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Influence of genotype and harvesting age on the mineral dynamics of *Brassica oleracea* var. *acephala* cultivated in South Africa

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Abstract

The concentration of desirable minerals in vegetables could influence consumers' preference for certain vegetable species especially in the recent times in South Africa where packaged vegetables are increasingly labelled with the details of their mineral constituents. The agronomic practices and the genotypes used by farmers could therefore influence the quality of their products. Six genotypes of *Brassica oleracea* var. *acephala* were cultivated in the Research and Experimental Farm of the University of Zululand, South Africa, to determine the influence of harvest age on the mineral dynamics of the vegetable types. The results of this study indicated that harvesting of *B. oleracea* fifteen weeks after transplanting could help to capture the highest levels of nutrient concentrations. Analysis of the comparative performance of the six genotypes tested in the study also suggests that genotype 2 showed superior performance in terms of nutrient accumulation. However, an improvement programme via hybridization of genotypes 2 and 3 could help increase the level of zinc to balance iron-to- zinc ratio in both genotypes. Intraspecific hybridization programme that aims to breed for zinc improvement on genotype 2 could also be of advantage in a situation where food based strategy is the main target to addressing iron and zinc deficiencies especially among financially challenged population groups. The current study is the first literature on the mineral dynamics among genotypes of *B. oleracea* var. *acephala* in northern KwaZulu-Natal, South Africa.

Key words: Harvesting age, mineral dynamics, *Brassica oleracea* var. *acephala*, genotypes, South Africa.

Introduction

The need to increase food production to meet the ever growing world population has promoted over-reliance of urban and rural populations on limited number of staple foods mainly produced by commercial farmers [1]. This has narrowed down the diet base of many people both in developed and developing countries. For instance in South Africa, studies have shown that when both exotic and wild vegetable species are taken into account, the amounts of vegetables consumed have been far below the recommended daily intakes [2]. There is also enough evidence that this scenario of reduced balanced diet is a major source of chronic diseases in modern world's society; especially among less vegetable based diet populations [3,4]. Vegetables are cheap sources of macro- and micronutrients which could be efficiently produced with limited resources [5]; and the inclusion of vegetables in human diets has been identified as a major means of promoting balanced diets across populations of various income brackets [6]. However, malnutrition and disease are still major problems among marginal income Africans and the disadvantaged population of South Africa is still faced with this challenge [3,7-9].

Many previously disadvantaged South Africans are unemployed probably due to lack of formal education that normally limits their access to the labour market and many individuals go to bed hungry; with many households reported to be food insecure [10]. Most of these financially challenged individuals live in rural communities with high potential for vegetable crop production. *Brassica oleracea* var. *acephala* has been well documented to be rich in several essential minerals, protein and vitamins [11,12] that could alleviate hidden hunger in marginal income populations and several communities in Kenya (east Africa) and Zimbabwe (southern Africa) currently use this vegetable as standard staple in their diets. The KwaZulu-Natal (KZN) Province of South Africa is described as having a Mediterranean climate with mild cold winter season and very hot summer; typical of the European climate where this vegetable is mainly cultivated. Currently there is no information in literature on the leaf maturity age when *B. oleracea* could trap the optimum level of essential nutrients (minerals, protein and vitamins) in the Mediterranean northern KwaZulu-Natal climate of South Africa.

Genetic differences in mineral nutrient uptake have been reported for most world economically important crop species [13]. These include the *Brassica* genus whose genotypes have been reported to be particularly diverse in mineral contents [11-16]. However, the remarkable differences in the concentration of different minerals reported by previous studies on *B. oleracea* make further investigations on the species highly important [11,12]. This is particularly relevant to better understand the mineral dynamics of this species in the farming systems of northern KwaZulu-Natal. The objective of this study was to document the mineral dynamics in the leaf of *B. oleracea* during the winter season of northern KwaZulu-Natal, South Africa, as affected by leaf age of six genotypes of the species.

Materials and Methods

Field study and harvesting procedure: Seedlings of *Brassica oleracea* were raised in the nursery for two weeks prior to transfer to the field. Soil preparation was conducted using mechanized equipment to pulverise the soil thereby ensuring good establishment. The genotypes were transplanted in the Research and Experimental Farm (28°51'S and 31°50'E) of the Department of Agriculture, University of Zululand in KwaZulu-Natal Province, South Africa, during the winter season of year 2009. The experiment was laid out in a randomised complete block design (RCBD) and replicated three times. Each replicate had ten plants. Harvest samples were drawn randomly from five plants. All leaf samples harvested for chemo-analysis where previously tagged with labels two weeks after transplanting for ease of identification of appropriate age of leaf used for subsequent analysis. Samples were collected in paper bags and immediately transferred to the laboratory for drying. The six genotypes were subjected to the same cultural practices for the purpose of uniformity.

