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Genetic Variability in Protein and Amino Acid Composition in Leaves of some *Gongronema latifolia* Clones

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ABSTRACT

Analysis of leaves of *Gongronema latifolia* Benth. identified clone EBS 015 with 58.38% protein and nine essential amino acids. The clone was significantly different from other clones with respect to its protein content. The dominant amino acids identified in the clones used for study were glutamic acid (13.48 g/100 g protein), leucine (9.66 g/100 g protein) and aspartic acid (9.25 g/100 g protein); those in moderate amounts were arginine, lysine, alanine, valine, isoleucine, phenylalanine and glycine while those in low concentrations were histidine, methionine and cysteine. Even though significant differences existed in protein and amino acid concentrations in the clones, all of them, irrespective of their local area of collection had comparable levels in the quality of protein and amino acids. The availability of methionine and cysteine, which are pre-cursors of taurine in crop species, supports the wide use of the plant in the treatment of diverse ailments. A positive and significant correlation (r = 0.922: n =21) between lysine and serine and between essential and non-essential amino acids suggest complimentary relationship in the synthesis and availability of the amino acids in the species. Furthermore, the phenotypic and genotypic variances estimated indicate that there was a minor environmental influence on the expression of the clones suggesting higher genetic influence on the results obtained. The amino acid levels in the crop species have potential value in maintaining good health and for use in the manufacture of varying food supplements that will compare favorably with supplements from soybeans.

Keywords: dominant, environment, essential amino acids, food supplements, non-essential amino acids

INTRODUCTION

The composition of the human body depends to a large extent on what the person has consumed. Selective breeding of plants by man due to selection of the best plant for replanting has eliminated many natural and important plant foods from our diet perhaps because of taste preferences. In the recent times, there has been a shift towards evaluating the chemical composition and nutritive value of tropical plants including the traditionally known medicinal plants. Chickpea, cowpea, lentil, green pea, fluted pumpkin leaves and *Mucuna flagellipes* contain 24, 24.9, 26.1, 24.9, 22.4, and 24.9% protein, respectively (Glew *et al*. 1997; Akwaowo *et al*. 2000; Ajayi *et al*. 2006; Igbal *et al*. 2006). Okafor (2005) and Eleyinmi (2007) reported wide varying results of 62.6 and 27.7%, respectively for the protein content of *Gongronema latifolia.*

G. latifolia is a non-wood forest product commonly available in virgin forests of Nigeria and other sub-Saharan Africa (Nielsen 1965). The plant is of the Asclepiadaceae family and of West African origin (Nielsen 1965). It is a climber from a tuberous base and mostly grown on a small scale under shade near fences (Etukudo 2003). The plant can be propagated vegetatively by stem cuttings (Agbo and Obi 2006). Seeds developed from flowering clones are good sources of commercial propagation of the species (Agbo and Obi 2007) and have a high potential for genetic variability in the species. It is called *utazi* and *arokeke* in southeastern and Southwestern Nigeria, respectively, used as a leafy vegetable and is a good source of proteins, vitamins, iron and minerals (Okafor 2005). The leaves are eaten raw and in any quantity to treat diabetes and hypertension (Etukudo 2003). The plant is also useful in the treatment of cough, malaria, constipation, throat pain, mouth odour, catarrh/cold, typhoid and topical sexually transmitted diseases like gonorrhea (Agbo *et al*. 2005). Burkhill (1985) had earlier enumerated the use of the plant in new-born babies and children in Sierra Leone and Ghana. The stem extracts of the plant have been shown to contain five bioactive compounds namely, alkaloids; saponins, tannins, flavonoids, and glycosides (Gamaniel and Akah 1996).

Amino acids are the most important nutrients and the basic structural units of proteins. Richard Smayda (2002) suggested the use of nutritional and manual approaches as the best option in a successful preventive medicine programme. Amino acids have been shown to be very useful in diverse medical treatments in humans as well as precursors of other amino acids that are useful in the human body, like taurine.

Protein and amino acid composition of different clones of the crop species from southeastern Nigeria have not been studied in detail although protein contents of 62.6 and 27.2% have been reported (Okafor (2005) and Eleyinmi (2007), respectively). The need to select accessions (clones) rich in protein and amino acids based on wide varying results by Okafor (2005) and Eleyinmi (2007) at different places motivated this research. The objectives of the study were to determine the genetic variation in the biological value of the protein component and to quantify amino acid levels of different clones of *G. latifolia*.

