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Original Research Article

Genetics of Four Mineral Micronutrients Contents in Edible Leaves of Cowpea (Vigna Unguiculata L. Walp.) Genotypes

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Abstract: The objective of this study was to determine the genetic mechanism of copper, iron, magnesium and zinc concentration in fresh leaves of fifteen dual-purpose cowpea pure lines and 28F$_1$ hybrids derived from an 8x8 half-diallel mating, under the environmental conditions of the high savannah guinea zone of Cameroon. Significant differences amongst the pure lines for Cu (9.68 to 13.68 mg.kg^{-1}), Fe (370.52 to 770.45 mg.kg^{-1}), Mg (0.33 to 1.03 mg.kg^{-1}) and Zn (35.35 to 44.40 mg.kg^{-1}) contents indicated the presence of diversity in the materials. Diallel analysis done using DIAL 98 microcomputer software showed that the effects of the general combining ability of the eight parents and the specific combining ability of the 28 F$_1$ hybrids were highly significant (p<0.01). Additivity and non-additive gene effects were significant in the genetic control of these traits with preponderance of non-additive genes for Cu and Zn. These traits were controlled mainly by partial dominance model and genes are asymmetrically distributed in the parental genotypes. Recessive alleles exerted overall a positive effect, suggesting the advantage of delaying selection to later generations. The broad-sense heritability values obtained for these traits were very high (0.94-0.98) showing the preponderance of the genetic component in their expression. Moderate heterosis over the better parent (HB = 23% for Cu and Zn) was recorded for few combinations exhibiting positive SCA values. For these traits, recurrent selection in later generations might be a useful breeding strategy. This study would be of immense importance in enhancement of these minerals in cowpea foliage as well as mitigating nutritional deficiency prevalent among the poor population in developing countries.

Keywords: Vigna unguiculata, leafy vegetable, copper, iron, magnesium, zinc, diallel analysis.

INTRODUCTION

Cowpea is an important protein-rich legume crop in many parts of the world, particularly in warm regions of Africa and Asia, where it plays a major role in the diet and economy of millions of farmers (Singh *et al.*, 2003; Pottorf *et al.*, 2012). In facts, cowpea is cultivated mainly by subsistence peasants for its edible seeds and young leaves consumed as dried or fresh leafy vegetables prepared as salad or eaten as relish along with other food meals (Imungi and Potter, 2006; Pottorf *et al.*, 2012). It is also grown for immature pods as well as hay for livestock feed (Ahenkora *et al.*, 1998). In arid and semi-arid tropical regions of Africa, cowpea is among the top four leafy vegetables used by farmers and represents cheaper source of plant-protein and minerals especially in these areas where food insecurity and human malnutrition are recurrent (Ahenkora *et al.*, 1998). In dual-purpose varieties where seeds and leafy vegetables are harvested, the developed leaves are first collected during flowering and then the seeds are taken at maturity (Singh *et al.*, 2003). In many African markets, fresh and dried cowpea leaves are sold mainly by resources-poor people (Noubissié *et al.*, 2011). The nutritional composition of fresh cowpea leaves is comparable to that of other tropical leaf vegetables with high levels of carbohydrates, proteins, vitamins and minerals especially phosphorus, zinc and iron (Ohler *et al.*, 1996; Sebetha *et al.*, 2010).

Green vegetables have long been recognized as the cheapest and most abundant sources of protein, vitamins and minerals (Omokanye *et al.*, 2003). Minerals are elements required by living organisms, both animals and plants, to support various physio-

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biochemical processes (Welch and Graham, 2002). They are essential components of food quality and have major effects on health (Omokanye *et al.*, 2003; Okonya and Maass, 2014). In human being and plants, iron (Fe) is incorporated into the heme complex which mediates redox reactions and oxygen transport (Okonya and Maass, 2014). Fe is also an integral part of many enzymes such as nitrogenase and hydrogenase, and mediates electron transport during photosynthesis and terminal respiration, and reduction and accumulation of nitrate and sulphate (Okonya and Maass, 2014). Zinc (Zn) is part of the structure of several enzymes such as carboxylpeptidase, alcohol dehydrogenase and carbonic anhydrase, which also mediate leaf formation and auxin synthesis. Zn ions are considered as neurotransmitters and its deficiency in humans, causes hair loss, skin lesions, diarrhea, loss of memory, weak eyesight, as well as taste and smell abnormalities (Santos and Boiteux, 2013). Copper (Cu) is an integral part of cytochrome oxidase, plastocyanin, superoxide dismutase and many other enzymes and proteins. Cu also assists in carbohydrate metabolism and biological nitrogen fixation (Santos and Boiteux, 2013; Okonya and Maass, 2014). Magnesium (Mg) is an important co-factor of many regulatory enzymes, particularly the kinases, and is fundamental in the energy transfer reactions involving high energy compounds like ATP and creatine phosphate and thus muscle contraction and pregnancy-induced hypertension and preeclampsia (Arivagalan *et al.*, 2013). A diversified diet with adequate micronutrients is prerequisite for human health. Adequate dietary intake of iron, zinc, copper and magnesium is essential to human health. In many regions in the world, the diets do not contain enough nutriments, and mineral elements deficiencies are common (Pottorf *et al.*, 2012).

