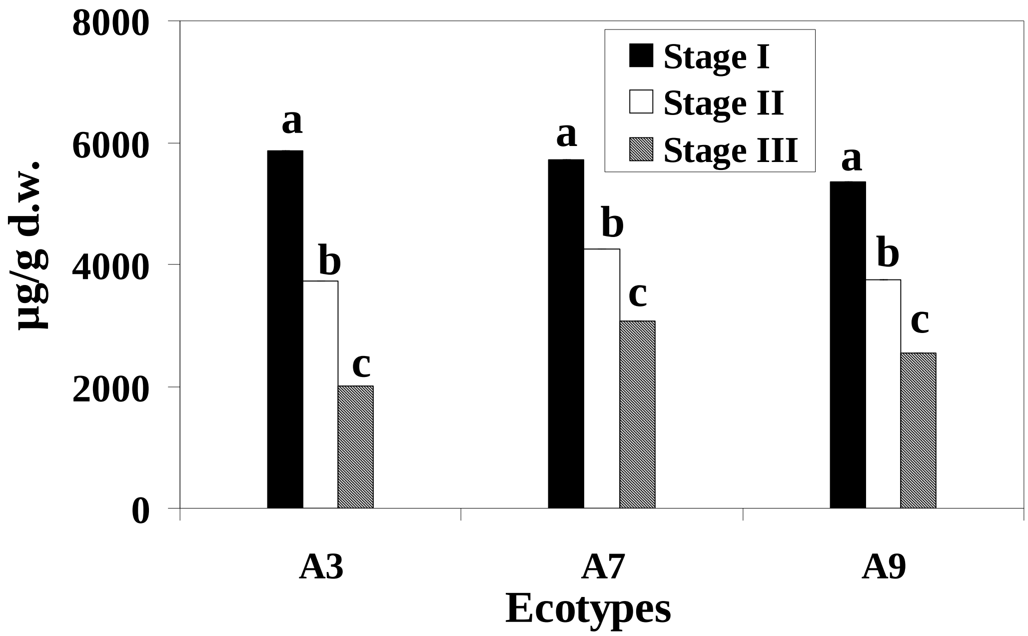

Figure 2: P content of leaves at different growth stages of Roselle

Magnesium content There was no significant difference of Mg content in leaves of Roselle between vegetative and flowering stages for the three ecotypes (Fig. 3). However, the Mg content of the leaves decreased significantly ($p<0.05$) from flowering to maturity: 28% for A3, 23% for A9 and 14% for A7 (Fig. 4). At maturity, the Mg content of the leaves was higher for A7 compared to that of ecotypes A3 and A9.

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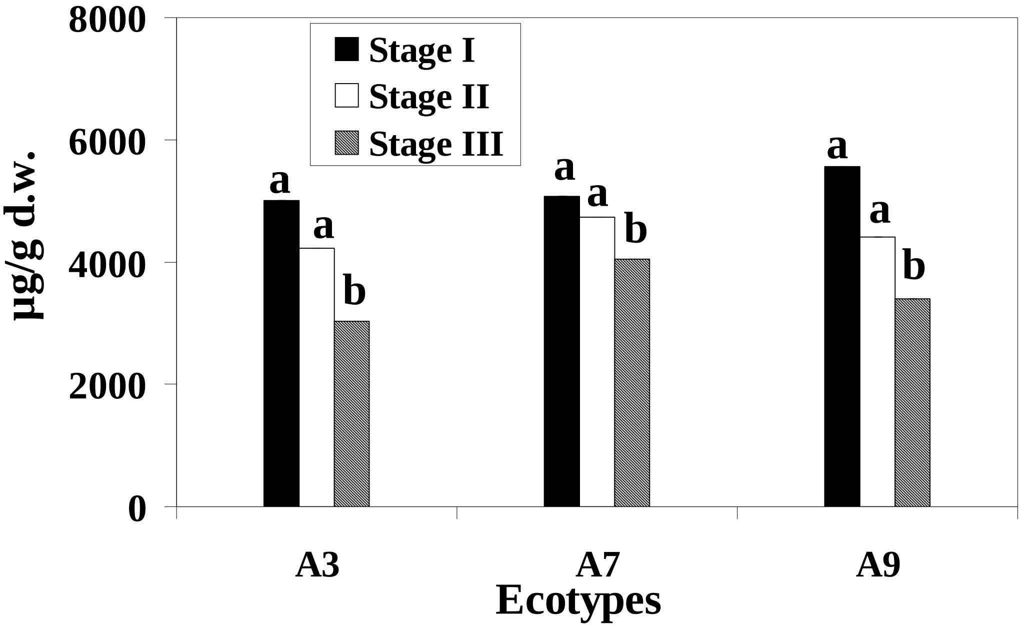