MATERIALS AND METHODS

Stem cuttings of 46 different accessions (clones) of *G. latifolia* obtained from different locality forests of different states of south eastern Nigeria were established in Department of Crop Science Research Farm, University of Nigeria, Nsukka. The establishment was done following the method for vegetative propagation of the species as described by Agbo and Obi (2006). The plants were staked with bamboo of about 2 m high, weeded periodically and manured with poultry droppings at a rate of 10 tons/ha. Data were collected on the quantitative characters of the accessions (clones)

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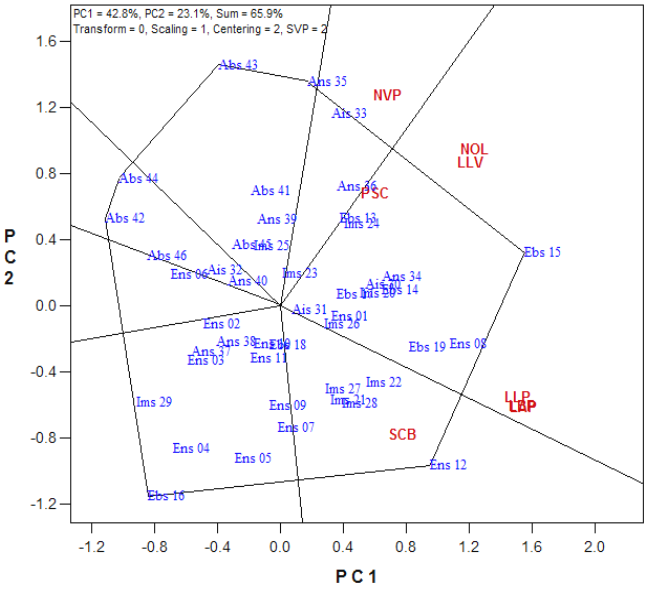

Fig. 1 GGE-biplot analysis of the 46 clones based on their vegetative data in 2004. NVP = Number of vines per plant; NOL = Number of leaves per plant; PSC = Percentage of surviving cuttings; LLV = length of longest vine per plant; LLP = Leaf length per plant; LAP = Leaf area per plant; SCB = Size of cordate base; Letters and numbers 01-46 represent the clones from different states of Southeastern Nigeria.

after three years of growth. Such data included: leaf length, breadth and area, number of vines and leaves/plant. The data collected were subjected to genotype + genotype × trait interaction (GGE) as put forth by Kang and Yan (2003). The GGE biplot analysis showed seven cluster groups of the 46 clones in the germplasm garden (**Fig. 1**). Accessions AIS 33, EBS 15 and IMS 28 were high in number of leaves and vines/plant. On the other hand, ANS 38, ENS 06, ABS 42 and ABS 43 were low in number of leaves and vines/plant. Seven accessions (clones) identified as contrasting in their number of vines and leaves and leaf area were selected each from a cluster (four clones at the vertex of the polygon and three clones within the polygons to increase variance) and used for the determination of protein and amino acid composition.

Amino acid determination

Amino acid composition of the dried leaves was determined according to the method of Spackman *et al.* (1958). An amino acid analyzer (model DNA-0209, Technicon Auto Analyser 1973) with the columns packed with TSM C-3 bed resin at 47°C (acid-neutral) and 63°C (basic) was used. The buffer pH ranged between 2.70 to 6.00 and the flow rate was 0.45 ml min^{-1}.

Protein determination

The protein content of the dried leaves of the clones was determined following the official methods of AOAC (1980).

Data were collected in three replicates per accession(clone) giving a total of 21 samples and were subjected to analysis of variance following the procedures outlined for Completely randomized design (CRD).The data were analysed using computer software, Genstat Discovery edition 2.0 (2005). Least significant difference as outlined by Obi (2002) was used to separate the means. Principal component analysis (PCA) was conducted on the amino acid data of the seven clones using Genstat DE 2.0 to identify discriminant amino acids for differentiating the clones. Also genetic and phenotypic variances of the amino acids were estimated.

RESULTS

Analysis of the dried leaves showed high levels of protein for most of the clones (**Table 1**). Clone EBS 015 with protein level of 58.38 g/100 g dry sample, is significantly higher than other Clones including those of fluted pumpkin

Table 1 Protein, methionine and lysine content of the leaves of *Gongronema latifolia* clones and some plant food stuff (values given for 100 g of edible portion).