In developing countries in particular, many people do not eat a balanced diet and rely on a staple diet of cereals which is low in Zn and Fe. To reduce these deficiencies, enrichment and fortification programs added high-cost factitious nutriments in foods (Santos and Boiteux, 2013). However, these nutriments are found in some local natural resources. Cowpea leaf is an excellent food complement for babies and young children, and helps them to satisfy their daily needs in energy (Ahenkora *et al.*, 1998). Many investigations revealed the presence of variable concentration of Fe, Mg, Cu and Zn content in Vigna unguiculata leaves (Ohler *et al.*, 1886; Ahenkora *et al.*, 1998; Megueni *et al.*, 2011; Belane and Dakora, 2012; Igbatim *et al.*, 2014). The mineral elements concentration in cowpea leaves varied with genotypes, agro-ecological conditions and plant stage (Sebetha *et al.*, 2010; Megueni *et al.*, 2011; Santos and Boiteux, 2013; Chikwendu *et al.*, 2014; Yoka *et al.*, 2014; Gerrano *et al.*, 2015). Young expanded leaves usuallyare harvested at the end of vegetative and during the reproductive phases, then during pod-filing, nutrients are transferred from the leaves to other organs of the plant to be used in its developments (Ohler *et al.*, 1886). Presently, to meet the needs of the rural population, the research tends to develop dual-use cowpea varieties that produce leafy vegetables and dry seeds of better nutritional qualities respectively at flowering and maturity stages (Hall *et al.*, 2003; Imungi and Potter, 2006).

Studies on cowpea leaves as vegetables have been very little discussed in research despite its increasing importance in Africa (Noubissié *et al.*, 2011; Belane and Dakora, 2012; Igbatim *et al.*, 2014). In general, many efforts have been laid emphasis in grain, while sources of leafy vegetables have been largely overlooked (Singh *et al.*, 2003). Only little information is available on the genetic mechanisms governing leaf mineral contents in cowpea (Gerrano *et al.*, 2015). Previous studies namely in the Guinea savannah zone, have focused on the genetic architecture of some leaf yield components, protein content, dietary fiber and vitamin C contents (Noubissié *et al.*, 2011; Ndogonoudji *et al.*, 2018). Plant breeding to enhance the nutrient quality of food crops holds promise for a low-cost and sustainable approach to alleviate the problem of micronutrients malnutrition among the poorest segments of the population in developing countries (Welch and Graham, 2002; Santos and Boiteux, 2013). The production biofortified plants can be done by selecting varieties with broad genetic background. The minerals metabolism is a complex mechanism regulated by many genes and involving processes of mobilization, uptaking, translocation and accumulation (Welch and Graham, 2002; Megueni *et al.*, 2011). The diallel mating system developed and advocated by Hayman (1954) is used to investigate the genetic control of polygenic traits especially in autogamous plants like cowpea. Because of dual-purpose cowpea increasing popularity and health benefits of consuming mineral-rich foods, this study was conducted to access the level and nature of variability of selected cowpea genotypes for four leaf mineral contents, and investigate their genetic properties through analysis of diallel crosses. Information obtained would be exploited in cowpea breeding program in the Guinea savannah zone to produce mineral-rich genotypes.

MATERIALS AND METHODS

Experimental Site

Field experiments were conducted during 2016 main cropping season, at the University of Ngaoundéré experimental farm, at Dang (latitude 7°28' N; longitude 13°34' E; altitude 1115 m at sea level), in the Adamawa region (Cameroon). Adamawa belongs to the high Guinea savannah zone which is characterized by a ferruginous soil type with relatively high fertility, a Sudano-guinean climate, an average annual rainfall of 1480 mm distributed over the 6 months wet season (April to September) (Noubissié *et al.*, 2011; Ndogonoudji *et al.*, 2018). The high elevation lends a relatively cool climate with annual average temperature of 22°C and an annual hygrometry of about 70% (Noubissié *et al.*, 2011). Soil analysis on pre-planting surface (0–15 cm) showed a pH value of 5.3; with 9.6 mg kg^{-1} organic matter, total N content of 0.09% and Bray P content of 34 mg kg^{-1} (Ndogonoudji *et al.*, 2018).

Genotypes

The tested genotypes was composed of fifteen dual-use cowpea fixed lines consisting of registered varieties 24-130 and VYA from the National Institute of Agricultural Research for Development (IRAD, Cameroon); six lines from the International Institute for Tropical Agriculture (IITA, Nigeria) namely IT81D98, IT97K-573-1-1, 24-125B, IT98K-205-8, IT93K-693-2 and IT97K-819-118; five registered genotypes originated from Burkina and widely cultivated in Chad: Gorom, KVX414-22-2, KVX61-1, TVX32-36 and Vita 5; variety B301 selected in Botswana; and line TN5-78 from the National Institute of Agronomic Research of Niger (INRAN). Seeds of these fully homozygous varieties were obtained from the IRAD-Maroua station.