Samples preparation: Six genotypes (Bo1– Bo6) of *Brassica oleracea* were used for this study. Leaf harvesting was conducted periodically starting from twelve weeks after transplant and subsequent harvests were conducted at one week intervals. The moisture contents of the samples were determined according to the standard method of the Association of Official Analytical Chemists [17] where moisture loss after oven-drying divided by the initial weight of the sample, multiplied by 100 gives the percentage moisture content in the sample. The dried samples were separately milled, passing through a 0.5 mm sieve, using a Fritsch pulverisette 141 Rotor-Speed mill (Fritsch GMBH, Laborgeraetebau, Germany). The milled samples were stored in labelled air-tight containers prior to the determination of their mineral compositions. All reagents used for these analyses were of analytical grade.

Chemical analyses: A mixture of concentrated H_2SO_4, a catalyst (selenium), salicylic acid and hydrogen peroxide were used as reagents for sample digestion. Selenium–sulphuric acid mixture was prepared by dissolving 3.5 g selenium powder in 1000 ml of concentrated sulphuric acid. The mixture was then heated at 300°C until the original blackish colour of selenium suspension turns via green/blue to light yellow. A digestion mixture was also prepared by dissolving 7.2 g salicylic acid in 100 ml of selenium–sulphuric acid mixture. Thereafter, 0.3 g sample was added to 2.5 ml of the digestion mixture, heating at 110°C for 1 h. Hydrogen peroxide was later added; heating now at 330°C until the colour turned colourless or light yellow. The digest was cooled and made up to 50 ml. Subsequently, phosphorus, calcium, magnesium, sodium, potassium, zinc, copper, iron and manganese in the digests were determined [18]. The mineral element composition was determined using the Unicam Solaar Atomic Absorption Spectrophotometer (AAS) — (Model 969 Mk II; Unicam Ltd., Cambridge, UK). Potassium, sodium, magnesium and calcium contents were determined by reading their absorbance at 766.5, 589.0, 285.2 and 422.7 nm wavelengths, respectively, while the copper, manganese, zinc and iron contents were measured at 324.8, 279.5, 213.9 and 248.3 nm wavelengths, respectively. Total phosphorus was obtained using the ascorbic acid blue colour procedure [18] by reading the absorbance at a wavelength of 880 nm on a Helios Gamma spectrophotometer (Thermo Spectronic; Helios Gamma, UK).

Statistical analysis: All determinations were replicated three times. The data obtained were subjected to analysis of variance (ANOVA) [19]. Analysis was conducted in a factorial arrangement where harvesting and genotypes were the main factors at 4 and 6 levels, respectively; and differences within and between factor means were tested using Duncan's multiple range test (DMRT).

Results and Discussion

Leaf maturity showed significant ($p<0.05$) variation in the mineral compositions of the six genotypes of *B. oleracea* (Table 1). The results of the current studies indicated that the choice of harvesting date for all the genotypes tested in this study depends mainly on the specific mineral elements considered to be more important in the diet of a target population. For instance, harvest age one (12 weeks after transplant) showed significant advantage over subsequent harvests for Ca, K, P, Na and Cu while harvest 3 (14 weeks after transplant) was the best for Zn, Mn and Fe. Although harvest 3 was more superior to the other harvests for Zn, the result of the current study did not show any significant ($p>0.05$) difference among the harvest dates for this element. Unlike previous studies [20, 21] on other vegetables (*Amaranthus blitum, Amaranthus gangeticus, Spinacia oleracea, Vigna unguiculata* and *Momordica balsamina*) where Zn was found to increase with increasing age, the Zn content of all the genotypes of *B. oleracea* in the present study were initially low and peaked at harvest stage 3 with a slight drop in the concentration during the last harvest. Calcium and magnesium showed similar marked shift in concentration between stage 1 and 3. At stage 1, both elements recorded significantly ($p<0.05$) higher concentration than harvest stage 2 and then peaked again at stage 3 with Mg reaching its previous (harvest 1) peak again at stage 4 (Table 1). The superior concentration of calcium at stage 1 of the harvest can only be explained by the initial influx of minerals to actively growing juvenile plant parts followed by a redistribution of minerals to newly formed leaves and floral parts preparation for flowering.