G. latifolia clones	Protein (g/100 g of dry sample)	Methionine (g/100 g)	Cysteine (g/100 g)
ENS 006	41.30	1.30	1.12
EBS 015	58.38	1.36	1.23
IMS 028	21.60	1.04	1.05
AIS 033	25.29	0.95	0.92
ANS 038	21.63	1.20	1.20
ABS 042	30.55	1.30	0.77
ABS 043	34.67	0.85	0.79
LSD $_{(P< 0.05)}$	7.14	0.15	0.08
Other plant food stuff			
Wheat a	12.20	0.94	0.159
Maize a	9.50	0.12	0.097
Beans (white) a	22.10	0.234	0.188
Soy (grains) a	33.70	0.58	0.59
Peanuts (fresh) a	23.60	0.338	0.366
Spinach (leaves) a	2.50	0.43	0.036
Fish meal b	-	3.12	0.036
Soybean meal c	-	1.22	1.7
Sesbania aculeata c	-	1.03	0.70
Mucuna seeds b	-	0.83	1.13

a from McCance and Widdowson (1991), FAO (1970) and Souci *et al.* (1994)
b from Siddhuraju and Becker (2001b)
c from Hossain and Becker (2001)
ENS 006=Clone number 6 from Enugu State
EBS 015=Clone number 15 from Ebonyi State
IMS 028=Clone number 28 from Imo State
AIS 033=Clone number 33 from Akwa-Ibom State
ANS 038=Clone number 38 from Anambra State
ABS 042=Clone number 42 from Abia State
ABS 043=Clone number 43 from Abia State

leaves and soybeans. The amino acid methionine and cysteine were significantly lower in clone ABS 043 and significantly higher in clone EBS 015 that also had higher leaf sizes. In the same vein, clone EBS 015 had significantly higher levels of arginine, phenylalanine and isoleucine (**Tables 1, 2**). The methionine level in clones ENS 006, EBS 015 and ABS 042 compares favorably with those reported for soybean meal, *Sesbania aculeata* and *Mucuna* seeds (**Table 1**). All the clones contain nine essential amino acids at significantly varying levels (**Table 2**). The amino acids lysine, and methionine that are always found lacking in green vegetables and fruits are present in all the clones evaluated. However, clones ANS 038 and EBS 015 have higher levels of lysine and methionine respectively, when compared to other ones. Leucine was the most dominant of all the essential amino acids followed by arginine while methionine had the least level in all the clones. In the same vein, glutamic acid was the most dominant of all the non-essential amino acids and was followed by aspartic acid while cysteine was the least in all the clones.

The correlation matrix of the amino acids identified in the crop species are shown in **Table 3**. Cysteine had the highest, positive and significant correlation with lysine and serine (r =0.922 and 0.909: n =21, respectively). On the other hand, glutamic acid had the least correlation with leucine (r = -0.006: n = 21). Essential amino acid leucine had positive and significant relationship with threonine (r = 0.819: n = 21) while tyrosine and alanine (both non-essential amino acids) had positive and significant relationship (r = 0.773: n =21). Also, high relationship existed between an essential and non-essential amino acids such as alanine and isoleucine (r = 0.866: n = 21).

The estimates of variance component (genetic and phenotypic) and heritability of the amino acids in the different clones are presented in (**Table 4**). In all cases, the phenotypic variance was slightly higher than the genotypic variance. Glutamic acid, aspartic acid and leucine had higher levels of genotypic variance among the clones. Broad sense heritability estimate was high in all amino acids especially in aspartic acid, glutamic acid, leucine and

Table 2 Essential and non-essential amino acid content of the leaves of *G. latifolia* clones (values given for g/100 g protein).