To evaluate the variability of the pure lines for these mineral elements, an early field trial was conducted during 2013 wet season. For crossings, eight varieties namely B301, IT98K-205-8, IT81D-98, TN5-78, TVX32-36, VYA, 24-125B and IT97K-573-1-1 were selected as parents and sowed in pots from May to September 2014. The 8x8 half-diallel crosses comprised 36 combinations consisting of 28 F_1 hybrids and the eight pure lines.

Field Trials

All 15 lines and the 28 F_1 hybrids were arranged in randomized complete block design (RCBD) with three replications during the rainy season. Sowing was done on May 6, 2016. Each experimental unit was constituted by a row of 5m length x 0.5m broad, spaced 0.3m apart. Three seeds were sown and one seedling was retained after thinning. The distance between holes was 20cm; which give a density of 154000 plants per ha. Before the sowing, the NPK fertilizer (7% N, 14% P_2O_5, 7% K_2O) was spread on the plots at rate of 6 g per square meter. All standard agronomic practices were adopted uniformly and the weeding was done three times, at three weeks after plant emergence, during the vegetative stage and close to the crop maturity. The plants were protected from insects through spray of cypermethrin insecticide 25 and 35 days after planting (DAP). Three young expanded leaves per plant were harvested on ten randomly selected plants in the middle row of each plot on June 17, 2016, at 40 days after planting corresponding to flowering stage. As noted by Belane and Dakora (2012), leaf concentration of micronutrients was much greater in cowpea leaves up to flowering stage than close to physiological maturity.

Determination of Mineral Micronutrient Content

The leaves were oven dried, ground into fine powder, and 1.2g samples were drawn for analysis. The chemical analysis was carried out in the Laboratory of Soils and Water Analysis of the University of N'Djamena, Chad. The analysis involved the determination of the amount of four mineral elements (Cu, Fe, Mg and Zn) by using an atomic absorption spectrophotometer equipped with a D_2 lamp background correction system (AAS, model-Varian Spectra AA 220 FS, Australia) as recommended by AOAC (2002).

Statistical and Genetic Analysis

Estimates of genetic variability for the four leaf mineral elements among the fifteen studied pure lines were done by the analysis of variance (ANOVA) using STATGRAPHICS PLUS statistical package program. The least significant difference at 5% level of probability (LSD 0.05) was used to compare the means showing statistically significance.

DIAL 98 microcomputer software developed by Ukai (1998) was used to conduct the diallel analysis. Griffing's method 2 (excluding reciprocal F_1 crosses), model 1 (fixed effects) was used to analyze the general combining ability (GCA) of lines and the specific combining ability (SCA) of crosses (Griffing, 1956). GCA and SCA estimates of parents and hybrids were obtained as:

$$GCA_i = X_i - X$$
$$SCA_{ij} = X_{ij} - X_i - X_j + X$$

where, X is the general mean of the populations, X_i is the mean of the hybrids derived from parent i, X_j is the mean of hybrids derived from parent j, and X_{ij} is the value of the cross from parents i and j. Student's t-test was used to test the hypotheses that GCA and SCA effects equal to zero.

The effects of additive and dominance components were obtained according to Walters and Morton's analysis of variance (Walters and Morton, 1978). The variation was divided into the additive effects (a) and the dominance effects (b) which were decomposed into components b_1 measuring directional dominance, b_2 examining the difference between selfs and crosses among parents, and b_3 measuring residual dominance variation (Walters and Morton, 1978). Genetic parameters for estimating the gene effects and parameter ratios in the parents and F_1s of the diallel cross were estimated as by Hayman (1954). The correlation coefficient R derived from the regression of the covariance values between the parents (Vp) and their offspring in the r^{th} array (Wr) against variance values of the r^{th} array (Vr) was used to test the additive-dominance model (Griffing, 1956) as:

$$R = Wr / (Vr + Vp)^{1/2}$$

If R<1, the scatter points are within the limiting parabola $Wr^2 = Vr \times Vp$ showing the adequacy of simple additive-dominance model (Griffing, 1956).

