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RESEARCH ARTICLE

Genetic and Chemical Analyses of six Cowpea and two *Phaseolus* Bean species Differing in Resistance to Weevil Pest

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Abstract

The objective of this work was to evaluate the genetic variability among six cowpea (*Vigna unguiculata*) cultivars differing in their resistance to *Callosobruchus maculatus* (F.) weevil. Two resistant bean cultivars were used to compare between the sensitive, moderate tolerant, and high tolerant cowpea cultivars. The differentiations were performed by using random amplified polymorphic DNA (RAPD) fingerprinting, protein concentration and organic and non-organic components in seed coat. Six polymorphic primers were identified, resulting in different informative bands. Based on polymorphic profiles, three clusters were formed. Clustering was mainly affected by the resistance to weevil pest. The sensitive cowpea cultivars were separated in one group, the moderate tolerant and high tolerant cultivars came in separate groups, and finally, the resistant bean cultivars separated clearly in one distinct group. The most interesting result was represented by concentration of total protein in the seed coat. The protein concentration in the resistant bean cultivars were approximately 50% less than concentration in each of the moderate tolerant and sensitive cultivars of cowpea. Ferric ions were about 25% less than the moderate tolerant and sensitive cultivars. The concentrations of calcium and potassium in seed coats were higher in the resistant beans than in cowpea cultivars. Cobalt was about four times higher in resistant bean than in the sensitive and moderate tolerant cowpea cultivars, which may play a major role in seed resistance to weevil.

Key words: *Vigna unguiculata*; *Callosobruchus maculates* (F.); RAPD fingerprinting; total protein; seed coat..

Abbreviations: Abs, absorbance; CTAB, cetyltrimethylammonium bromide; d.w., dry weight of sample, EDTA, ethylenediaminetetraacetic acid; h, hour; min, minute; TBE, Tris/Borate/EDTA; RAPD, Random Amplified Polymorphism DNA; U, unit; Vex, volume of extraction; Vsample, volume of sample.

Introduction

Old World legume cowpea, *Vigna unguiculata* (L.) Walp. is a tropical grain legume which plays an important nutritional role in developing countries of the tropics and subtropics, especially in Sub-Saharan Africa, Asia, Central and South America (Singh et al. 1997). Because of its high protein content (20-25%), cowpea has been referred to as “poor man's meat.” Cowpea young leaves, pods, and peas contain vitamins and minerals which have fuelled its usage for human consumption and animal feeding (Nielsen et al. 1997). On the other hand, the New World legume common bean (*Phaseolus vulgaris* L.) and lima bean (*Phaseolus lunatus*) are a widely cultivated legume originating in the New World which have been domesticated both in Mesoamerica and South America and are currently cultivated in many tropical regions of the World (Smartt 1990). *Callosobruchus maculatus* F. (Coleoptera: Bruchidae), a pest of the seeds of *Vigna unguiculata* (L.) Walp. (Cowpea), do not attack the seeds of *Phaseolus vulgaris* (Applebaum et al. 1970). Bruchids are major threats to

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stored cowpea grains (Singh and Ishivaku 2000), and infestations by the most prominent species, *C. maculatus* and *C. chinensis* are responsible for grain losses estimated at 20-60% (Tarver et al. 2007).

In Egypt and in many places the seeds of both legumes (*Vigna unguiculata* and *Phaseolus spp*.) are stored in the same conditions, the major losses of these stored seeds occur only in cowpea. *C. maculatus*, which is associated with *Vigna unguiculata*, completely destroys cowpea seeds within a very short time after infestation and it does not attack (*Phaseolus spp*.) seeds (Simmonds et al. 1989). An explanation for this apparent specificity was given by Janzen (1977), who suggested that *C. maculatus* larvae die shortly after passing through the testa of wild *Phaseolus spp*. due to the high levels of HCN originating from the hydrolysis of the glucoside linamarin. Simmonds et al. (1989) showed that *C. maculatus* larvae die on entering the cotyledons of *P. lunatus* and suggested that this happens due to the delayed consequences of the ingestion of toxins present in the testa. Thirty years ago Janzen (1977) demonstrated that the seed coat can be a barrier to bruchid infestation and suggested that this could complicate the analysis of the toxicity of seed contents, emphasizing the need to analyze seed contents separately from seed coats.