The current trend in the dynamics of calcium in genotypes of *B. oleracea* was reported in early works [21-23]. Mineral dynamics in *Brassica* species are quite complex with almost every study showing interesting variability in the accumulation of nutrients in the leaf with increasing age.

For instance, magnesium was earlier reported to accumulate with age [23]. The Mg content of the genotypes in the present study showed rather interesting pattern of significant drop in concentration between stage 1 and 2 but later picked at stage 3 reaching its peak at harvest stage 4. This result is in contrast to an earlier report [24] where a significant drop in Mg level was recorded with increasing leaf age. For P, harvest stage 1 undisputedly performed significantly ($p<0.05$) better than the other stages. There was no significant ($p>0.05$) difference in the concentration of N across all growth stages. This result is in contrast with a previous finding where the concentration of nitrogen in the leaves of *Brassica oleracea* var. *acephala* increased with leaf age [25]. Copper recorded a significant drop in concentration at stage 2 and picked again during harvest stage 3. Harvest stage 1 and 3 did not show any significant difference ($p>0.05$) in the concentration of copper (Table 1), an indication that harvesting the genotypes at stage 1 will not make any significant difference in the contribution of the element to human dietary need compared with stage 3. Harvest stage 3 distinctively showed significant ($p<0.05$) superiority to

Table 1. Effect of harvesting age on mineral dynamics of six genotypes of *B. oleracea.*

Harvest age (Weeks)	Minerals (Mean±SD)									
	Ca (%)	Mg (%)	K (%)	P (%)	N (%)	Na (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
1 (12)	1.87±06[a]	0.48±01[a]	5.17±02[a]	0.50±03[a]	3.68±06	63.64±02[a]	28.28±09	2.73±08[a]	17.94±42[b]	158.94±62[b]
2 (13)	1.58±03[b]	0.41±04[b]	4.79±06[ab]	0.45±02[b]	3.64±04	53.01±04[b]	29.72±31	2.22±15[bc]	20.22±18[ab]	125.39±56[b]
3 (14)	1.85±08[a]	0.46±02[ab]	5.17±02[a]	0.45±02[b]	3.08±01	56.53±03[ab]	31.44±42	2.52±23[ab]	23.61±32[a]	374.50±48[a]
4 (15)	1.79±02[ab]	0.48±01[a]	4.70±04[b]	0.40±07[c]	3.19±05	53.06±07[b]	29.11±57	2.16±09[c]	23.50±26[a]	172.61±36[b]

Means with the same letter along the same column are not significantly different at p<0.05; values with no letters along same column are not significant at p<0.05.

other harvest dates for Fe and Mn (Table 1). Although the concentration of Mn only dropped slightly at stage 4, there was no significant change in the level of accumulation of this mineral compared with Fe at stage 3; which showed a sharp increase in concentration between stage 2 and 3 and dropped almost 50% of its concentration in a period of one week during stage 4 (Table 1). The results of these two minerals (Fe and Mn) were in conformity with previous studies where the concentrations of Fe and/or Mn were found to increase steadily up to certain stage and then began to drop at later stages of maturity [14, 21, 26]. Early studies suggest that the tapering off in the concentrations of Fe and Mn during the latter stage of maturity may be attributed to the redistribution of these minerals for flower development [20, 21]; a condition that was observed for *B. oleracea* during our study. Considering the overall mineral dynamics of the genotypes tested as affected by increasing age, it is strongly suggestive that it will make nutritional and economic sense for famers to harvest this species at harvest stage 3. At this stage (stage 3), the leaves of the genotypes would have almost doubled in size and weight thereby increasing income to farmers and also provide an optimum nutritional benefit to consumers. The current study indicates that, of the ten minerals listed in the analysis, only phosphorus showed a significantly ($p<0.05$) higher level of concentration at stage 1 compared with stage 3. Nitrogen also had the highest concentration at stage 1 but there was no significant difference in concentration of the elements across all harvest stages considered in the study.