The gene action was tested by the correlation between parental values (Pr) and recessive factor (Wr+Vr) (Walters and Morton, 1978). Heritability was estimated for each trait differently. Broad-sense heritability (h^2) was evaluated as the proportion of genetic variance ($\delta^2 g$) on the phenotypic variance ($\delta^2 p$), while narrow-sense heritability ($h^2 n$) was estimated as the proportion of additive variance (δ^2_a) on the overall phenotypic variance (Allard, 1960; Mather and Jinks, 1982). Heterobeltiosis (HB) was quantified as deviation of F_1 value (F_1) from the better parent (BP) as highlighted by Fonseca and Paterson (1968) as:

$HB (\%) = [(F_1 – BP)/BP] \times 100$

RESULTS AND DISCUSSION

Genotypic Variability

The mean values for the concentration of the four micro-mineral elements in cowpea fresh leaves are shown on Table 1. Highly significant differences ($p<0.01$) were observed among the fifteen pure lines for the concentration of these micro-mineral elements. The leaf Cu concentration ranged from 9.68 to 13.68 mg.kg^{-1} (mean = 11.86 mg.kg^{-1}) with genotypes 24-125B, 24-130, IT93K-693-2 among the highest. For Fe concentration, values ranged from 370.52 to 770.45 mg.kg^{-1} (mean = 554 mg.kg^{-1}) and genotype that ranked high were IT97K-573-1-1 and IT81D-98. Mg appeared as the minor mineral in cowpea leaves (range of 0.33 to 1.03 mg.kg^{-1}; (mean = 0.60 mg.kg^{-1}) and varieties IT97K-573-1-1, VITA5 and B301 showed the highest concentrations. The coefficient of variability for Mg (37.41%) was maximum than those of all other minerals studied. The rates of Zn were relatively high particularly for lines IT97K-208-5 and VYA, and varied from 35.35 to 44.40 mg.kg^{-1} (mean = 39.47 mg.kg^{-1}). The coefficient of variation for Zn (6.62%) was least among all the minerals analyzed.

Significant differences amongst the pure lines for Cu, Fe, Mg and Zn contents in cowpea indicated the presence of diversity in the material. The coefficient of variation ranged from 6.66% for Zn to 37.42% for Mg, showing that these characters are quantitative in nature and exhibit considerable degree of interaction with gene. The existence of a wide genetic variability is a prerequisite to effective selection for traits of interest in plant breeding program (Allard, 1960). Thus, this variability offers a wide scope for the selection of potential parents for the improvement of leaf mineral contents. The overall average values for Cu, Fe, Mg and Zn were close similar to the magnitude of these elements reported by Mamiro *et al.*, (2011) in Tanzania and Gerrano *et al.*, (2015) in South Africa. On cowpea leaves, Yoka *et al.*, (2014) also reported close results for Cu (13.50 86 mg.kg^{-1}) and for Zn (44.10 mg.kg^{-1}). The Fe content in the current findings was in the range of what Chikwendu *et al.*, (2014) reported in cowpea leaves in Nigeria. These values were relatively higher than those of Okonya and Maass (2014) in Uganda (176-387 mg.kg^{-1}). These variations might be due to genotypic differences as well as differences in the soil and in the climatic conditions (Gerrano *et al.*, 2015; Mohammad and Mukhtar, 2017). Mohammad and Mukhtar (2017) noted that cowpea plants cultivated during the rainy season possessed more minerals than in the dry season, and combined application of liquid nitrogen fertilizer and benzyl aminopurine (BAP) increased the absorption of nutrients by the plants and delayed senescence. Therefore, mineral elements contents in plants show variation depending to their concentration in soil and their availability to plants. High genotypes x environment interaction for mineral elements levels, which affect rank of genotypes across locations, have been previously reported (Ohler *et al.*, 1996; Gerrano *et al.*, 2015). According to Okonya and Maass (2014), the nutritional quality of cowpea leaves was more influenced by the cultivars comparing to the environmental conditions. The highest average mean value was recorded in Fe compared to Zn, Cu and Mg. With the very high level of Fe and Zn deficiency in Africa (Nielsen *et al.*, 1994; Belane and Dakora, 2012) and its associated health problems, genotypes with high Fe and Zn concentrations like IT97K573-1-1 and IT81D-98 can be very valuable cultivars in supplying these nutrients. As noted by Belane and Dakora (2012), the levels of trace elements were sometimes two to 20-folds greater in leaves than grain suggesting that cowpea genotypes can be selected for high mineral accumulation for human nutrition and health. Therefore, these genotypes are good candidates for use as parental lines in cowpea improvement program for minerals. High mineral elements contents recorded in the young leaves of these genotypes, although does not necessary indicate their bioavailability, is an indication of their potentials to contribute in meeting the daily dietary requirements of essential minerals (Ahenkora *et al.*, 1998; Singh *et al.*, 2013).

Table 1: Variability for the concentration of four micro-mineral elements in the leaves of 15 cowpea pure lines

