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EVALUATING TWELVE WORLD ACCESSIONS OF PEARL MILLET FOR THEIR AMINO ACID PROFILES AND AGROECONOMIC TRAITS

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Twelve pearl millet accessions representing different regions of the world were studied for their amino acid profiles in the leaf samples of 10 day old seedlings using an automatic amino acid analyzer. In most of the accessions, proline, tryptophan and serine were more prominent. The variation for tryptophan was very high ranging from as low as 1 μ mole to as high as 39 μ moles. Irrespective of the origin and source, total amino acids ranged between 50 to 90 μ moles in the early maturing ones as compared to the 50 to 135 μ moles in the late ones reflecting the accumulation of more amino acids with a delay in flowering period. When the twelve accessions were categorised into three height groups (220,260 and 290 cm), glutamine was higher in the very tall accessions and glycine was confined to the talls around 240 cm. The distribution of lysine, tryptophan and anginine was independent of plant height giving a scope for their improvement in plants of all height groups; however, contents of alanine and tryptophan decrease with increase in grain size. Insignificant correlations were recorded with panicle characters. Some of the accessions are rich as regard to the proportion of essential amino acids in the total and prove more useful in quality improvement of the green fodder yield in breeding programmes. Positive correlations of essential acids like leucine with morphological traits suggest that selection of those traits indirectly improves the contents of these amino acids. Although there was considerable variation in the amino acid patterns, marked differences to differentiate the accessions were very few, possibly be due to events like multiple domestications and post-domesticational differentiations.

Keywords : Amino acid profiles; Pearl millet; World accessions.

Introduction

Pearl millet is an important cereal crop that comes next to *Sorghum* in area of cultivation and consumption and is often a food crop for people hardpressed for their susteinance. Earliest literature reveals that pearl millet had occurred in the semi-arid tropics of Africa before 1100 A.D. and has reached India via sea and land from western Africa and Iran[1]. The huge morphological diversity in western Africa, south of Sahara desert[2,3] and the occurrence of wild progenitor in this region suggested its domestication 3000 years ago along the lake edges of Sahara[4]. The various pearl millet accessions of this crop form good sources of disease resistance[5] and provide genes for introgression into cultivars. Over grazing and destruction habitats are threatening the wild and weedy forms demanding the need for their collection, conservation and further characterization. In fact ICRISAT has assembled as many as 671 accessions from all over the world[6] and have stressed the need for proper characterization and systematic evaluation over a wide range of agro-climatic conditions. One reason is that some of the accessions possessing other desirable traits may often be either very tall or very late or less superior qualitatively at one location might prove more useful under other conditions[6]. Such a situation amply indicates the need for multi-location evaluation for various important morphological and biochemical traits. The outcome of such studies reflect the relative performance of the desired accessions and thus aid in an easy management in breeding programmes. It is in this context, the present investigation is aimed at evaluation of twelve accessions of this crop from different regions of the world for their amino acid profiles and to further estimate the degree and direction of correlations of these with other useful agroeconomic traits; the data has also been used to focus on the concepts about the origin of this crop and its further distribution to other regions of the world.

Materials and Methods

All the twelve pearl millet accessions studied here were obtained from ICRISAT. Leaf samples were collected from 10 day old seedlings of these accessions and free amino acids were extracted from the fresh leaves using 70% ethanol as the solvent. The samples were purified following the procedure used earlier in our laboratory[7]. Each sample was finally dissolved in a sample buffer (acetate buffer pH 2.2) and 0.5 ml of it was injected into an LKB 4101 automatic amino acid analyzer. The quantities of individual amino acids were determined by comparing the peak area of the amino acid in the sample with the peak area of the amino

acid in the standard mixture by using the formula.

C/A = Cx/Ax

Where A = Peak of the amino acid in the standard mixture.

C = its concentration (per 0.5 ml)

Ax = Peak area of the corresponding amino acid in each sample

Cx = Its amount in the given sample.

