Electronic Journal of Plant Breeding, 9(4): 1484-1496 (Dec 2018)
ISSN 0975-928X
DOI:10.5958/0975-928X.2018.00184.9

Research Article

Characterization of amaranth genetic resources for agro-morphological and nutritional traits in submontane Himalayan region of India

Salej Sood[*$], Ramesh Singh Pal, Anubhuti Sharma, Lakshmi Kant and Arunava Pattanayak
ICAR-Vivekananda Parvatiya Krishi Anusanthan Sansthan, Almora – 263601, Uttarakhand, India.
[$] Present Address: ICAR-Central Potato Research Institute, Shimla, 171001, Himachal Pradesh, India
***E-Mail**: salej1plp@gmail.com; salej.sood@icar.gov.in

(Received: 27 Oct 2018; Revised:10 Dec 2018; Accepted: 26 Dec2018)

Abstract
Grain amaranth is a promising crop because of its wide adaptation, nutritional importance and multiple uses. Forty eight grain amaranth accessions were evaluated in augmented block design along with 4 checks for 10 quantitative traits in two growing seasons and the grain samples of 52 along with three additional checks were analyzed for nutritional parameters. Analysis of variance for morphological traits resulted in significant differences among treatments for both the years. Adjusted mean values of quantitative traits for each year and mean values of nutritional traits were used for correlation and multivariate analysis. There were 24 and 16 positive significant associations in the years 2013 and 2014, respectively among various grain yield components. Grain yield per plant was significantly positively associated with plant height (0.459, 0.574), leaf length (0.615, 0.321), and petiole length (0.726, 0.381) during both the years. HCA of morphological data showed that national check variety Durga occupied a separate cluster as a singleton in 2014andin 2013, where IC 42346-6 was also in the same cluster. The protein, TPP, DPPH and TEAC content of amaranth accessions varied from 8.99±0.31 to 15.26±0.30%, 2.44±0.08to 4.86±0.13 mg GAE/gDW,4.72 to 8.47 uMTE/gDWand 15.21 to 33.63 mg GAE/gDW, respectively. Wide range of variation was also observed for iron (66.67-83.19 ppm) and zinc content (28.47-42.98 ppm). Correlation among nutritional traits revealed highly significant positive association of TPP with DPPH and TAA. PCA analysis of nutritional traits revealed that PC1 distinguished those accessions that had high values for TPP, DPPH and TAA, whereas PC2 distinguished accessions with high protein and zinc content. Scatter plot of PCA1 and PCA2 showed overlapping of Indian Himalayan accessions with Russian origin accessions. Results of cluster analysis were similar to that of PCA for nutritional traits. It was observed that high yielding check varieties of amaranth were low in protein, TPP, DPPH, TEAC, iron and zinc content in comparison to the accessions with high values for both the traits. The accessions identified for nutritional traits could be used in breeding programme for the improvement of nutritional traits in adapted varieties.

Keywords
Grain amaranth, germplasm, phenotypic diversity, nutritional analysis, PCA, correlation, cluster analysis

Introduction

Amaranthus L. is a genus of Amaranthaceae family with around 70 different species distributed worldwide including pigweeds, water hemps and grain amaranths(Sauer, 1967). There are two types of Amaranth species, grain type (*A.caudatus, A. cruentus* and *A. hypochondriacus*) and vegetable type (*A.dubius ,A.tricolor* and *A.cruentus*). Grain Amaranth species are of new world origin, *A. caudatus* from Andean Peru and Ecuador, *A. cruentus* and *A. hypochondriacus* from Mexico and Central America (Sauer, 1950; Drzewiecki, 2001). This has been classified under the group of crops called pseudocereals, which have unique qualities and nutritional importance. Nowadays, the grain amaranths are cultivated from the temperate to tropical zone. It displays wide morphological diversity and has high stress tolerance. The superior nutrition, drought tolerance, disease and pest resistance, high yield in production, and increasing rate of consumption have made this crop more attractive for cultivation. The crop is known for its excellent nutritional value. Compared with other crops, this pseudocereal is rich in protein (17–19% of dry weight) with double the amount of essential amino acids than wheat grain protein (Becker *et al*., 1981; Bressani *et al*., 1987a).

Despite being a self-pollinated crop, varying amounts of outcrossing and frequent inter-specific and inter-varietal hybridization have brought wide variation in amaranth genotypes. However, selection is the major breeding methodology followed in Amaranth improvement in India. Although inter-varietal crosses have been found to be successful, little progress has been made due to minute flowers which are difficult to emasculate and un-availability of precise crossing

DOI:10.5958/0975-928X.2018.00184.9

technique. Thus, germplasm collection, conservation and evaluation are being regularly practiced in this crop for identification of new potential genotypes. Most of the germplasm evaluation attempts have focussed on agro-morphological data particularly of one year without nutritional quality information. Therefore, in view of the present increased consciousness among people about the nutritional quality of amaranths there was a need to characterize the available accessions for nutritional quality vis a vis for quantitative traits. Thus, 48 accessions along with four checks were evaluated for two consecutive seasons and for important agro-morphological as well as nutritional parameters.