Figure 3: Mg content of leaves at different growth stages of Roselle

Calcium content For the three ecotypes, the Ca content of the leaves remained relatively constant from the vegetative to the flowering stage (Fig. 4). However, the differences between ecotypes were statistically significant: at the vegetative stage, the Ca content ranged from 13,600 µg/g of d.w. for A3 to 20,200 µg/g d.w. for A9. At flowering stage, the Ca content of the leaves varied from 12,700 µg/g for A7 to 21,400 µg/g d.w. for A9. From flowering to maturity stage, the Ca content of the leaves of the three ecotypes increased markedly, to about 150% for A7, 85% for A3 and 50% for A9 (Fig. 4). At maturity, the Ca content of the leaves was about the same for the three ecotypes, namely about 32,000 µg/g d.w.

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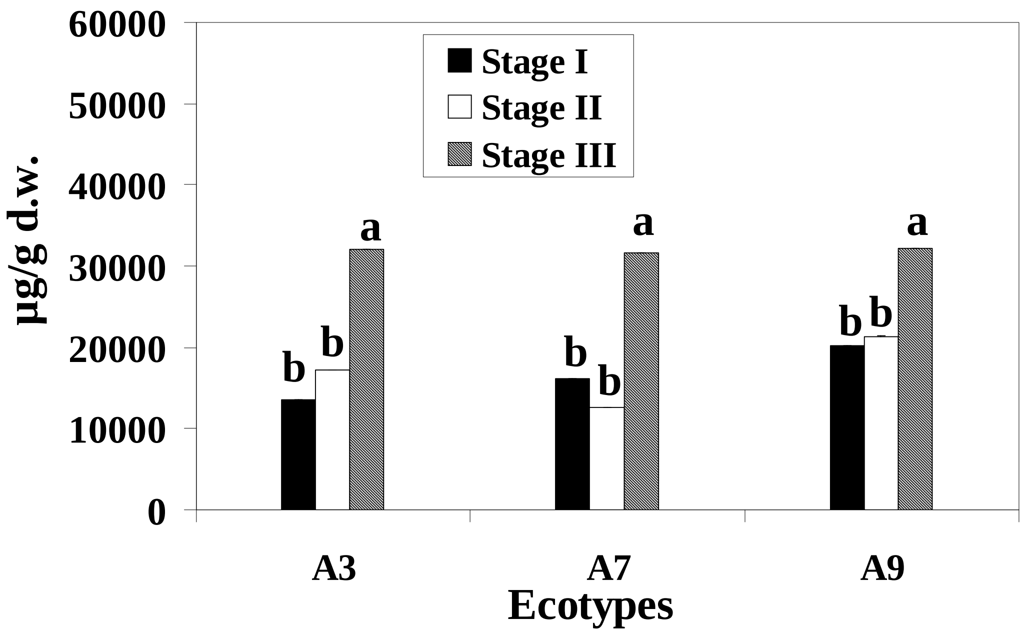

Figure 4: Ca content of leaves at different growth stages of Roselle

Potassium content At the vegetative stage, A3 and A9 had significantly ($p<0.05$) higher K content in leaves than A7 (Fig. 5). From the vegetative stage to flowering, the leaves' K content decreased significantly ($p<0.05$) for all three ecotypes. The decrease was greater for ecotypes A3 and A9 (62% and 67%, respectively) compared to A7 (44%). At maturity, the K content of the leaves was halved for all ecotypes, compared to those of vegetative stage.

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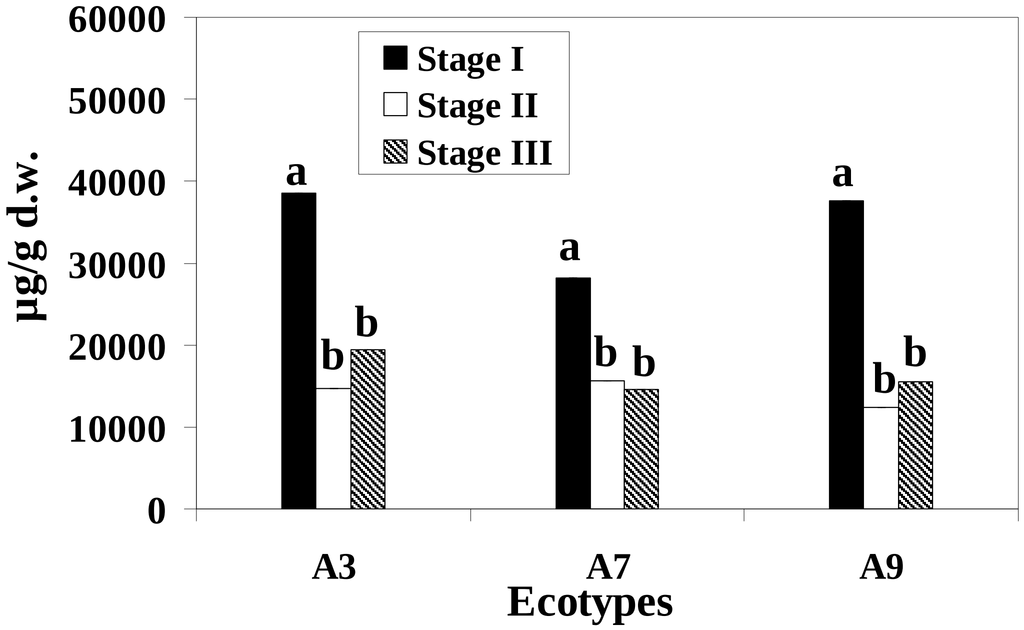