The aminograms of two of the accessions were presented in Figures 1A and 1B.

Observations

The quantities of individual amino acids in each accession (as obtained from their corresponding aminograms) were presented in the table 1 A and 1B. Higher quantities were recorded for IP 9135 from India and IP 9357 from Ghana followed by other like IP 9833 etc. Least quantities were also seen in the accessions from the same location Ghana as in IP 9297 and IP 9401. In most of the accessions, the amino acids proline, tryptophan and serine were more prominent; however, it was altogether unseen in one accession of Ghana (IP 9401) while higher in two of the accessions from the same region. The variation for tryptophan was very high ranging from as low as 1 μ mole to as high as 39μ moles.

A closer observation of the contents of the three amino acids cysteine, valine and methionine that were eluted successively on the aminogram, the first two were absent in two accessions, the next two were absent in two more accessions while cysteine and methionine were unseen in four of the accessions. However, only in one accession (IP 9018), all the three were absent. γ-aminobutyric acid, an unusual amino acid of the profile, was missing only in IP 8947 while its quantity was variable to different extents in all other accessions. Of the nineteen amino acids eluted on the aminogram, isoleucine and leucine elute as one pair, tyrosine and phenyl alanine as a second pair while histidine and tysine being eluted as the third pair apart from the ammonia peak as a buffer artefact. Considering the first pair, leucine was 1.5 to 2 times higher the content of isoleucine while the differences in the contents of the second pair were almost negligeable. As regards to the third pair, histidine was lesser than lysine in two, greater in some and equal to lysine in others indicating the impact of location on this pair of amino acids.

Among the four accessions from Ghana that have been studied here, three are from commercial market of that region; yet they differed in plant height, seed weight and maturity that is very much reflected in their amino acid profiles also. For instance, IP 9566 and IP 9357 were both taller (270 cm) but were late to very late in days to flowering; proline was 60 times higher in the latter while glycine, valine and tyrosine were confined only to IP 9357. Among these four stocks, flowering period and plant height and to a limited extent seed size appeared to correlate with quantities of aspartic acid, alanine and leucine. A similar conclusion can also be drawn from a comparison of the two accessions of Togo (IP 8947 and IP 9297) either alone or in combination with those from Ghana. Irrespective of the origin and source, total free amino acids ranged between 50 to 90 μ moles in the early maturing ones as compared to the 50 to 135 μ moles in the late ones reflecting the accumulation of more amino acids with a delay in flowering period. In addition alanine was lesser in early ones while tryphophan was higher in the late ones.

When these twelve accessions were categorized under three height groups (220, 260 and 290 cm), glutamine was higher in the very tall accessions and glycine was confined mostly to the talls around 240 cm. Alanine was positively related to plant height in most of the accessions. Similarly histidine was higher in plant height between 260 to 290 cm. The distribution of lysine, tryptophan and arginine was independent of plant height giving a scope for their improvement in plants of all height groups including the dwarfs. Contents of both alanine and tryptophan decrease with increase in grain size. Insignificant correlations were recorded between amino acids and panicle characters like length and width.

The total essential amino acids ranged from about 17 μ moles to 34 μ moles with their values nearer to the mean (29 μ moles) in most of the accessions (see tables); yet their proportion in the total free amino acids is highly variable. Thus accessions with more or less similar quantities of the essential acids (IP 9566 and IP 8947) may differ in their proportion in the total. In fact it was as much as 50% in IP 9566 and was more superior qualitatively compared to accessions like IP 9795 and IP 9357. At the same time, those with very high amounts of total amino acids were infact deficient in one or the other essential amino acids or with traces, if at all they were present. All these observations indicate the need for giving a priority to the contents of the essential amino acids in choosing the accessions, especially in the improvement of the green fodder yield.