Material and Methods

A set of 48 accessions representing Indian as well as exotic collections (received from ICAR-NBPGR, New Delhi) along with four check varieties namely Durga, Annapurna, PRA2 and PRA3 were evaluated for agronomic characters. For nutritional analysis, three more samples including one local check variety VL *Chua*44 were also analyzed. In our study, all of the accessions were of *A. hypochondriachus* species except one exotic accession of *A. edulis*. The crop was raised from June to October in 2013 and 2014 at the experimental farm of the ICAR-Vivekananda Institute of Hill Agriculture (79°39'E latitude and 25°35'N longitude, 1250 m above msl). A single row of each accession was planted in an augmented design in both the years. The row length was 3 m with row to row spacing of 50 cm. Thinning was practiced within a month of sowing to maintain plant to plant spacing of 15 cm within the rows.

The crop received fertilizer 60:40:20 (N: P: K) Kg/ha, where the entire amount of phosphorous and potassium and half of the nitrogen was applied as basal dose during field preparation. The remainder half of the nitrogen was applied as top dressing after 45 days of sowing after second weeding. Earthing up was done 40 days after sowing when the plants attained 15-20cm height.

Data were recorded on 10 quantitative traits. Five individual plants of each accessions were used for recording the data, except for days to flowering and days to maturity, which were recorded on a plot basis. Grain yield was also recorded on plot basis. However, for grain yield per plant. the plot yield was divided by the number of plants.

The protein content was estimated by Bradford method (Bradford, 1976).Seed samples were oven dried to a moisture content of 10-12 per cent were used for extraction. Each sample (1.00 g) was extracted by stirring in20 mL 80% methanol at 25 °C, 150 rpm for 12 h and filtered through What man filter paper No. 1. The extraction was repeated again as described earlier. The extracts were mixed, filtrated and diluted upto 50 mL with methanol and double distilled water separately. The extract solutions were stored in amber bottles at 4°C, which served as the working solution (20 mg/mL) for determination of total phenolics and antioxidant activity.

Total polyphenol was estimated by a spectrophotometric assay, as described by Singleton and Rossi (1965) with minor modifications. Briefly, 1.0 mL of extract (10 mg/mL) was taken and 1 mL of Folin and Ciocalteu's phenol reagent was added into it. After 3 min, 1.0 mL of 20 % Na_2CO_3 solution was added to the reaction and the final volume of the reaction was adjusted to 10 mL with distilled water. The reaction tubes were kept in the dark for 90 min and then the absorbance was taken by spectrophotometer at 725 nm. Gallic acid was used to calculate the standard curve (1-80µg/mL). The results were mean values ± standard deviations and expressed as mg of gallic acid equivalent (GAE)/g DW.

Radical scavenging activity(RSA) on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was determined by spectrophotometric method as described by Brand-Williams et al. (1995). The stock solution containing 24 mg of DPPH in 100 mL of methanol was prepared and stored at -20^0C and the working solution was prepared by mixing 10 mL stock solution with 45 mL methanol to get an absorbance of 1.17 ± 0.02 units at 515 nm. Amaranth seed extract (150 µL) was allowed to react with 2850 µL of DPPH working solution for 24 h in the dark and absorbance was read at 515 nm. A standard curve of trolox (10-100 µM) was prepared and radical scavenging activity on DPPH was expressed as µM trolox equivalent.

The total antioxidant activity (TAA) of extracts was estimated using the phosphomolybdenum method (Prieto, *et al.,* 1999) based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of specific green phosphate /Mo (V) compounds. A 0.3 ml aliquot of extract solution combined with 2.7 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was capped and incubated in a boiling water bath at 95°C for 90 min. Samples were allowed to cool at room temperature and absorbance was measured at 695 nm. For the blank, 0.3 ml methanol was mixed with 2.7 ml of the

DOI:10.5958/0975-928X.2018.00184.9

reagent. A standard curve of gallic acid (10-100µg) was prepared and total antioxidant activity was expressed as mg gallic acid equivalent per gram dry weight.

The oven-dried samples were ground to pass through a 0.2 mm sieve. For estimation of zinc and iron,0.5 g sieved samples were digested with a mixture of nitric acid and perchloric acid in the ratio of 10:4 (v/v) (10 ml) on hot plates. After complete digestion, samples were cooled at room temperature and appropriately diluted. Total zinc and iron were analyzed by atomic absorption spectrometry (AAS vario 6, Analytik Jena).

The agro-morphological data of each year was analyzed in augmented block design separately to work out the adjusted mean values. The adjusted mean values of all the agro-morphological traits were used for correlation analysis, factor analysis and hierarchical cluster analysis. Similarly, the mean values of nutritional parameters were also used for similar analysis. Nutritional parameters were recorded for the grain samples of year 2014 only. ANOVA for augmented design was done using SAS in ICAR-IASRI server. SPSS (SPSS version 20, SPSS Inc., Chicago, IL, USA) was used for correlation analysis, whereas, DAR win 6.0 was used for factor and hierarchical clustering of morphological data. JMP 9.0 was used for PCA and hierarchical cluster analysis of nutritional data.