Genotypes	Cu (mg.kg^{-1})	Fe (mg.kg^{-1})	Mg (mg.kg^{-1})	Zn (mg.kg^{-1})
24-125B	13.48±0.04^{gh}	370.52±28.55^{a}	0.330±0.028^{a}	37.74±0.34^{bc}
24-130	13.68±0.25^{h}	425.63±35.33^{b}	0.370±0.028^{a}	36.67±0.25^{abc}
B301	12.29±0.30^{e}	625.12±35.44^{g}	0.835±0.021^{f}	36.31±0.27^{a}
Gorom	11.64±0.19^{d}	440.29±14.55^{bcd}	0.455±0.007^{b}	40.63±0.18^{ef}
IT81D-98	11.74±0.34^{d}	755.08±7.44^{i}	0.960±0.014^{g}	39.60±0.14^{e}
IT93K-693-2	13.26±0.37^{g}	470.33±28.11^{d}	0.545±0.007^{c}	38.25±0.35^{cd}
IT97K-573-1-1	12.66±0.27^{f}	770.45±28.74^{i}	1.030±0.028^{h}	40.30±0.28^{ef}
IT97K-819-118	10.69±0.28^{c}	525.05±35.98^{e}	0.525±0.035^{c}	41.30±0.28^{fg}
IT98K-205-8	10.26±0.35^{b}	435.17±21.00^{bc}	0.460±0.056^{b}	44.40±0.14^{i}
KVX414-22-2	12.38±0.18^{ef}	545.68±7.77^{ef}	0.425±0.035^{b}	39.52±0.03^{de}
KVX61-1	12.28±0.32^{e}	465.11±21.88^{cd}	0.335±0.021^{a}	36.64±0.19^{ab}
TN5-78	9.68±0.25^{a}	660.05±14.50^{h}	0.640±0.014^{d}	42.34±0.23^{gh}
TVX32-36	10.53±0.04^{bc}	637.38±17.66^{gh}	0.545±0.007^{c}	35.35±0.21^{a}
VITA5	12.63±0.18^{f}	625.95±35.43^{g}	0.855±0.007^{f}	39.74±0.33^{e}
VYA	10.74±0.34^{c}	565.67±21.62^{f}	0.730±0.028^{e}	43.29±0.23^{hi}
Mean	**11.86±1.23**	**554. 67±12.14**	**0.602±0.225**	**39.47± 2.63**
LSD (0.05)	**0.29**	**32.55**	**0.041**	**1.28**
CV (%)	**10.39**	**21.87**	**37.42**	**6.66**

LSD (0.05): Least significant difference at 5% level. CV: Coefficient of variation. For each parameter, averages with the same letter are not significantly different at p<0.05.

Diallel Analysis

Mean squares for general and specific combining abilities among parents and their crosses for the traits analyzed are presented in Table 2. Highly significant mean squares ($p < 0.01$) were observed for GCA and SCA for all traits, showing the importance of both additive and dominance effects (Hayman, 1954). To weigh the relative importance of GCA and SCA in the expression of the different traits, the proportion of GCA and SCA variances were calculated. The σ^2GCA/σ^2SCA ratios were higher than unity for Fe and Mg (1.43 and 1.64 respectively); but these values were lower than unity for Cu and Zn (0.60 and 0.48 respectively). These ratios showed that SCA variance was higher than GCA variance component for Cu and Zn, while GCA was more important in the expression of Fe and Mg. This agrees with the findings of Liu *et al.*, (1996) that GCA variance was higher than SCA for Fe content in cabbage. In contrast, Ndogonoudji *et al.*, (2018) noted GCA/SCA rations were less than unity for protein and dietary fiber contents in cowpea leaves showing preponderance of dominant gene action.

Table 2: Mean squares for general and specific combining abilities for four minerals of cowpea leaves in an 8x8 half-diallel crosses

Source of variation	df	Mean squares			
		Cu	Fe	Mg	Zn
Replication	2	1.66	3.88	0.13	2.74
GCA	7	32.53**	128.67**	11.91**	148.08**
SCA	20	8.86**	99.33**	8.64**	46.25**
Error	15	1.90	22.53	0.69	5.34
σ^2GCA/σ^2SCA		**0.60**	**1.43**	**1.64**	**0.48**

df :Degree of freedom; σ^2GCA: Variance of general combining ability; σ^2SCA: Variance of specific combining ability; ** indicates significance at p<0.01

In diallel analysis, some of the eight studied parents showed significant positive or negative GCA effects for these traits (Table 3). Only improved lines IT97K-573-1-1 showed positive significant GCA effect for all the four traits, while other had positive GCA for some traits and negative GCA for others. However, 24-125B and IT81D-98 had relatively large and positive GCA values for Cu and Fe, respectively, while VYA exhibited large and positive GCA for and Zn and Mg. Genotypes with negative and significant GCA effect were TN5-78, IT98K-205-8 and TVX32-36 for Cu; 24-125B and IT98K-205-8 for Fe and Mg; TVX32-36; B301 and 24-125B for Zn. Information on GCA is helpful for ranking the lines on the base of their potential as best sources among the genotypes used. Generally, there exist a fairly good relationship between the GCA effects and the mean values of the parents. In this case the per se values could be used to select parents for hybridization.