Discussion

Even though only three of the accessions investigated here, come under the category of early flowering, the positive correlation of this trait on amino acids like aspartic acid, alanine, leucine and to some extent on tryptophan levels suggests that selection for this trait indirectly improves the contents of these amino acids. Interestingly, the leucine being an essential amino acid paves way for the quality improvement as well. All the twelve accessions appear to have undergone considerable domestication in their local regions after migration followed by frequent introgressions from wild populations. Inspite of

Table IA. Quantities of free amino acids in the leaf samples (μ moles/gram leaf material) in six Pearl millet accessions.

* essential amino acids.

Accession number	IP 8947	IP 9297	IP 9372	IP 9566	IP 9401	IP 9357
Origin	Togo	Togo	Ghana	Ghana	Ghana	Ghana
Source	Institute	Institute	CM	CM	Farmer's Field	CM
Plant Height(cm)	230	240	220	270	220	270
PanicleLength(cm)	26	24	15	20	17	20
Panicle Width(mm)	29	23	30	17	35	26
Maturity	Early	late	early	late	early	late
1000 Grain Weight(gm)	14.1	11.8	13	7.9	15.6	12.2
Amino acid						
Asp	1.018	4.545	5.454	1.515	4.242	2.045
Thr*	5.303	4.91	1.272	2.454	2.272	3.606
Ser	3.904	8.773	4.761	6.845	5.714	8.214
Glu	—	2.142	1.607	1.714	1.821	3.571
Pro	18.75	2.41	38.101	1.667	—	67.083
Gly	3.515	0.046	0.031	—	—	0.046
Ala	2.142	5.357	1.392	4.821	1.785	4.714
Cys*	2.5	1.25	—	1.667	4.167	—
Val*	3.36	1.1	—	—	—	2
Met*	1.5	0.025	0.025	—	—	—
Iso*	7.083	3.402	6.667	2.778	1.112	2.223
Leu*	2.545	1.363	1.09	5.303	1.818	3.334
Tyr	1.875	2.5	2.812	—	—	1.437
Phe*	2.734	3.515	6.562	0.031	—	2.578
γ-amino	—	0.047	1.214	1.667	6.071	2.083
His*	0.075	0.06	1.515	6.362	6.06	1.939
Lys*	2.083	1.25	7.859	8.752	6.668	2.857
NH-3	8.684	2.807	1.381	3.421	7.894	3.421
Trp	11.112	5	1.112	8.334	1	4.5
Arg*	2.812	4.167	5.625	9.375	5.625	3.645
Total of all	80.995	54.669	88.48	66.707	56.249	119.296
Total essential Amino Acids	29.995	21.042	30.609	36.723	27.722	22.162
Their percent in Total	39	38.5	34.6	55.1	49.3	18.6

Table 1B. Quantities of free amino acids in the leaf samples (μ moles/gram leaf material) in the following six accessions. *** essential amino acids.**

Accession number	IP 9135	IP 9018	IP 9666	IP 9833	IP 9795	IP 9584
Origin	India	Mali	Nigeria	Tanzania	USA	Yemen, Republic of
Source	farmer'sfield	Institute	Institute	farmer's field	Institute	Institute
Plant Height(cm)	280	230	290	230	260	265
PanicleLength(cm)	15	20	26	20	32	25
Panicle Width(mm)	22	22	18	28	26	28
Maturity	late	late	late	late	late	Late
1000 Grain Weight(gm)	6.1	8.4	5	8	9.1	7
Amino acid						
Asp	2.272	7.575	9.91	1.606	—	7.878
Thr*	4.01	2.181	1.325	3.727	5.454	4.667
Ser	6.845	6.43	4.93	8.053	8.511	6.16
Glu	6.428	1.607	6.428	1.071	—	—
Pro	57.41	34.91	9.91	2.334	22.41	22.874
Gly	0.093	—	1	0.046	4.687	5.468
Ala	7.232	8.035	6.785	2.375	2.5	2.375
Cys*	—	—	3.334	—	—	—
Val*	5.6	—	2.2	—	0.06	0.06
Met*	—	—	4.5	0.025	—	—
Iso*	2.334	4.583	1.722	1.913	9.722	8.75
Leu*	4.545	8.181	2.212	1.545	1.568	1.5
Tyr	1.875	0.093	1.054	3.75	2.812	2.812
Phe*	3.125	0.093	1.335	3.75	2.812	2.812
γ-amino	1.75	7.857	7.5	1.428	0.089	1.071
His*	3.94	6.818	8.636	1.818	1.318	1.6
Lys*	2.393	6.43	3.631	1.334	4.286	5.417
NH-3	7.368	1.052	1.754	3.315	2.013	2.456
Trp	11.916	1.667	33.344	33.344	33.344	38.89
Arg*	4.687	8.334	1.718	3.552	9.166	9.166
Total of all	133.823	105.846	113.227	74.986	110.752	123.956
Total essential Amino Acids	30.634	36.62	30.613	17.664	34.386	33.972
Their Percent in Total	22.9	34.6	27.00	23.5	31	27.4