The results of the mineral dynamics across the six genotypes of *B. oleracea* are presented in Table 2. Overall, the six genotypes showed selective diversity in mineral concentrations. For instance, while genotypes 2 and 3 outperformed the other genotypes for most minerals studied, genotype 4 recorded slightly higher concentrations than the other genotypes for Ca, Mg and Na; although there was no significant difference between this genotype (4) compared with genotype 2 for these elements. Genotype 5 also recorded the highest concentration for P compared with other genotypes but fails to produce any significant ($p<0.05$) yield difference with genotype 2. As far as genotype is concerned, our focus was mainly drawn to two (Fe and Zn) of the ten minerals listed in the study. This becomes important due to early reports that the deficiencies of iron and zinc are the most prevalent micronutrient malnutrition in the world [27]. Genotype 2 showed significantly ($p<0.05$) higher concentration for Fe compared with the others; while genotype 3 recorded significant ($p<0.05$) yield difference compared to genotypes 1, 2 and 4 for Zn. Considering the distinctive performance of these two genotypes in these key minerals, a hybridization programme to fuse the genes responsible for high Zn and Fe contents in both genotypes (*Bo* 2 & *Bo* 3) could help in the improvement of landraces of *B. oleracea* to be considered for release into the farming systems of northern KwaZulu-Natal. Similar suggestions to improve *Brassica* genotypes through hybridization have been documented [15, 28]. An improvement programme for this species becomes more important due to the possible challenge to absorption of these two micronutrients that could be experienced following the remarkably high iron-to-zinc ratio found in the genotypes of *B. oleracea*. Early reports indicate that the actual bioavailability of Fe and Zn could be affected by the interactions between these two micronutrients [29] and an iron-to-zinc ratio above 2 has been documented to inhibit the absorption of zinc [30, 31]. In the current study, the iron-to-zinc ratio at harvest stage 3 was 11.9 among the genotypes while ratio comparison between the two micronutrients was 8.7 for genotypes 2 and 3.

Based on the outcome of this study, a hybridization programme aimed at increasing the zinc level of genotype 2 could remarkably guarantee an improvement in the bioavailability of iron and zinc within a target population. Also, in a continent ravaged by serious infant and adult mortality due to the interaction of iron deficiency and the malaria parasite [32, 33] a cheap source of iron like *B. oleracea* must be encouraged to form part of nutritional programmes that could turn the tide against micronutrient malnutrition especially in the diet of marginal income populations in northern KwaZulu-Natal, South Africa, where there is a growing incidence of malaria due to its proximity to Mozambique. The current study is the first literature on the mineral dynamics among genotypes of *B. oleracea* in northern KwaZulu-Natal, South Africa.

Table 2. Mineral contents among six genotypes of *B. oleracea.*

Genotypes	Minerals (Mean±SD)									
	Ca (%)	Mg (%)	K (%)	P (%)	N (%)	Na (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
Bo 1	1.78±02[ab]	0.45±06	4.90±01[ab]	0.42±04[cd]	3.27±08	59.88±49[ab]	27.33±42[cd]	2.29±21	20.08±41	171.25±28[b]
Bo 2	1.88±04[a]	0.47±02	5.29±02[a]	0.47±02[ab]	3.48±23	61.19±23[ab]	28.75±38[bcd]	2.44±13	22.50±72	295.00±56[a]
Bo 3	1.73±01[ab]	0.44±03	4.66±04[b]	0.45±06b[c]	3.40±10	51.34±52[b]	33.83±12[a]	2.53±26	19.92±34	219.25±32[b]
Bo 4	1.97±01[a]	0.48±01	4.98±02[ab]	0.39±03[d]	3.14±26	64.29±36[a]	25.08±64[d]	2.17±17	21.92±25	201.25±40[b]
Bo 5	1.76±03[ab]	0.46±04	5.12±03[ab]	0.50±01[a]	3.55±13	51.36±09[b]	30.92±23[abc]	2.51±20	21.67±27	202.92±18[b]
Bo 6	1.52±02[b]	0.44±02	4.80±01[ab]	0.47±06[ab]	3.55±02	51.36±43[b]	31.92±52[ab]	2.50±18	21.83±48	157.50±36[b]

Means with the same letter along the same column are not significantly different at p<0.05; values with no letters along same column are not significant at p<0.05 and *Bo* = *Brassica oleracea*.

Conclusions

The results of this study indicated that harvesting the leaves of *B. oleracea* fifteen weeks after transplant could help to capture the highest level of nutrient concentrations. Analysis of the comparative performance of the six genotypes tested in the study also suggests that genotype 2 showed superior performance in terms of nutrient accumulation. However, an improvement programme via hybridization of genotypes 2 and 3 could help capture and increase the level of zinc to balance iron-to-zinc ratio. Intraspecific hybridization programme that aims to breed for zinc improvement on genotype 2 could also be of advantage in a situation where food based strategy is the main target to addressing iron and zinc deficiencies especially among financially challenged population groups.

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