Results and Discussion

Morphological characterization is important for identification of accessions with desirable traits to be employed directly as cultivars or trait donors for use in crop improvement programmes. Analysis of variance for morphological traits resulted in significant differences among the treatments for both the years, suggesting that sufficient variability is present in the germplasm for exploitation in crop improvement. The accessions with a wide range of variation for agronomic characters had the potential to be determined as the superior genotypes for different environments. The mean values and range of variation of different traits is given in Table 1. Wide variability for morphological traits have been observed in amaranth by Akin-Idowu *et al.* (2016) earlier also. Height of plants and length of inflorescence have been found to be useful traits for grain yield and mechanical harvesting (Janovska *et al.*, 2012). Taller genotypes are useful to develop varieties for feed, and/or as barrier crops growing around fields. While shorter genotypes can be used as a source to develop varieties adapted to high density production or windy environments.

There were 24 and 16 positive significant associations in the years 2013 and 2014, respectively among various yield components. Grain yield per plant was significantly positively associated with plant height(0.459, 0.574), leaf length(0.615, 0.321), and petiole length(0.726, 0.381) during both the years. In addition, leaf width (0.633), inflorescence length(0.311) in 2013 and days to flowering(0.404) in 2014 also showed significant positive association with grain yield per plant. Days to flowering and days to maturity were significantly positively associated with each other during both the years. Only one significant negative association was observed between days to flowering and inflorescence length (-0.287) in the year 2014 (Table 2).Akin-Idowu et al. (2016) also observed significant positive correlation of grain yield with leaf length, leaf width and plant height. These results indicated that grain yield in amaranth can be improved upon by improving the petiole length, leaf length, plant height, inflorescence length, leaf width and days to flowering. It may be inferred that these traits represent higher biomass which provides more photosynthetic area and in turn results in higher grain yield.

Hierarchical cluster analysis of morphological data showed two major clusters of accessions based on phenotypic data observed on different accessions for two years. The separation was very clear and the check variety Durga was a single genotype cluster in 2014 while in 2013, IC 42346-6 was included in the same cluster as Durga (Fig. 1).It is evident from the morphological data that separation of genotypes was mainly based on grain yield and its component traits. Durga was the leading genotype in terms of grain yield and its component traits during both the years and its yield levels were much higher than the other checks as well as accessions and therefore, it appeared as a separate cluster in the analysis. None of the accessions were better than Durga for grain yield as well as its components and currently there is no replacement available for Durga. Therefore, Durga should be included as one of the parents in breeding programme of amaranth in North Western Hills of India.

Amaranth is known for its nutritional value especially quality protein with balanced amino acid profile. Breeding for nutritional quality wherein the future varieties itself will have higher nutrient content is the best strategy to deliver it to masses particularly in the

DOI:10.5958/0975-928X.2018.00184.9

areas which are malnutrition affected. To breed nutritionally rich varieties, identification of specific nutrient rich donors are required for the development of potential genotypes for use in pharmaceutical or food industry. The foundation of this work requires nutritional profiling of germplasm sets for all major parameters of importance. The results of nutritional evaluation for major traits are presented below.

Amaranth seeds as a good source for high-quality proteins are a valuable raw material for the preparation of protein concentrates (APCs). The protein content of 55 amaranth accessions varied from 8.99±0.31 in IC42397 to 15.26±0.30 % in IC93946 with a mean value of 11.58±0.5%. The checks Annapurna (14.26±0.43), PRA2 (13.96±0.59), IC95326 (13.94±0.83), IC95322 (13.88±0.89) and VL *Chua* 44 (13.60±0.50) were also better for protein percentage (Table 3). The variation for protein content was huge however, the widely adaptable high yielding Amaranth variety Durga (National Check) expressed comparatively low protein percentage (12.34±0.44). Four exotic accessions of Russia were intermediate in protein content. In earlier studies protein content in 14 genotypes of 4 amaranth species (*A. caudatus, A. hybridus, A. cruentus,* and *A. hypochondriacus*) varied from 12.5% to 16.0% (Bressani *et al.,* 1987b), a 13 to 21% variation was observed in wild and cultivated forms of amaranth. Among the miscellaneous foods analyzed, *A. paniculatus* grainshad a protein content of 22.0 g/100 g (Rajyalakshmi and Geervani, 1994).*A. molerosa* grains were studied in biochemical experiments with various cultivars of 12 cereal species, one buckwheat and one amaranth, and it was determined that amaranth had 15.4% protein of a favourable amino acid composition with the highest content of Met., Lys., and Arg..Among four varieties of *A. hypochondriacus* (Tulyehualco, Nutrisol, DGETA and Gabriela) grown in the Mexican Highlands zone, Gabriela contained the highest protein content (17.3%), but all varieties had an adequate balance of essential amino acids (Barba de la Rosa *et al.,* 2009). Similarly, the analysis of 48 *A. hypochondriacus* and 11 *A. caudatus* lines revealed that *A. caudatus* lines had higher protein content than *A. hypochondriacus* lines (Kaur *et al.,* 2010). Eight groups of *A. cruentus* and *A. hypochondriacus* grain samples grown in Hungary and Austria were studied and it was found that a difference between the lowest (14.23%) and highest (17.40%) protein content was relatively large andwe too observed large variation suggesting that breeding might be a potential means for increasing the protein content of new varieties of Amaranths (Tomoskozi *et al.,* 2009).