Table 3: Estimates of general combining ability effects (GCA) for mineral leaf traits among eight cowpea parents

Parents	**Cu(mg.kg^{-1})**	**Fe (mg.kg^{-1})**	**Mg(mg.kg^{-1})**	**Zn (mg.kg^{-1}).**
24-125B (P_1)	1.04**	-7.50**	-0.23**	-1.15**
B301 (P_2)	0.71**	-0.17	0.08	-2.19**
IT81D-98 (P_3)	-0.02	8.00**	0.15**	-0.30
IT97K-573-1-1 (P_4)	1.07**	5.67**	0.12*	1.60**
IT98K-205-8 (P_5)	-0.78**	-4.83**	-0.11*	2.18**
TN5-78 (P_6)	-1.39**	3.50*	0.02	0.91**
TVX32-36 (P_7)	-0.46*	3.33*	-0.08	-2.73**
VYA (P_8)	**-0.13**	**0.09**	**0.50****	**1.69****

*, ** Significantly different at 0.05 and 0.01 probability levels respectively

Estimates of SCA effects for the 28 F_1 hybrids for the traits evaluated are provided in Table 4. Positive and significant SCA effects were observed for Cu content in ten hybrids, with the largest values in the crosses 24-125B x B301 and IT98K-205-8 x TN5-98. For this trait, significant and negative SCA values were noted in seven crosses. Positive and significant SCA effects were noted on twelve combinations for Fe content with largest values in the crosses B301 x TN5-78, IT81D-98 x IT98K-205-8 and TN5-78 x IT81D-98. Only five crosses showed positive and significant SCA effects for Mg, with high values noted on combinations B301 x IT97K-573-1-1 and 24-125B x VYA. For the Zn content ten hybrids showed positive and significant SCA effects and crosses B301 x IT81D-98, 24-125B x TN5-78 and IT97K-573-1-1 x IT98K-205-8 showed the highest values. Positive values of SCA effects were associated with percentages of heterobeltiosis (HB%).

Indeed, for most hybrids with positive and significant SCA effects, positive and moderate percentages of HB were also noted (Table 4). HB values were mainly negative for all studied traits. HB values were positive in seven combinations for copper; nine crosses for iron, four hybrids for magnesium and eight combinations for zinc. Best combinations SCA performance might be considered as a criterion for selecting the best crosses. The low x low or low x moderate general combiners exhibiting high SCA effects suggested gene dispersion and genetic interaction between favourable alleles contributed by both parents (Griffing, 1956; Gupta *et al.*, 1993). In contrast, some combinations showed low SCA effects despite their parents had high GCA effects. These weak SCA effects could be explained by absence of interactions between the parents' favourable alleles (Gupta *et al.*, 1993). These results prove that the effects of SCA would be greater than those GCA in the hybrid constitution for the high mineral content of cowpea leaves. In this study, moderate magnitudes of HB in certain crosses demonstrate that some hybrids can be exploited only as basic material for breeding purposes. According to Mather and Jinks (1982), HB could be linked to the accumulated action of favourable dominant genes dispersed amongst two parents; to the complementary interaction of additive genes at different loci; or to the intra or inter locus interactions referred to as overdominance. It is noteworthy that the crosses, showing consistently positive SCA, also exhibited positive significant HB values. The association suggested that HB could be useful for the choice of potentially more segregating populations in situation where SCA cannot be estimated. Thus, hybrid crosses with high per se values and positive SCA estimates, involving at least one of the parents with positive and significant GCA, would be indicated to increase the concentration of favourable alleles. In maize, Chen *et al.*, (2007) noted that Fe concentration of the ear-leaf was controlled by additive gene effects and heterosis was high, indicating that hybrid breeding can be used for improvement. Sala *et al.*, (2015) also recorded high heterosis for Fe and Zn contents in segregating populations of rice. High values of heterosis were also noted for mineral elements contents in single cross-hybrids of cabbage (Bhargava *et al.*, 2013). Attention should be focused on the development of F_1 hybrids showing positive and large SCA effects with high amplitude of HB values.

Table 4: Estimates of specific combining ability (SCA) and heterobeltiosis (HB) among 28 F_1 hybrids for four microelements in cowpea leaf