Clones	Essential amino acids contents (g/100 g protein)								
	Isoleucine	**Leucine**	**Lysine**	**Methionine**	**Phenylalanine**	**Threonine**	**Valine**	**Arginine**	**Histidine**
ENS 006	4.34	9.66	5.05	1.30	4.19	3.78	4.65	5.26	2.32
EBS 015	4.56	8.21	4.85	1.36	4.35	3.51	4.51	5.53	2.62
IMS 028	3.20	7.92	4.67	1.04	3.60	3.00	3.96	4.66	1.79
AIS 033	3.59	9.49	4.05	0.95	3.96	4.04	4.39	4.44	3.18
ANS 038	4.00	7.30	5.25	1.20	3.90	2.97	4.06	4.98	2.34
ABS 042	3.49	8.30	3.80	1.30	4.26	2.87	4.10	4.98	2.53
ABS 043	3.67	8.05	3.88	0.85	4.11	2.78	4.24	4.31	1.89
$LSD_{(p<0.05)}$	0.17	0.14	0.10	0.15	0.25	0.13	0.25	0.06	0.13
Clones	**Non-essential amino acids contents (g/100 g protein)**								
	Alanine	**Aspartic acid**	**Cysteine**	**Glutamic acid**	**Glycine**	**Proline**	**Tyrosine**	**Serine**	
ENS 006	4.70	8.93	1.12	13.48	3.98	3.66	3.33	3.47	
EBS 015	4.54	8.51	1.23	11.89	4.31	4.10	3.14	4.21	
IMS 028	3.26	8.93	1.05	12.27	3.38	3.96	2.06	3.38	
AIS 033	3.67	8.99	0.92	10.16	3.46	3.80	2.98	3.06	
ANS 038	3.90	7.92	1.20	12.16	4.10	3.31	3.02	3.71	
ABS 042	3.52	9.25	0.77	10.75	2.87	2.65	2.69	2.82	
ABS 043	4.21	7.04	0.79	11.36	3.81	2.95	2.69	2.92	
$LSD_{(p<0.05)}$	0.22	0.07	0.08	0.12	0.21	0.15	0.06	0.05	

ENS 006=Clone number 6 from Enugu State
EBS 015=Clone number 15 from Ebonyi State
IMS 028=Clone number 28 from Imo State
AIS 033=Clone number 33 from Akwa-Ibom State
ANS 038=Clone number 38 from Anambra State
ABS 042=Clone number 42 from Abia State
ABS 043=Clone number 43 from Abia State

Table 3 Correlation matrix of the amino acid profile of the clones used for study.

	Ala	**Arg.**	**Asp**	**Cys**	**Glu**	**Gly**	**His**	**Iso**	**Leu**	**Lys**	**Meth**	**Phe**	**Pro**	**Ser**	**Thr**	**Try**	**Val**
Ala	-																
Arg	0.516*	-															
Asp	-0.307	0.347	-														
Cys	0.387	0.661**	0.065	-													
Glu	0.501*	0.519*	-0.044	0.619**	-												
Gly	0.716**	0.436*	-0.485*	0.706**	0.527*	-											
His	0.054	0.172	0.440*	0.020	-0.524*	-0.036	-										
Iso	0.866**	0.753**	-0.120	0.644**	0.478*	0.789**	0.242	-									
Leu	0.306	0.027	0.484*	-0.116	-0.006	-0.108	0.503*	0.187	-								
Lys	0.376	0.617**	0.016	0.922**	0.786**	0.686**	0.134	0.563**	-0.158	-							
Meth	0.299	0.865**	0.440*	0.498*	0.372	0.149	0.199	0.565**	0.033	0.453*	-						
Phe	0.615**	0.453*	-0.018	-0.055	-0.078	0.156	0.291	0.620**	0.269	-0.179	0.432*	-					
Pro	0.610**	0.299	0.274	0688**	0.307	0.461	0.170	0.319	0.242	0.533*	0.126	0.251	-				
Ser	0.515*	0.757**	-0.038	0.909**	0.499*	0.798**	0.094	0.762**	-0.191	0.803**	0.518*	0.159	0.644**	-			
Thr	0.338	0.225	0.461*	0.315	0.099	0.234	0.712**	0.395	0.819**	0.200	0.139	0.118	0.608**	0.261	-		
Tyr	0.773**	0.530*	-0.024	0.373	0.202	0.573**	0.563**	0.846**	0.448*	0.347	0.430*	0.614**	0.091	0.441*	0.580**	-	
Val	0.721**	0.391	0.094	0.245	0.232	0.463*	0.390	0.693**	0.666*	0.153	0.217	0.515*	0.353	0.314	0.660**	0.729**	-

* = significant at 5%; ** = highly significant at 1%; Asp = Aspartic acid, Arg = Argine, Ala = Alamine, Cys = Cysteine, Glu = Glutamic acid, Gly = Glycine, His = Histidine, Iso = Isoleucine, Leu = Leucine, Lys = Lysine, Meth = Methionine, Pro = Proline, Phe = Phenylalanine, Ser = Serine, Thr = Threonine, Thr = Tyrosine, Val = Valine

serine.