The seed coat plays a vital role in the life cycle of plants by controlling the development of the embryo and determining seed dormancy, germination as well as tolerance/or resistance to weevil pest. The seed coat synthesizes a wide range of novel compounds that may serve the plant in diverse ways, including defense and control of development. Edde and Amatobi (2003) revealed that seed coat has no value in protecting cowpea seed against attack by *C. maculatus* (F.). In contrast, Akintola and Oyegoke (2004) indicated that seed coat texture plays significant role in inducing ovipositional response. Non-preference was suspected to be the resistance mechanism. Vincenzo et al. (2005) revealed that seed coat tannins must also be considered in biochemical defence mechanisms

Molecular markers reveal differences of natural sites at the DNA level. These variations are not seen in the phenotype and each might be a single nucleotide difference in a gene or a piece of repetitive DNA (Johns et al. 1997). Thus, they are much more numerous than morphological markers and do not disturb the organisms physiology. One of the most widely used PCR-based marker techniques is Random Amplified Polymorphic DNA (RAPD). RAPD marker is generated by PCR amplification of random genomic DNA fragments with single oligonucleotide primers of arbitrary sequence.

The seed coat synthesizes a wide range of novel compounds that may serve the plant in diverse ways, including defense and control of development (Moïse et al. 2005). Chemical analysis of seed coat in cowpea and common bean has been studied by many investigators (Gayan et al. 2006; Macedo et al. 1993, 1995; Moraes et al. 2000; Sales et al. 2000; Seifelnasr 1991; Silva et al. 2004; Xavier-Filho et al. 1989, 1996; Yunes et al. 1998).

In a previous study (Obiadalla-Ali et al., 2007), we screened 21 cultivars of cowpea for resistance to weevil pest based on pest development assessment including number of adults emerged, percentage of damaged seeds, developmental period (days), average life span of female (days), number of eggs laid on seeds, mean of egg laid per female, percentage of adult emergence, and percentage of loss in seed weight. The assessed cowpea cultivars were classified into three groups, sensitive (Dokki 331 and Creamy 7), moderate tolerant (Black eye and IT 90 K 2840-2), and high tolerant (IT 93 K 12904 and IT 81 D-1064). Herein we attempted to elucidate the differential resistance to weevil pest of each cowpea group comparing with two *phaseolus* beans in relation to seed-coat component of some organic and non-organic compounds. In addition, a confirmatory RAPD marker-based genetic relationship was constructed for the studied plant materials.

Materials and Methods

Seed Materials

As mentioned in the introduction, we selected these six cultivars out of twenty-one cowpea cultivars which screened for their resistant to weevil pest (Obiadalla-Ali et al. 2007). Sensitive (Dokki 331 and Creamy 7), moderate tolerant (Black eye and IT 90 K 2840-2), and high tolerant (IT 93 K 12904 and IT 81 D-1064) cowpea (*Vigna unguiculata*) cultivars to weevil infection were used in addition to two resistant bean cultivars [(Lima bean, *Phaseolus lunatus*) and (Common bean, *Phaseolus vulgaris* L.)].

Data of resistance characters

Preference and non-preference tests were conducted according to Messina and Renwick (1985), antibiosis tests were conducted according to Van Emden (1987) and Ofuya (1987) and tolerance tests were conducted as described by Van Emden (1987) and Nakhla (1988). Seed resistance to cowpea weevil (*Callosobruchus maculatus*), has been explained in details previously (Obiadalla-Ali et al. 2007).