The content of phenolic compounds could be used as an important indicator of antioxidant capacity. It has been reported that the antioxidant activity of plant materials is well correlated with the content of their phenolic compounds (Pan et al., 2008).The phenolic contents of the different accessions of Amaranth were expressed as mg gallic acid per gram of dry weight (Table 3). The range of variation for TPP in different amaranth accessions varied from 2.44±0.08mg GAE/g DW in IC 95315 to 4.86±0.13 mg GAE/g DW in IC 95326 with average value of 3.29±0.10 mg GAE/g DW. We observed high values of TPP in few accessions (IC 95326-4.86; IC 95249 - 3.84; IC 93946 – 4.73; IC 42358 – 4.33; IC 42351 – 4.32; IC 95302 –4.25; IC 95286 – 4.19mg GAE/g DW) which was much higher than the check varieties PRA2, PRA3, VL 44 and Durga (Table 3). The check variety Annapurna exhibited significantly higher value of TPP among checks. The variability available in the accessions can be utilized in breeding programmes for improved nutritional quality and such accessions should be included in the list of core panels for genomics assisted breeding for mapping of nutritional traits. Pasko et al. (2009) observed TPP in the range of 2.95 – 3.0 in two amaranth varieties.

The free radical scavenging activity against DPPH and gallic acid equivalent anti-oxidant capacity (GEAC) showed a wide range of values for different amaranth accessions. DPPH in micro mole trolox equivalent per g dry weight varied from 4.72 in IC 95315 to 8.47 in IC 95249 with an average of 6.40 micro mol TE/g DW. Significant differences were observed among different accessions of amaranths for AOA. GEAC varied from 15.21 mg GAE/ gDW in IC 95315 to 33.63 mg GAE/ gDW in IC 95321 with an average of 27.60 mg GAE/ gDW (Table 3). Among the checks Annapurna had highest radical scavenging and antioxidant activity. High yielding best check variety Durga had intermediate values for DPPH and TAA. Such differences in TAA activity among different accessions could be due to inherent levels of compounds responsible for variation in TAA activity.Several studies have shown that amaranth seed or oil may benefit those with hypertension and cardiovascular disease, regular consumption reduces blood pressure and cholesterol levels, while improving antioxidant status and some immune parameters (Castelano-Sousa and Amaya-Farfán, 2012).The range obtained for TAA was higher in Amaranth accessions in comparison to major cereal grains (Asao and Watanabe, 2010). It has been observed that all pseudo-cereals have stronger radical scavenging ability than cereals, quinoa being the highest followed by amaranth (Asao

DOI:10.5958/0975-928X.2018.00184.9

and Watanabe, 2010).This indicates that amaranth is a potential source for antioxidants among grains.

Iron and zinc are two important mineral elements supposed to be very important for nutritional security (Goudia and Tom Hash, 2015). Major population in India is aneamic and has zinc deficiency too. This is also known as hidden hunger. Amaranth grains are an important source of iron and zinc. In general, the major cereals (rice, wheat and maize) are deficient in iron. Amaranth accessions evaluated in the present study showed a range of variation from 66.67 ppm in IC 94656to 83.19 in IC 82625 for iron content and 28.47 ppm in IC 95339 to 42.98 ppm in the check variety PRA 3 for zinc content (Table 3). In general, all the accessions recorded high iron and zinc content in them. All the high yielding varieties i.e. Durga and VL *Chua* 44, Annapurna, PRA2 and PRA 3 recorded high iron and zinc content in comparison to all the accessions included in the study. This indicates that amaranth varieties have been unknowingly selected for high iron and zinc content. Higher range for iron content (72-174 ppm) has been observed earlier by Becker et al. (1981). However, the zinc content observed by Becker et al. (1981) was found to be in lower range in comparison to our study.

Correlation among nutritional traits revealed highly significant positive association of total phenols with DPPH and TAA. DPPH and TAA were also significantly positively associated with each other. None of the other nutritional traits was correlated with each other, neither they showed any significant positive or negative association with any other traits. The results indicate that DPPH, TAA and TPP which are correlated with each other, can be improved by targeting any one of these traits. Whereas, Fe, Zn and protein content are independent of each other as well as the other traits and require independent approach for their improvement. It was also observed that since there was no negative correlation between these traits, improvement in one trait will not influence the other traits. A strong positive correlation between iron and zinc has been reported by Burger *et al*. (2014). High correlation between Zn and Fe have been reported in pearl millet (Velu *et al.,* 2007; Govindaraj *et al.,* 2009; Bashir *et al.,* 2014) and wheat (Gomez-Becerra *et al.,* 2010a; Velu *et al*., 2012; Zhang *et al*., 2010). A strong correlation between total polyphenols content and antioxidant activity was observed (TPP vs. TAA, r = 0.703: TPP vs. DPPH, r = 0.771) and these findings indicate that total polyphenols content is a good predictor of in vitro antioxidant activity (Table 4). Total polyphenols content and antioxidant activity studied in amaranth grains indicated that total polyphenols content increases antioxidant activity in grains and there is a linear correlation between total phenols content and antioxidant activity (Gorinstein *et al.,* 2007; Zielinski and Kozłowska, 2000).