The three component axes retained in the principal component analysis (PCA) showed distinct amino acid concentration in the clones (**Table 5**). PRIN 1 which explained about 43% of the total variability in amino acid composition evaluated showed that isoleucine and serine had the highest eigen vector values on the axis. Similarly, leucine and histidine weighted highest on PRIN 2 (which accounted for about 21% variability). PRIN 3 revealed that aspartic acid and proline could explain about 14% variability in amino acid composition of *G. latifolia* species.

DISCUSSION

Gongronema latifolia has been harvested from forests in West Africa and other sub-Saharan Africa as a leaf vegetable, and the nutritional properties of the leaves have been scantly reported (Okafor 2005; Eleyenmi 2007). The results of the present investigation show that the leaves of the plant are an excellent source of high quality protein as it contains nine essential amino acids in high proportions. The results indicated high genetic variability in levels of protein and amino acids in the crop species. There is then need for further germplasm collection and analysis for selection of high protein and amino acid clones. Any selected clone could thus be perpetuated without fear of segregation. The proteins and amino acids of the species are higher and richer when compared to other leafy vegetables like fluted pumpkin leaves (22.4% protein content), spinach leaves (2.5% protein content) and grain legumes like white beans, peanuts and soybeans (Akwaowo *et al*. 2000; Ajayi *et al*. 2006; Igbal *et al*. 2006). They also compare favorably with the WHO (1985) recommendation on dietary allowance (RDA) for children and adults as well as FAO (1973) recommendations for amino acids requirements. The rich amino acid in the results of the present study which is an indicator of the quality of protein in the crop species could be exploited for use in the manufacture of food supplements that will compare favorably with those from soybeans. The presence of methionine and cysteine which are pre-cursors of taurine in the crop species supports the wide usage of the plant in treatment of diverse ailments like diabetes mellitus, hypertension, typhoid, malaria and many others as had been reported by (Burkhill 1985; Etukudo 2003; Okafor 2005; Agbo *et al*. 2005). Richard Smayda (2002) had earlier enumerated the roles of the amino acid taurine in the control of

Table 4 Estimates of variance components and heritability of the amino acid composition of the different *G. latifolia* clones.

Amino acid	δ^2_e	δ^2_P	δ^2_g	PVC (%)	GCV (%)	H^2_{bs} (%)
Asp	.0015	.6076	.6061	9.155	9.147	99.8
Arg	.0020	.1916	.1936	9.016	8.969	98.9
Ala	.0015	.2969	.2811	13.791	13.351	94.6
Cys	.0018	.0365	.0347	18.889	18.417	95.0
Glu	.0043	1.1906	1.1862	9.309	9.292	99.6
Gly	.0136	.2543	.2407	13.625	13.256	94.6
His	.0052	.2227	.2171	19.819	19.569	97.4
Iso	.0091	.2422	.2331	12.829	12.586	96.2
Leu	.0061	.7344	.7283	10.179	10.136	91.1
Lys	.0043	.3516	.3473	13.156	13.075	98.7
Meth	.0067	.0433	.0366	18.205	16.737	84.5
Phe	.0201	.0784	.0583	6.908	5.957	74.3
Pro	.0072	.2967	.2888	15.589	15.398	97.5
Ser	.0007	.2401	.2394	14.614	14.592	99.7
Thr	.0054	.2490	.2436	14.627	14.585	97.8
Tyr	.0010	.1731	.1721	14.627	14.585	94.4
Val	.0200	.0777	.0577	6.523	56.21	74.2

δ^2_e = Error variance, δ^2_P = Phenotypic variance, δ^2_g = Genotypic variance, PCV = phenotypic coefficient variance, GCV = genotypic coefficient variance; H^2_{bs} = heritability in broad sense, Asp = Aspartic acid, Arg = Argine, Ala = Alamine, Cys = Cysteine, Glu = Glutamic acid, Gly = Glycine, His = Histidine, Iso = Isoleucine, Leu = Leucine, Lys = Lysine, Meth = Methionine, Pro = Proline, Phe = Phenylalanine, Ser = Serine, Thr = Threonine, Thr = Tyrosine, Val = Valine

Table 5 Eigen vector values for principal components of the amino acid composition of the different *G. latifolia* clones.