DNA extraction and RAPD assay

Total genomic DNA was extracted from fresh young leaves following the cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle 1990; Poresbski et al. 1997). The quality of the DNA was checked by electrophoresis in 1% agarose gelscontaining ethidium bromide (0.5 mg ml^{-1}) in $^1/_2$ x TBE (89 mM Tris-HCl, 89 mM boric acid, and 2 mM EDTA). Twenty-three RAPD primers (decamer oligonucleotides obtained from Operon Technologies, U.S.A.) were tested as single primers for the amplification of a genomic sequence of cowpea. Of these, six primers (Table 1) produced polymorphic band patterns

Table 1. RAPD primers generating polymorphic bands, total number of fragments detected by each primer, and polymorphism found among cultivars studied.

RAPD Primer Name	Sequences	Amplified bands		Polymorphic bands
		Total number of fragments	Number of polymorphic fragments	
OPA-2	5'-TGCCGAGCTG-3'	10	9	90%
OPA-3	5'-AGTCAGCCAC-3'	12	9	75%
OPA-13	5'-CAGCACCCAC-3'	16	16	100%
OPB-9	5'-TGGGGGACTC-3'	7	7	100%
OPC-18	5'-TGAGTGGGTG-3'	12	11	91.7%
OPI-9	5'-GGACACCACT-3'	15	13	86.7%
Total		72	65	
Mean		12	10.8	90%

among genotypes. PCR reaction mixes consisted of 2.5 µl of 10_PCR buffer, 0.5 µl of dNTPs (0.2 mM) (Promega, Madison, USA) 2 µl of primer (25 pmol), 0.3 µl of *Taq* DNA polymerase (0.3 U) (Promega, Madison, USA), 4 µl of $MgCl_2$ (25 mM), 9.7 µl of sterile ultrapure deionized water and 1 µl of 100 ng DNA template, all reaction volumes were 20 µl. A negative-DNA control was performed by adding 1 µl of sterile ultrapure deionized water.

Amplification was carried out in the (Primus 25 Thermal Cycler, Germany). The Thermal Cycler was programmed by: 1 cycle (an initial denaturing step) of 4 min at 90 °C, 35 cycles of 1 min at 90 °C (denaturation step), 1 min at 33 °C (annealing step), 2 min at 72 °C (elongation step) and 10 min at 72 °C (final extension), then kept at 4 °C. The amplification products were electrophoresed at 60 V for 2 h in 1.6% agarose (Himedia, India) gels containing 0.2 µl Ethidium Bromide (0.5 mg ml^{-1}) in $^1/_2$ x TBE. The amplified fragments were visualized under ultraviolet light (BXT-20-M, France) and photographed by digital camera (Olympus SP-510UZ).

DNA banding pattern analysis

The DNA banding patterns generated from RAPD experiments were analyzed by computer program, Gene Profiler (version 4.03). The presence (1) or absence (0) of each band was recorded for each line for the six primers used. Genetic similarity estimates were determined using Jaccard's coefficient (Jaccard 1908). Dendrograms were generated with the unweighted pair group method with arithmetic mean (UPGMA) algorithm using the computational package MVSP version 3.1.

In order to detect patterns of genetic relationship in the genotypes, data analysis on the means of the resistance to weevil pest characters was initially performed based on the Euclidean distance matrix. The output was analysed using an agglomerative hierarchical clustering method with complete linkage strategy. First, the data was subjected to analysis to produce a matrix of dissimilarity values and the phenotypic distance between each pair of genotypes was estimated as Euclidean distance. Second, cluster analysis was conducted on the Euclidean distance matrix with unweighted, pair-group method based on arithmetic averages (UPGMA) to develop a dendrogram using computer program NTSYS-pc version 2.1 (Rolhf 2000).

Combining the Euclidean distance and RAPD distance

The Mantel test is a statistical test of the correlation between two matrices. The matrices must be of the same rank .The similarity matrix of RAPD was converted to dissimilarity matrix. A cophenetic matrix was derived from each matrix to test goodness of fit of the clusters by comparing the two matrices using the Mantel test (Mantel 1967). Finally, the correlation between each distance pair using computer program NTSYS-pc version 2.1 was calculated.

Chemical analysis of seed coat

Protein assay The protein contents were colorimetrically determined according to Lowery et al. (1951). A bovine serum albumin was used for making standard curve and the data was expressed as mg/g d.w. The following equation was used in calculating of protein concentration:

Concentration of protein = Abs. x 0.5 x V_{ex} / V_{sample} x d.w. = mg g^{-1} (d.w.)