Interrelationships among the different nutritional parameters were studied using principal component analysis (PCA). The first three PCA components provided a reasonable summary of the data and explained 77.6 % of the total variation (Table 5).The first principal component (PC1), was the most important, and explained 40 % of the total variance. PC1 was attributed to TPP, DPPH and TAA for largest positive loadings. As a result the first PC differentiated the accessions mainly associated with the contribution of high values for TPP, DPPH and TAA. The second PC explained an additional 19.6 per cent of the total variance and was attributed to positive loadings of protein and zinc content. The third component basically identified the accessions with high values for Fe content.

Similar observations were found in the PC1 and PC2 biplot where TPP, DPPH and TAA were on the one side of the plot whereas protein and zinc content were almost in right angle to them. Iron content was in obtuse angle to TPP, DPPH and TAA (Fig. 2). This indicates that the traits TPP, DPPH and TAA are highly correlated to each other, protein and zinc too had high correlation however, iron content was negatively correlated to TPP, DPPH and TAA. There was no clear cut separation of accessions based on nutritional traits data in PC1-PC2 biplot. However, two groups were observed in the biplot. One group of accessions was better for TPP, DPPH and TAA whereas, the second group was better for protein, zinc and iron content. The red dots in the biplot shows the five check varieties already in cultivation in India. All these check varieties were good for protein iron and zinc content with lower values of TAA, DPPH and TAA. Blue dots indicate the exotic lines of Russia which were again at par with the checks for nutritional trait values. IC 95315 was one of the outliers in the biplot which showed minimal values for TPP, DPPH and TAA but has good protein, iron and zinc content.

Principal component analysis (PCA) is a way of identifying patterns in data, and expressing the data in such a way as to emphasize their similarities and differences. It compresses the data, that is, by reducing the number of dimensions without much loss of information based on their similarities and differences, and define a limited ‘principal

DOI:10.5958/0975-928X.2018.00184.9

components' which describe independent variation structures in the data. When more than three variables have been measured, visualization of the data by various plotting systems is then possible (Kamal-Eldin and Andersson, 1997). Therefore, PCA indicates relationships among groups of variables in a data set and show relationships that might exist between objects. Principal component analysis revealed that PC1 distinguished those accessions that had high values for TPP, DPPH and TAA. Similarly PC2 distinguished accessions with high protein and zinc content. Scatter plot of PCA1 and PCA2 showed overlapping of Indian Himalayan accessions with Russian origin accessions. A possible explanation for the same could be similar geographical conditions of Indian Himalayan region to that of Russia.

Cluster analysis of accessions for nutritional data showed two major clusters. First cluster contained 41 accessions including check varieties as well as four exotic accessions also. First cluster was subdivided into four sub-clusters based on trait values. Second major cluster contained 14 accessions of Indian origin including one check variety Annapurna. This cluster was further subdivided into two clusters (Fig. 3). The first cluster was mainly characterized by lower values of TPP, DPPH and TAA whereas the accessions in the second cluster had higher values of all the nutritional traits. Thus the accessions from the second cluster could be utilized in breeding programme for improvement of nutritional traits in Amaranth particularly antioxidant activity and mineral elements.

The results on the present set of amaranth accessions indicate that during breeding of improved genotypes, emphasis has been given mainly on grain yield and its components only while giving less emphasis on quality traits. The evaluation of these 55 accessions including check varieties identified putative donors for nutritional quality traits could be involved in breeding for nutritional quality high yielding genotypes/ varieties of amaranth for future cultivation.

Acknowledgements
Authors are thankful to Dr. J. C. Rana, Bioversity International and Dr. Mohar Singh, Incharge, ICAR-NBPGR, RS, Shimla for providing the germplasm and their passport information. Thanks are due to Mr. G.S. Bisht, Technical officer, ICAR-VPKAS, Almora for assistance in field evaluation of amaranth accessions.

References

Bürger,A. Høgh-Jensen, H.,Gondah, J.,Hash, C.T. and Haussmann,B.I.G. 2014. "Micronutrient density and stability in West African pearl millet: Potential for biofortification." Crop Science **54**: 1709-1720.

Akin-Idowu, P.E., Gbadegesin, M.A., Orkpeh, U., Ibitoye, D.O. and Odunola, O.A. 2016. Characterization of Grain Amaranth (*Amaranthus* spp.) Germplasm in South West Nigeria Using Morphological, Nutritional, and Random Amplified Polymorphic DNA (RAPD) Analysis. Resources **5:** 6; doi:10.3390/resources5010006.

Asao, M. and Watanabe, K. 2010. Functional and Bioactive Properties of Quinoa and Amaranth Food Science and Technology Research 16: 163-168.

Barba de la Rosa, A.P., Fomsgaard, I.S., Laursen, B., Mortensen, A.G., Martinez, L.O., Silva-Sanchez, C., Mendoza-Herrera, A., González-Castañeda, J. and León-Rodríguez, A.D. 2009. Amaranth (*Amaranthus hypocondriacus*) as an alternative crop for sustainable food production: Phenolic acids and flavonoids with potential impact on its nutraceutical quality. Journal of Cereal Science **49**(1): 117-121. http://dx.doi.org/10.1016/j.jcs.2008.07.012.