Amino acid	PRIN 1	PRIN 2	PRIN 3
Asp	-0.28958	-0.04528	0.33843
Arg	-0.29862	0.04484	-0.05719
Ala	-0.02923	-0.24415	-0.47712
Cys	-0.29418	0.22808	-0.22288
Glu	-0.21538	0.28093	-0.02673
Gly	-0.28647	0.18942	0.16069
His	-0.09709	-0.40945	-0.14199
Iso	-0.34748	-0.02008	0.19665
Leu	-0.09706	-0.43195	-0.11172
Lys	-0.26917	0.28856	-0.19257
Meth	-0.22505	-0.001541	-0.08672
Phe	-0.15459	-0.23633	0.39576
Pro	-0.19960	0.04422	-0.41251
Ser	0.31183	0.19991	-0.09453
Thr	-0.19989	-0.33856	-0.26403
Tyr	-0.28873	-0.21674	0.20776
Val	-0.25324	-0.26876	0.11685

Asp = Aspartic acid; Arg = Arginine, Ala = Alanine, Cys = Cysteine, Glu = Glutamic acid, Gly = Glycine, His = Histidine, Iso = Isoleucine, Leu = Leucine, Lys = Lysine, Meth = Methionine, Pro = Proline, Phe = Phenylalanine, Ser = Serine, Thr = Threonine, Tyr = Tyrosine, Val = Valine

most human ailments.

The significant differences in the clones in their composition of proteins and amino acids is an indication of the genetic contributions of the different clones in the synthesis of amino acids and proteins as the clones have grown in the same environment for three years. Clones EBS 015 and ENS 006 with higher levels of both the essential and non-essential amino acids are important clones for production and breeding purposes. The dominant amino acids are glutamic acid, leucine and aspartic acid. The result agrees with earlier report by Eleyinmi (2007) who identified leucine, valine and phenylalanine as the dominant amino acids in his study. The occurrence of essential amino acid leucine as a dominating one in the two studies is a strong indicator of the high quality of protein available in the crop species.

The positive and high relationship that exists between the essential and non-essential amino acids and within essential or non-essential themselves indicated complimentary relationship. It suggests that every clone of the crop species will have a potential of a mixture of high level of both the essential and non-essential amino acids. This is also expressed further by the high level of glutamic and aspartic amino acids in the species which Herbone (1973) showed to represent a storage form of nitrogen in plants. The abundance of both storage form of nitrogen in the crop species indicates the potential of the crop species to synthesize and contain other amino acids. Significant positive correlation between glutamic acid and lysine (r = 0.786: n = 21) could further explain the potentiality for high amino acid synthesis in the species. Similarly, some closely related amino acids may be synthesized by similar genetic code in the plant. For example, glycine and alanine with high correlation (r = 0.716: n = 21) are both classified as neutral amino acids. Also, phenylalanine and tyrosine with high correlation (r = 0.614: n = 21) are both classified as aromatic amino acids.

The slightly higher phenotypic variance when compared with genotypic variance in the amino acids indicated the relatively low impact of environmental factors to the level of different amino acids in the clones. Similarly, the higher level of genotypic variance among the clones with respect to glutamic acid, leucine and aspartic acid showed that genetic effects of the clones had higher influence on the composition of the three amino acids when compared to their effect on others. The results of the PCA revealed that the essential amino acids isoleucine, leucine, histidine and non-essential amino acids serine, aspartic acid and proline could be used as discriminant amino acids in differentiating clonal composition and for selection for breeding for higher qualities in the species. The amino acids level in the crop species have potential value in maintenance of good health and for use in the manufacture of different food supplements that will have higher amino acid quality than ones from soybeans.

CONCLUSION

The study identified nine essential amino acids in the crop species. The amino acids are highly concentrated in the species when compared to soybeans and other vegetables. The dominant amino acids were glutamic acid (13.48 g/100 g protein in clone ENS 006), leucine (9.66 g/100 g protein in clone ENS 006) and aspartic acid (9.25 g/100 g protein in clone ABS 042). The moderate ones were arginine, lysine, alanine, valine, isoleucine, phenylalanine and glycine while the low concentrated ones are histidine, methionine and cysteine.

The positive and significant correlation of (r = 0.922: n = 21) between lysine and serine and similar ones between essential amino acids suggest complimentary relationship. This implies that every clone of the crop species will have a potential of a mixture of high level of both the essential and non-essential amino acids. Furthermore, the phenotypic and genotypic variances estimated indicated that there was a minor environmental influence on the genetic expression of the clones and that there was room for selection of clones with higher levels of amino acids for consumption and improvement purposes. The eigen vector analysis suggest that some essential and non-essential amino acids could be used to classify *G. latifolia* species into quality groups.

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