Crosses	Cu		Fe		Mg		Zn	
	SCA	HB (%)	SCA	HB (%)	SCA	HB (%)	SCA	HB (%)
P_1 x P_2	0.46**	14.9	-4.33**	-16.2	-0.09*	-17.1	0.39	4.2
P_1 x P_3	0.34**	6.3	-4.50**	-35.7	0.01	-15.3	-0.36*	-6.4
P_1 x P_4	0.23*	4.4	-2.17*	-48.0	-0.09*	-12.2	-1.28**	-48.1
P_1 x P_5	-0.13	-43.1	5.33**	-5.5	0.16**	1.7	-0.34*	-22.3
P_1 x P_6	-0.41**	-41.6	0.91	-4.9	0.08*	-6.6	1.54**	13.4
P_1 x P_7	-0.18*	-18.2	3.17*	-1.7	-0.01	-7.6	0.86**	2.7
P_1 x P_8	-0.31*	-31.8	5.50**	5.1	-0.05	-10.1	-0.80**	-24.1
P_2 x P_3	-0.28*	-49.1	-3.83**	-33.0	0.04	-11.3	1.68**	19.0
P_2 x P_4	-0.47**	-54.1	5.50**	-17.1	0.12**	0.0	-0.57**	-7.7
P_2x P_5	0.24*	-8.7	0.81	8.2	-0.15**	-23.2	-1.91**	-40.7
P_2 x P_6	-0.23*	-74.2	6.67**	7.8	0.01	-8.4	-1.52**	-26.4
P_2 x P_7	0.06	-65.0	-2.17*	-5.2	0.06	-2.5	1.27**	23.0
P_2 x P_8	0.23*	-8.7	2.17*	1.1	0.02	2.2	0.67*	-1.9
P_3 x P_4	0.25*	-2.5	4.33**	7.4	0.08*	-7.9	-0.87**	-39.0
P_3 x P_5	-0.39**	-37.6	6.83**	1.8	0.01	-15.6	0.88**	-1.4
P_3x P_6	0.17*	15.2	5.55**	15.1	-0.05	-20.9	0.37	-3.8
P_3x P_7	-0.06	-4.2	-4.35**	-13.8	-0.05	-20.7	-0.50*	-20.1
P_3x P_8	-0.03	-1.0	1.01	-9.2	-0.02	-17.2	-1.20**	-29.3
P_4 x P_5	0.04	-54.3	-6.83**	-30.2	-0.03	-15.2	1.43**	3.2
P_4x P_6	-0.35*	-42.9	-4.17*	-27.9	-0.04	-7.3	-0.12	-17.4
P_4 x P_7	0.17*	-9.7	1.01	-2.1	-0.10**	-4.3	0.51*	-10.6
P_4 x P_8	0.13	-14.4	4.33**	12.4	0.06	0.8	0.90**	6.8
P_5x P_6	0.45**	23.3	-5.67**	-67.8	-0.03	-8.5	0.12	-1.3
P_5x P_7	-0.19*	-27.5	4.50**	6.3	0.04	6.1	-0.71**	-29.3
P_5x P_8	-0.02	-11.5	-3.17*	-8.1	0.01	-0.8	0.52*	5.0
P_6x P_7	0.28*	14.7	3.17*	-3.9	0.07*	-7.5	-0.86**	-47.2
P_6 x P_8	0.09	2.8	-4.50**	-60.8	-0.02	-33.8	0.48*	-4.0
P_7x P_8	**-0.09**	**-4.0**	**-5.33****	**-56.3**	**0.01**	**-11.9**	**-0.56****	**-41.6**

P_1: 24-125B; P_2: B301; P_3: IT81D-98; P_4: IT97K-573-1-1; P_5: IT98K-205-8; P_6: TN5-78; P_7: TVX32-36; P_8: VYA; *; **: Significant at 0.05 and 0.01 probability levels respectively

Mean squares resulting from analysis of variance of Walters and Morton (1978) for additive (a) and dominance (b) and dominance components b_1, b_2, and b_3 for Cu, Fe, Mg and Zn contents were presented in Table 5. The analysis showed that the additive and dominant effects were highly significant ($p < 0.01$). The components of the dominant effects (b_1, b_2 and b_3) were also significant ($p < 0.05$) for these traits except the mean dominance effect b_1 for Cu and Fe. The significance of b_1 showed that the dominance was unidirectional for Fe and Mg, but it was bidirectional for Cu and Zn (Walters and Morton, 1978). Considering b_2, the dominant genes were asymmetrically distributed among the parents, with some of studied genotypes harboring considerably dominant alleles than others (Walters and Morton, 1978). The presence of specific dominance or combining ability in some combinations was confirmed by the significance of the residual dominance (b_3) for these characteristics.

Table 5: Mean squares from analysis of variance for additive and dominance effects and dominance components for four leaf traits of cowpea

Source	df	Mean square			
		Cu	Fe	Mg	Zn
Replication	2	0.04^{ns}	1.22^{ns}	0.02^{ns}	0.09^{ns}
Additive effect (a)	7	12.88**	187.50**	0.88**	78.00**
Dominance effect (b)	28	36.13**	134.39**	0.19**	184.89**
b_1	1	0.88^{ns}	22.39**	0.10**	0.03^{ns}
b_2	7	2.46**	19.77**	0.53**	6.27*
b_3	20	32.79**	73.23**	-0.53**	178.59**
Error	**25**	**1.98**	**82.52**	**0.01**	**97.23**

df: Degree of freedom; a: Additive effects of genes; b: Dominant effects of genes; b_1: Mean dominance effects; b_2: Additional dominance deviation due to the parents; b_3: Residual dominance effects; *, ** indicates significance at $p < 0.05$ and $p < 0.01$; ns: indicates non-significance at $p < 0.05$.