Becker, R., Wheeler, E.L., Lorenz, K., Stafford, A.E., Grosjean, O.K., Betschart, A.A., Saunders, R.M. 1981. A compositional study of amaranth grain. J Food Sci **46**:1175-80.

Bradford, M.M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Analytical Biochemistry **72**: 248-254

Brand-Williams, W., Cuvelier, M.E. and Berst, C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und Technologie**28**: 25-30.

Bressani, R., Gonzales, J.M., Elias, L.G. and Melgar, M. 1987a. Effect of fertilizer application on the yield, protein and fat content, and protein quality of raw and cooked grain of three amaranth species. Plant Food Hum Nutr **37**:59-67.

Bressani, R., Gonzales, J.M., Zuniga, J., Brauner, M. and Elias, L.G. 1987b. Yield, selected chemical composition and nutritive value of 14 selections of amaranth grain representing four species. J. Sci. Food Agric. **38**: 347-356.

Castelano-Sousa, V.M. and Amaya-farfán, J. 2012. State of knowledge on amaranth grain: a comprehensive review. Journal of Food Science**77**: 93-104.

DOI:10.5958/0975-928X.2018.00184.9

Drzewiecki, A. 2001. Similarities and differences between Amaranthus species and cultivars and estimation of outcrossing rate on the basis of electrophoretic separations of urea-soluble seed proteins. Euphytica**119** (3): 279-287.

Bashir,E.M.,Ali,A.M.,Melchinger, A.E.,Parzies, H.K. and Haussmann,B.I.G. 2014. "Characterization of Sudanese pearl millet germplasm for agro-morphological traits and grain nutritional values." Plant Genetic Resources **12**: 35-47.

Velu,G.,Rai,K.N.,Muralidharan,V.,Kulkarni,V.N.,Longvah, T. and Raveendran,T.S. 2007. "Prospects of breeding biofortified pearl millet with high grain iron and zinc content." Plant Breed**126**: 182-185.

Velu,G.,Singh,R.P.,Huerta-Espino, J. et al. 2012. "Performance of biofortified spring wheat genotypes in target environments for grain zinc and iron concentrations," Field Crops Research**137**: 261–267.

Gorinstein, S., Vargas, O. J. M., Jaramillo, N.O., Salas, I.A., Ayala, A.L.., Arancibia-Avila, P., Toledo, F., Katrich, E.and Trakhtenberg, S. 2007. The total polyphenolsand the antioxidant potentials of some selected cereals and pseudocereals. European Food Research and Technology**225**: 321-328.

Goudia, B. D. and Tom Hash, C. 2015 Breeding for high grain Fe and Zn levels in cereals. International Journal of Innovation and Applied Studies **12** (2) 342-354.

Gomez-Becerra,H.F. Yazici,A.,Ozturk,L. Budak,H. Peleg,Z.,Morgounov,A.,Fahima,T.,Saranga, Y. and Cakmak,I. 2010a. "Genetic variation and environmental stability of grain mineral nutrient concentrations in Triticum dicoccoides under five environments." Euphytica**171**: 39-52.

Kamal-Eldin, A. and Andersson, R. 1997. A multivariate study of the correlation between tocopherol content and fatty acid composition in vegetable oils. Journal of the American Oil Chemists' Society**74** (4): 375-380.

Kaur, S., Singh, N., and Rana, J.C. 2010. Amaranthus hypochondriacus and Amaranthus caudatus germplasm: Characteristics of plants, grain and flours. Food Chemistry**123**(4): 1227-1234. http://dx.doi. org/10.1016/j.foodchem.2010.05.091.

Govindaraj,M.,Selvi, M. and Rajarathinam,S. 2009. "Correlation studies for grain yield components and nutritional quality traits in pearl millet (Pennisetum glaucum (L.) R. Br.) germplasm." World Journal of Agricultural Sciences **5**: 686-689.

Pan, Y., Wang, K., Huang, S. Q., Wang, H. S., Mu, X. M., He, C. H., Ji, X. W., Zhang, J. and Huang, F.J. 2008. Antioxidant activity of microwave-assisted extract of longan (Dimocarpus Longan Lour.) peel. Food Chemistry **106**(3): 1264-1270. http://dx.doi. org/10.1016/j.foodchem.2007.07.033

Paśko, P., Bartoń, H., Zagrodzki, P., Gorinstein, S., Fołta, M., and Zachwieja, Z. 2009. Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. Food Chem **115**:994-8.

Prieto, P., Pineda, M. and Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of vitamin E. Analytical Biochemistry **269**: 337-341.

Rajyalakshmi, P. and Geervani, P. 1994.Nutritive value of the foods cultivated and consumed by the tribals of south India.Plant Foods Hum Nutr. **46**(1): 53-61.

Sauer, J.D. 1950. The grain amaranths: A survey of their history and classification. Ann. Missouri Bot. Gard. **37**:561-632.

Sauer, J.D. 1967. The Grain Amaranths and Their Relatives: A Revised Taxonomic and Geographic Survey. Annals of the Missouri Botanical Garden**54**(2): 103-137.

Singleton, V.L. and Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult. **16**: 144-158.