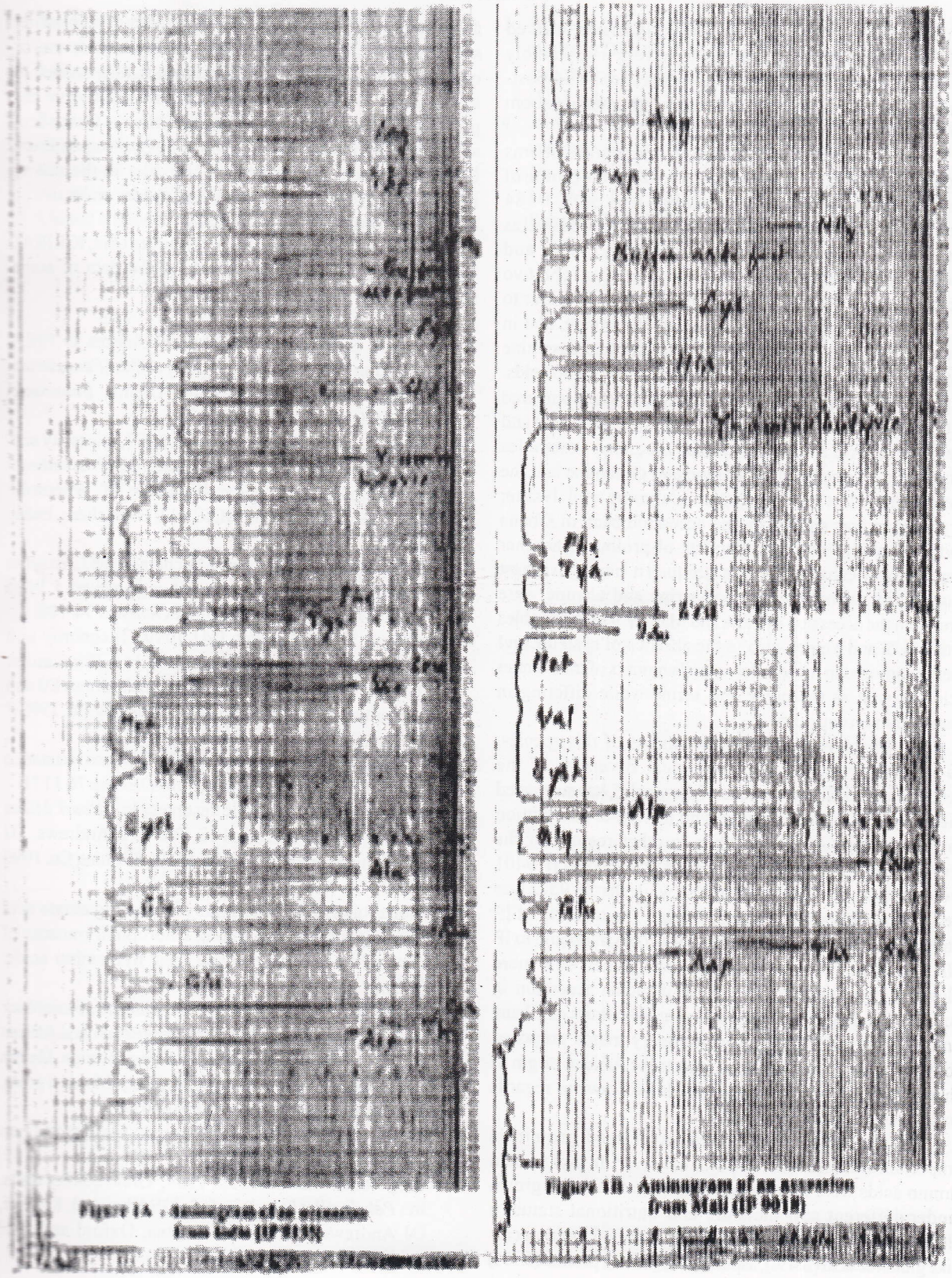

Figure 1B : Aminogram of an accession from Mali (IP 9018)

considerable variation in the amino acid patterns, marked differences among the accessions were very few, possibly be due to events like multiple domestications and post-domesticational differentiation through introgressions from wild species[8].

It is for this reason probably, the profile patterns of the different regions are comparable. Thus the profile of the US accession was much closer to that of IP 9584 (Yeman) than of IP 9666 from Nigeria; yet of total as well as contents of amino acids like tryptophan, lysine and phenylalanine reflect resemblance to the latter. The two accessions IP 9795 and IP 9584 were much more closer to IP 9833 from Tanzania in the absence of cysteine, and in equal quantities of leucine, serine, histidine and threonine but greatly differ in proline, glycine and other amino acids. Nigerian accession has a very different profile as compared to that of Tanzania, but resembles Mali in aspartic acid and γ-amino butyric acid. The Indian accession resembles Nigerian's on one hand, in alanine and glutamine and the Tanzanian's in threonine, phenylalanine and lysine; Tryptophan was similar in one accession each of Ghana and Togo with very high amounts of proline in another accession of Ghana but was unique in having highest quantity of valine. Quantity of serine and alanine were similar in the accession of India and Mali. IP 9135 resembles that of US and Yemen also in the absence of cysteine and methionine and in more or less equal amounts of total amino acids as well as threonine and serine while differing in many other amino acids.