The genetic parameters (average degree of dominance, direction of dominance, correlation between the degree of dominance and parental value) as well as the broad and narrow sense heritability values were presented in Table 6. Broad and narrow sense heritability for these characters ranged from 0.94 to 0.98 and from 0.34 to 0.56 respectively. Very high values of heritability in broad-sense ($h^2>0.9$) recorded for these traits suggested greater contribution of genotype rather than environment, and that the selection will be very effective and reliable. On cowpea, Gerrano *et al.,* (2015) also recorded very high values of heritability for Fe and Mg leaf content, but noted high and moderate values respectively for Zn (h^2 = 0.65) and Cu (h^2 = 0.46). High values of broad-sense heritability for these mineral elements were also reported by Arivalagan *et al.,* (2013) in Solanum melongena, Singh *et al.*, (2013) in Brassica oleracea var capitata, Spehar (1995) in Glycine; Bhargava *et al.,* (2013) in Chenopodium spp and Gerrano (2017) in immature fruits of Abelmoschus esculentus. A high heritability for these micro-mineral elements indicates that these traits are less influenced by the environmental factors. Thus, it may be possible to increase levels of these elements in cowpea leaves through a suitable and effective breeding approach. Narrow-sense heritability (h^2n) estimates were moderate and varied from 0.34 to 0.56 corresponding to 36.17 to 57.14% of the total genetic variation. These results confirm the implication of both additive and non-additive gene effects with preponderance of non-additive genes for Cu and Zn, and preponderance of additivity for Fe and Mg. In cabbage and in common bean seeds, the additive genes are responsible for the accumulation of Fe, Cu and Zn (Singh *et al.*, 2013; Mukamuhirwa *et al.*, 2015). In okra, Gerrano (2017) also noted the preponderance of additive genes for the accumulation of Fe and Zn in fruits. Accordingly, it would be difficult to adopt pedigree method to improve Cu and Zn contents when non-additive gene action predominates. The mean degree of dominance $(H_1/D)^{1/2}$ was less than unity and ranged from 0.62 for iron to 0.95 for magnesium, indicating the presence of partial dominance in their expression. Concerning the proportion of dominant genes (Kd), the studied parents had a moderate percentage for Cu, Fe and Zn, but a high proportion for Mg.

The coefficients of correlation (R) derived from the covariance values between the parents and their offspring in the rth array (Wr) against variance values of the rth array (Vr) were not significantly different from unity for Cu (0.91), Fe (0.89), and Zn (0.97), but this coefficient was largely different from unity for Mg (0.21). The coefficients of correlation derived from Wr/Vr regression indicated the adequacy of simple additive-dominance genetic model for Cu, Fe and Zn. However, for Mg, this coefficient was significantly different from unity suggesting the possibility of non-allelic interaction. The negative values of the average direction of dominance (h) for these traits indicated that the majority of favourable genes are recessive. This is in line with the findings of Spehar (1999) for mineral absorption in soybeans. The Pr/Wr+Vr correlation values were positive but non-significant for Cu (0.34), Fe (0.53), Mg (0.35) and Zn (0.44). The positive correlation between Pr and Wr+Vr for these traits indicated the preponderance of positive recessive alleles, suggesting that direct selection would be not effective. As highlighted by Singh et al (2009) in cabbage, the accumulation of these mineral elements in cowpea leaves could be improved by hybridization of selected genotypes followed by selection in later generation. Similar observations were highlighted by Ndogonoudji *et al.,* (2018) for the improvement of total protein, dietary fiber and vitamin C contents in leaves of cowpea.

Table 6: Genetic components estimates and heritability values for four mineral contents based on 8 x 8 half diallel

Genetic parameters	Cu	Fe	Mg	Zn
Degree of dominance $(H_1/D)^{1/2}$	0.74	0.62	0.95	0.73
Proportion of dominant genes (Kd)	0.40	0.49	0.87	0.45
Direction of dominance (h)	-0.65	-0.43	-0.21	-0.12
r (Pr, Vr + Wr)	0.34^{ns}	0.53^{ns}	0.35^{ns}	0.44^{ns}
Coefficient of correlation (R)	0.91	0.89	0.21	0.97
Broad-sense heritability (h^2)	0.97	0.98	0.96	0.94
Narrow-sense heritability (h^2_n)	0.38	0.56	0.52	0.34
h^2n/ h^2 (%)	**39.18**	**57.14**	**54.16**	**36.17**

r (Pr, Vr + Wr): Correlation coefficient between the degree of dominance of the parents (Wr + Vr) and the parental value (Pr), Vr: the variance on the rth array

CONCLUSION

Within in the range of dual-purpose cowpea germplasm used in this study, there exist substantial variability and high heritability in the minerals studied to warrant selection in the cowpea accessions for improvement. Thus, the content of these minerals could be genetically improved. These elements are controlled by both additive and non-additive genes. Recessive alleles tend to increase the concentration of these elements in cowpea leaves. In conventional breeding program for developing mineral-rich varieties of cowpea, recurrent selection procedure to allow favourable gene recombination in later generation before a final selection might be a useful strategy. Improved methods to predict genetic gain and evaluate these quantitative traits without the environmental influence are also needed. Quantitative traits loci (QTLs) may be targeted for future marked-assisted breeding strategies.

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