Tömösközi, S., Baracskai, I., Schönlechner, R., Berghofer, E. and Läsztity, R. 2009. Comparative study of composition and technological quality of amaranth I. Gross chemical composition, amino acid and mineral content. Acta Aliment Hung**38**:341-7.

Zhang,Y.,Song,Q., Yan, J., Tang,J.,Zhao,R.,Zhang,Y.,He,Z.,Zou, C. and Ortiz-Monasterio, I. 2010. "Mineral element concentrations in grains of Chinese wheat cultivars." Euphytica **174**: 303-313.

Zielinski, H. and Kozłowska, H. 2000. Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. Journal of Agricultural and Food Chemistry **48**: 388-393.

Electronic Journal of Plant Breeding, 9(4): 1484-1496 (Dec 2018)
ISSN 0975-928X
DOI:10.5958/0975-928X.2018.00184.9

Table 1. Mean and range of variation for agronomic traits in amaranth germplasm

Trait	Mean		Range		SD	
	2013	2014	2013	2014	2013	2014
Flowering days (DF)	51.0	44.4	42-62	38-55	3.95	3.44
Maturity days (DM)	87.4	80.5	81-98	73-85	3.87	2.94
Plant height (cm) (PH)	143.4	100.0	105-177	63-139	13.98	16.92
Inflorescence length (cm) (IL)	53.5	42.0	39-75	29-58	6.78	7.48
Leaf length (cm) (LL)	13.2	11.9	10.4-17.8	9.1-15.3	1.68	1.49
Leaf width (cm) (LW)	7.3	6.6	5.3-10.8	4.8-9.2	1.03	0.95
Petiole length (cm) PL)	7.5	6.68462	4.6-13.8	4.2-9.2	1.47	1.19
Grainyield/plant (g) (GYP)	11.3	8.10154	4.8-38.2	0.6-22.8	5.32	3.63
Plot yield (g) (PY)	228.7	128.846	98-688	30-405	104.64	63.68
1000 grainweight (g) (GW)	0.73	-	0.64-0.85	-	0.05	-

Table 2. Correlation among morphological traits in amaranth germplasm collections

	DF	DM	PH	IL	LL	LW	PL	GYP	PY	GW
DF		0.587**	0.393**	0.033	0.330*	0.056	0.163	0.231	0.164	0.201
DM	0.691**		0.465**	0.354*	0.220	-0.035	-0.018	0.221	0.247	0.128
PH	0.267	0.276		0.595**	0.665**	0.359*	0.433**	0.459**	0.384**	0.192
IL	-0.287*	-0.110	0.615**		0.432**	0.271	0.236	0.311*	0.316*	0.194
LL	0.090	0.431**	0.290*	0.229		0.675**	0.617**	0.615**	0.512**	0.173
LW	0.047	0.265	0.077	0.030	0.742**		0.673**	0.633**	0.569**	0.164
PL	0.170	0.291*	0.207	0.106	0.644**	0.677**		0.726**	0.648**	0.129
GYP	0.404**	0.282	0.574**	0.109	0.321*	0.217	0.381**		0.929**	0.080
PY	0.404**	0.247	0.564**	0.077	0.261	0.149	0.306*	0.815**		0.093

*-Significant at P < 0.05; **-Significant at P < 0.01 (1-tailed test)
The upper and lower diagonal shows correlations for the year 2013 and 2014, respectively

DOI:10.5958/0975-928X.2018.00184.9

Table 3. Nutritional evaluation of amaranth germplasm

Trait	Min	Max	Mean	Top five genotypes
TPP (mg GAE/g DW)	2.44±0.08	4.86±0.13	3.29	IC 95326 (4.86±0.13), IC 95249 (4.84±0.13), IC 93946 (4.73±0.09), IC 42358 (4.33±0.12), IC 42352 (4.31±0.11)
DPPH (micro mol TE/g DW)	4.72±0.32	8.47±0.15	6.40	IC 95249 (8.47±0.15), IC 42352 (7.84±0.15), IC 95286 (7.79±0.13), IC 107144 (7.78±0.26), IC 93946 (7.74±0.13)
TAA (mg GAE/g DW)	15.19±0.59	33.63±4.49	27.60	IC 95321 (33.63±4.49), IC 95249 (33.25±1.63), IC95299 (33.18±1.34), IC 95286 (33.12±1.53), IC 95302 (32.69±1.23)
Protein (%)	8.99±0.31	15.26±0.30	11.58	IC 93946 (15.26±0.30), Annapurna (14.26±0.43), PRA 2 (13.96±0.59), IC 95326 (13.94±0.83), IC 95322 (13.88±0.89)
Fe (ppm)	66.67±0.62	83.19±0.74	77.16	IC 82625 (83.19±0.74), PRA 3 (82.33±0.38), IC 95286 (82.15±2.14), IC 47436 (82.15±0.82), PRA 2 (81.58±1.10)
Zn (ppm)	28.47±0.92	42.98±0.59	38.00	PRA 3 (42.98±0.59), Annapurna (42.57±0.23), VL 44 (42.16±0.42), IC 42415 (42.00±0.21), IC 95301 (41.92±0.65)

Table 4. Correlation among nutritional parameters in amaranth germplasm collections