Non-organic component contents Plant samples (Coat of Seed) were separated mechanically and washed with diluted HCl and twice with water, dried in an aerated oven at 70 ºC until constant weight was reached. Then, seed coat tissues were ground in porcelain mortar and preserved for analysis. One half gram plant sample materials were digested using concentrated H_2SO_4 and $HClO_4$ (Pericloric acid) according to Black (1982). The digested sample was filtered and raised to 50 ml in volumetric flask. Plant digested was analyzed using atomic absorption spectrophotometer.

Statistical analysis All chemical data were statistically analyzed and treatment means were compared using the Duncan's Multiple Range Test (DMRT) at 0.05 probability level (Gomez and Gomez 1984).

Results and Discussion

Level of polymorphism

Six out of twenty-three primers used generated different degrees of genetic polymorphism among cultivars studied. Approximately 90% (65) of the 72 visible bands were polymorphic (Figure 1), with a mean of 10.8 bands per primer. This level of variation is much higher than that observed in Malawian Sorghum landraces (Nkongolo and Nsapato 2002). Nkongolo (2003) showed that about 80% of the scored bands were polymorphic in cowpea cultivars. In cowpea, about 54% polymorphism was found when applying six different primer combinations using AFLP technique (Fang et al. 2007).

The percentage of total polymorphic bands detected ranged

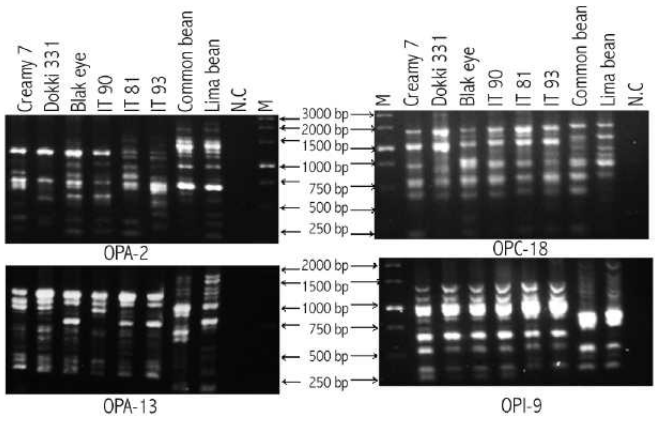

Fig. 1. RAPD profiles obtained for eight cultivars studied amplified with primers OPA-2, OPA-13, OPC-18, and OPI-9, N.C = Negative Control and M = 1000 bp ladder size marker.

Table 2. Similarity matrix calculated for cowpea and bean cultivars according to Jaccard's coefficient obtained from 72 RAPD fragments.

Cultivars	Creamy 7	Dokki 331	Black eye	IT 90 K 2840-2	IT 81 D-1064	IT 93 K 12904	Common bean	Lima bean
Creamy7	1.00							
Dokki 331	0.91	1.00						
Black eye	0.77	0.74	1.00					
IT 90 K 2840-2	0.79	0.81	0.92	1.00				
IT 81 D-1064	0.70	0.63	0.82	0.80	1.00			
IT 93 K 12904	0.65	0.63	0.77	0.74	0.89	1.00		
Common bean	0.18	0.15	0.21	0.19	0.25	0.22	1.00	
Lima bean	0.12	0.11	0.17	0.15	0.21	0.21	0.82	1.00

from 75-100% with an average of 90% (Table 2). Sarutayophat et al. (2007) used five primers and reported that polymorphic fragments percent ranged from 50-71.4% from yardlong bean and cowpea accessions. On the contrary, a low proportion of polymorphic bands 18.5% among 13 cowpea landraces was reported by Tosti and Negri (2002). The size of the amplified fragments ranged from approximately 200-2500 bp. Pooprompan et al. (1996) identified various varieties of yardlong bean by RAPD and reported that fragment sizes ranged from 500-2200 bp, while fragment sizes of 940-1100 bp were reported by Phansak et al. (2001). Also, Sarutayophat et al. (2007) obtained 225-1650 bp amplified bands in RAPD cowpea experiments. However, primers used by the three research groups were different from primers used in our experiment.