Figure 5: K content of leaves at different growth stages of Roselle

DISCUSSION

The main result of the present study was that P, Mg, K and protein contents in the leaves of Roselle were higher at vegetative stage; however, variations were recorded among ecotypes.

The protein content in leaves decreased significantly ($p<0.05$), mainly from the flowering stage to maturity. This indicated nitrogen remobilization from leaves to seeds filling. In oilseed rape, more that 48% of the total N which had been remobilized from vegetative tissues was ultimately recovered in the mature pods [11]. Also, in cereals, a large proportion of the N previously accumulated in the stems and leaves was mobilized to sustain grain filling when N uptake is limited by soil N availability and/or down-regulation of the N uptake mechanisms [12]. In addition, it has been shown in both herbaceous and woody species that N can be transiently stored as vegetative storage proteins, which can be hydrolysed to supply developing sinks such as seeds with N [13, 14].

No variation in the P content of the leaves was found between ecotypes at the vegetative stage. However, at the flowering and maturity stages, the highest P content was observed in A7. For all three ecotypes, the P content of the leaves decreased progressively from the vegetative stage to maturity. This finding confirms the results which indicated that P content, which was higher at the initial stage, decreased continuously until maturity in *H. sabdariffa*, *H. cannabinus*, *Amaranthus blitum*,

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Amaranthy gongecus and *Spineces olerecea* [15]. Moreover, the extant of the decrease in the leaves' P content reported by these researchers for *H. sabdariffa* and *Amaranthy gongecus,* were confirmed in the present study. Therefore, P content was higher at the vegetative stage in young leaves, where the growth rate is highest. This indicated that the consumption of young leaves of *H. sabdariffa* would provide a valuable source of phosphorus for humans. The P content of the leaves we fund at the vegetative stage was similar to that reported by other colleagues [4]. However, it is higher than those reporter for leaves either of *H. sabdariffa* (2970 µg/g d.w.) or of other leafy vegetables such as *Marua crassifolia* (1430 µg/g d.w.), *Moringa oleifera* (2500 µg/g d.w.) and *Leptadania hastata* (2300 µg/g d.w.) [16].

Magnesium, a constituent of chlorophyll, is also required by many enzymes that catalyze phosphate transfer reactions [6]. The leaves' magnesium content decreased for all three ecotypes during plant growth: from the vegetative stage to maturity, this decrease was about 20% for A7, 40% respectively, for A3 and A9. However, the decrease in the Mg content occurred mainly during the reproductive period. These results are in agreement with those of other colleagues who found a decrease in the Mg content of the leaves between 30 days and 45 days in four leafy vegetables including *H. sabdariffa* and *H. cannabinus* [15]. A decrease was also observed in the Mg and Ca contents of many leafy vegetables during this period of growth [17].

The calcium content of the leaves of Roselle increased progressively during plant growth, particularly from the flowering stage to maturity, thereby confirming other results which indicated a continuous increase in calcium content from three months to one year of age in *Chekurmenis,* a similar leafy- vegetable [18]. In *Amaranthy gongecus* and *Spineces olerecea,* the calcium content increased also in the leaves as the plant matured [15]. This may be due to the immobile nature of calcium and the inability to retranslocate from older parts of the plant to growing parts [15].

During plant growth, the K content of the leaves of Roselle decreased mainly during the vegetative period (from stage I to stage II). The K content of the leaves recorded in the present study is similar to that reported for Roselle by other colleagues [4, 16] and also for other leafy vegetables such as *Moringa oleifera*, *leptadania hastata* and *Brassica oleracea* [16, 19].

CONCLUSION

The results indicated that the Ca, N and P contents in leaves of Roselle were higher at the vegetative stage; however, differences were recorded among ecotypes. The Mn content in the leaves decreased from vegetative to mature stage, while the Ca content increased markedly from flowering to mature stage.

The data indicated that Roselle can contribute useful amounts of essential nutrients such as K, Mg, P and protein. Therefore, the recommended optimal harvest time of Roselle to maximise these nutrients is the vegetative stage corresponding to 25 days after sowing.

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