Moreover, evolutionary studies of this species, as evident from the morphological diversity of the accessions in different regions of the world[4], has suggested the African origin of this crop with its further distribution to the northern Africa and then to other regions of the world. Accordingly the quantities of IP 9566 and IP 9401 were comparable to IP 9018 of Mali while that IP 9372 was very much similar to IP 9297 of Togo. The quantity of IP 9666 from Nigeria was closer to IP 9357 of Ghana and to IP 9018 of Mali. The patterns of USA and Yemen were more closer to one another and to that of Indian accession in some amino acids but highly different from those of Ghana and Nigeria reflecting the evolutionary trends proposed for pearl millet[6,9] and further supports the African origin of this crop although no evidence could be drawn as regards to its secondary origin in India.

Amino acid composition of seed protein is important from nutritional point of view[10]. In fact protein amino acids were determined in different varieties grown under different soil moisture and nutritional status in different locations[11-14]. Several non-essential amino acids like glutamic acid (8.4%), aspartic acid (8.9%), proline (6.7%) and alanine (8.4%) are the major constituents of pearl millet seed proteins. Although proline is one of the major component among free amino acid profiles, they are rich in amino acids like tryptophan and arginine. Moreover, the patterns of amino acids were quite different in the leaf samples as compared to seed proteins suggesting different lines of approach to be followed in breeding for the quality improvement of grain yield and green fodder yield.

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