Cluster analysis of the cultivars based on RAPD analysis

RAPD similarity of Jaccard's (1908) coefficient matrix for six cowpea cultivars and two bean species was calculated and used for UPGMA cluster analysis. Similarity coefficient ranged from 0.11-0.92 between Dokki331 and Lima bean, and between Black eye and IT 90 K 2840-2, respectively (Table 2). The genetic distance values among accessions of cowpea varied from 0.09 to 0.59 (Nkongolo 2003). Sarutayophat et al. (2007) reported a similarity coefficient varying from 0.548-1.000 among cowpea cultivars.

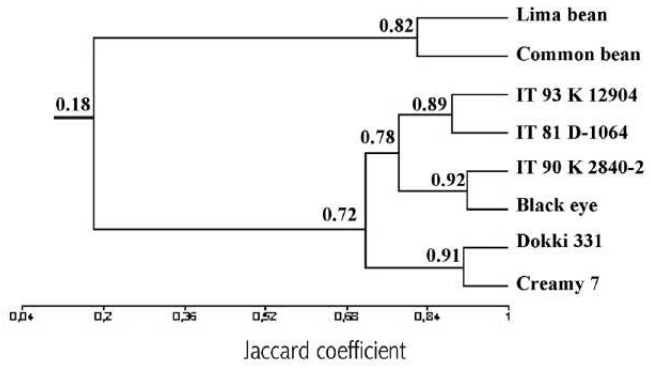

Fig. 2. Pairwise similarities between accessions were calculated using Jaccard's coefficient obtained from 72 RAPD fragments.

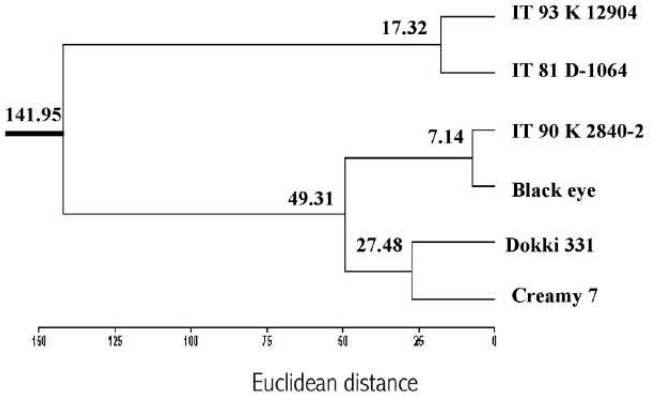

Fig. 3. Dendrogram based on UPGMA cluster analysis showing the genetic distances among cowpea cultivars.

The eight cultivars separated into two distinct clusters. The first cluster contains two resistant bean species (Lima bean and Common bean) with similarity coefficient 0.82 (Figure 2). The second cluster contains cowpea cultivars and it is subdivided into two sub-clusters, moderate tolerant and high tolerant cultivars grouped in first sub-cluster, and the second sub-cluster contains the two sensitive cultivars. The highest value of similarity coefficient (0.92) was observed between the moderate tolerant cultivars (Black eye and IT 90 K 2840-2), the similarities coefficient between the high tolerant (IT 93 K 12904 and IT 81 D-1064) and the sensitive cultivars (Dokki 331 and Creamy 7) were 0.89 and 0.91, respectively. It is worth noting that the results of RAPD fingerprint support the classification of the tested entries based on their genus and resistance to *C. maculatus* (F.) weevil (Obiadalla-Ali et al. 2007).

Cluster analysis based on resistance to weevil pest traits of cowpea

The dendrogram constructed on the basis of the genetic distances among cowpea cultivars was calculated based on the means of characters of resistance to *C. maculatus* (F.) that indicates the systematic relationships among cultivars studied (Obiadalla-Ali et al. 2007).

Table 3. Euclidean distance matrix of six cowpea cultivars using means of resistance characters to *Callosobruchus maculatus* (F.).

Cultivars	Creamy 7	Dokki 331	Black eye	IT 90 K 2840-2	IT 81 D-1064	IT 93 K 12904
Creamy7	00.00					
Dokki 331	27.48	00.00				
Black eye	61.68	35.47	00.00			
IT 90 K 2840-2	62.92	37.17	7.14	00.00		
IT 81 D-1064	181.25	155.40	123.26	124.07	00.00	
IT 93 K 12904	172.87	147.39	115.53	115.81	17.83	00.00

The Euclidean distance ranged from 7.14 between Black eye and IT 90 K 2840-2 cultivars to 181.25 between Creamy 7 and IT 81 D-1064 cultivars (Table 3). The range of Euclidean distance among the cultivars (7.14-181.25) was relatively wide. This result indicated that the amount of phenotypic variation among these cultivars was relatively high and reflects the genetic diversity of the genes controlling these characters. Bootstrap values on the dendrogram (Figure 3) indicated a high genetic variation pattern. The cultivars created two distinct clusters at high Euclidean distance of 141.95 between the two sensitive cultivars in the first cluster and the four other resistant cultivars that formed the second cluster (Figure 3). The second cluster created two sub-clusters, which were separated at 49.31 Euclidean distance (Table 3). The dendrogram also revealed that the morphological diversity was less than within all separated groups (Table 3). The results obtained based on the two dendrograms were similar. The agronomic characterization and RAPD based on dendrogram were somewhat similar, indicating that the agronomic characterizing information will continue to be useful to identify diverse germplasm in breeding programs of lupine and/or other species (Abd EL-Ghani et al. 2007). Pandey et al. (2008) showed that DNA markers are preferable to morphological ones because they relate variability directly at the genetic level and provide reliable and enormous data that permit a reproducible estimate of genetic diversity in the germplasm.

Correlation between the two distance matrices generated by mean of traits and RAPD marker was calculated. The correlation between Euclidean distance and RAPD distance was low (r = -0.80357, P = 0.0014). The low association between these traits and RAPDs markers was not surprising since the estimation of genetic relationship among different germplasm was based on different approaches. The lack of correlation between morphological traits and other genetic markers such as isozyme markers has been documented in cowpea and other crops (Doebley 1989; Vaillancourt et al. 1993). The correlation coefficient (r) between Euclidean distance and RAPD distance was - 0.40 (P = 0.11) in faba bean (Tanttawi et al. 2007). Schut et al. (1997) reported a correlation of -0.1 for AFLP and agronomic data in Barley varieties. RAPD markers are randomly distributed throughout the genome, but most regions of the genome (90%) are not expressed at the phenotypic level (Dahlberg 2000; Joyee et al. 1999; Williams et al. 1990). As a result, markers like RAPDs may accurately assay the degree of genetic change between two genomes, but they may not necessarily reflect the divergence in terms of changes in traits of agronomic importance. Pandey et al. (2008) showed that the comparison of Jaccard's similarity based on RAPD markers and average taxonomic distance based on quantitative characters was not significant (r = 0.0477) in Indian ash gourd.

Table 4. Organic and non-organic (heavy metals) component in coat seed of some cowpea and bean cultivars.

Susceptibility	Sensitive	Moderate tolerant	High tolerant	Resistant
Protein	1.294^{a}	1.146^{b}	1.019^{c}	0.492^{d}
Mg^{2+}	2082.6^{a}	2018.9^{a}	1910.2^{b}	1880.0^{b}
Ca^{2+}	3539.0^{d}	3791.5^{c}	5210.2^{b}	7173.2^{a}
Na^{+}	1421.6^{a}	1566.9^{a}	1378.6^{a}	1021.1^{b}
K^{+}	8282.3^{b}	495.8^{d}	5770.7^{c}	10203.7^{a}
Co^{2+}	2.227^{d}	5.251^{b}	2.877^{c}	8.198^{a}
Mn^{2+}	16.44^{a}	14.83^{b}	17.02^{a}	10.05^{c}
Fe^{2+}	450.13^{a}	367.13^{b}	378.87^{b}	97.67^{c}

Means within each rows followed by the same letter(s) are not significantly different at the 0.05 probability level.

Total Protein

The results in Table 4 clearly explain that protein concentration differed significantly in each group cultivar. Resistant bean species were the least in protein concentration (0.492 mg g^{-1}). While, sensitive cowpea cultivars were the greatest (1.294 mg/g). The most interesting result is that protein concentrations in the resistant bean cultivars were approximately half in comparison with both moderate tolerant and sensitive cowpea cultivars, indicating that the increase of protein concentration in seed coat may play a role towards susceptibility to weevil pest. Khokhar and Gupta (1974) revealed that high protein content was linked to susceptibility to the stored- product insect infection.

Magnesium (Mg^{2+})

Chemical analysis results (Table 4) clearly show that magnesium concentration differed significantly in resistant bean cultivars and sensitive cowpea cultivars. The magnesium concentrations were 1880.0 and 2082.6 ppm for resistant bean cultivars and sensitive cowpea cultivars, respectively, indicating that the presence of magnesium in seed coat may have a role in susceptibility to weevil pest.

Calcium (Ca^{2+})

Calcium concentration (Table 4) differed significantly in each group cultivars. Resistant bean cultivars had the greatest concentration calcium (7173.2 ppm), while sensitive cowpea cultivars had the least concentration calcium (3539.0 ppm). The most important result is that calcium concentrations in the resistant bean cultivars were approximately twice in comparison with both moderate tolerant and sensitive cowpea cultivars, indicating

that the presence of calcium in seed coat may have a major role in weevil pest tolerance/or resistance.

Sodium (Na^+)

The results of Sodium concentration analysis (Table 4) differed significantly in resistant bean cultivars and all group of cowpea cultivars. The concentration of sodium in seed coat was lower (1021.1 ppm) in the resistant bean cultivars than in all cowpea cultivars group (1566.9, 1421.6 and 1378.6 ppm) moderate tolerant, sensitive, and high tolerant cowpea cultivars, respectively.

Potassium (K^+)

Results in Table 4 show that potassium concentration differed significantly in each group cultivars. The concentration of potassium in seed coat was higher (10203.7 ppm) in the resistant bean cultivars than in all cowpea cultivars group (8282.3, 5770.7, and 495.8 ppm) sensitive, high tolerant, and moderate tolerant cowpea cultivars, respectively.

Cobalt (Co^{2+})

Resistant bean cultivars exhibited the greatest cobalt concentration (8.198 ppm, Table 4), while sensitive cowpea cultivars were the least in cobalt concentration (2.227 ppm). The Cobalt concentration was about four times higher in resistant bean than that in sensitive and high tolerant cowpea cultivars, which may play a major role in seed resistance to weevil.

Manganese (Mn^{2+})

The manganese concentration (Table 4) differed significantly in each group cultivars. The concentration of manganese in seed coat was lower (10.05 ppm) in the resistant bean cultivars than in all cowpea cultivars groups (17.02, 16.44 and 14.83 ppm), high tolerant, sensitive, and moderate tolerant cowpea cultivars, respectively.

Iron (Fe^{2+})

Resistant bean cultivars were the least in iron concentration (97.67 ppm, Table 4), while sensitive cowpea cultivars were the greatest (450.13 ppm). The most interesting result is that ferric ion in the resistant beans was about 25% less than that in the moderate tolerant, and high cultivars, indicating that iron concentration in seed coat may play a major role towards resistant weevil.

Gayan et al. (2006) showed that Na, K, Mg, and Ca were the major metal elements in cowpea seeds. These minerals were rich in the husk portion of the seed; therefore decrease in above properties may be due to formation of emergence holes in the husk by the C. maculatus larvae. In conclusion, the Ca, K, and Cu metals were higher in resistant been than those in cowpea cultivars. But the protein, Mn, and Fe metals were less in resistant been cultivars than those cowpea cultivars which may play a role in seed resistance to weevil.

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