	DPPH micro mol TE/g DW	TAA mg GAE/g DW	Protein (%)	Fe ppm	Zn ppm
TPP (mg GAE/g DW)	0.771**	0.703**	-0.105	-0.164	-0.039
DPPH micro mol TE/g DW		0.629**	-0.042	-0.068	0.026
TAA mg GAE/g DW			0.049	-0.039	0.064
Protein (%)				0.096	0.156
Fe ppm					-0.078

**-Significant at P < 0.01 (1-tailed test)

Table 5. Percent contribution of principal components analyzed for nutritional traits

Number	Eigenvalue	Per cent	Per cent	Cum Percent	ChiSquare	DF	Prob>ChiSq
1	2.43	40.42		40.42	95.35	20.00	<.0001**
2	1.18	19.63		60.05	52.60	14.00	<.0001**
3	1.06	17.54		77.59	41.43	9.00	<.0001**
4	0.78	12.96		90.55	24.78	5.00	0.0002**
5	0.37	6.14		96.69	5.07	2.00	0.079
6	0.20	3.31		100.0	0.00	0.00	

**-Significant at P < 0.01

DOI:10.5958/0975-928X.2018.00184.9

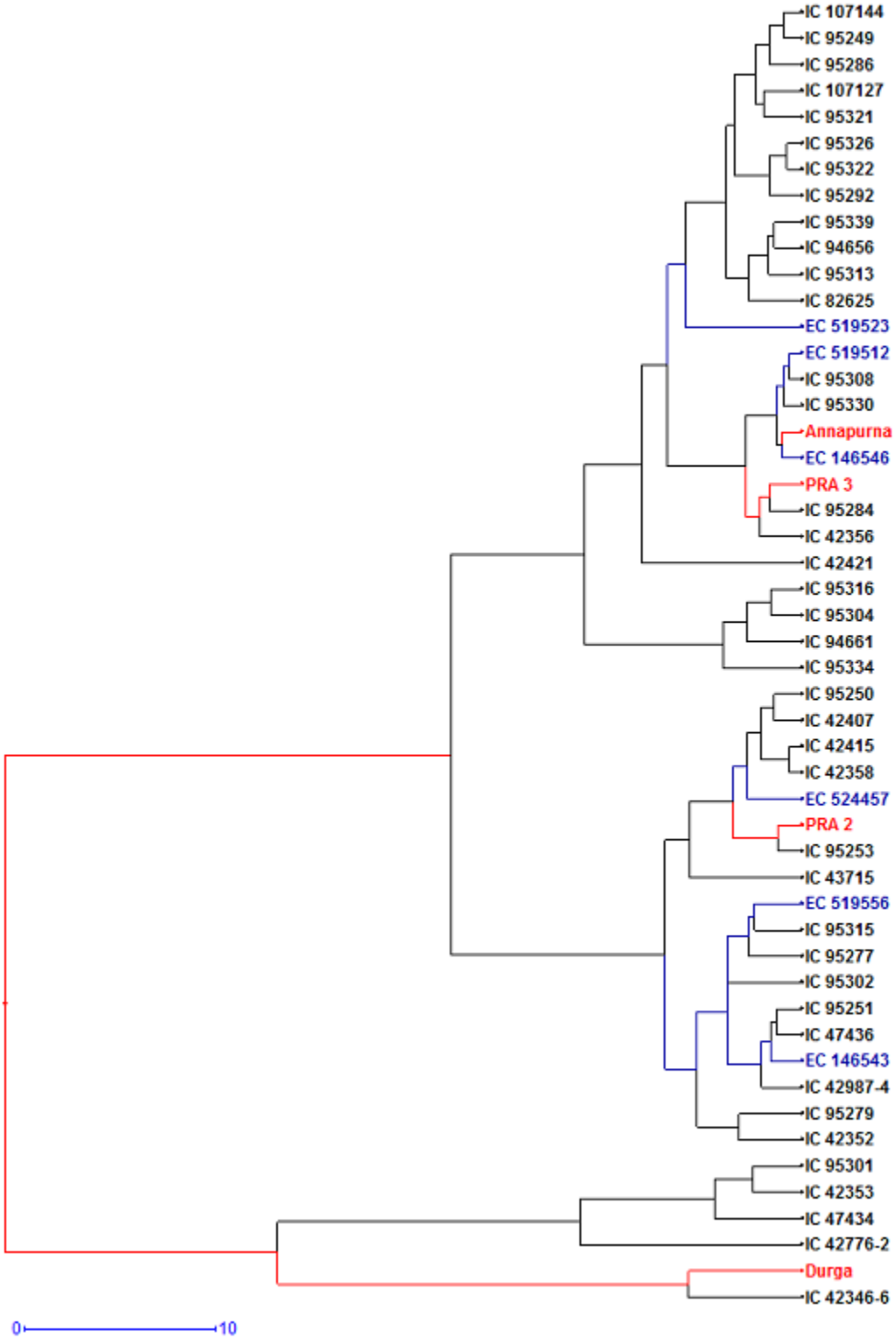

Fig. 1a. Cluster analysis of amaranth accessions based on agro-morphological data for the year 2013

DOI:10.5958/0975-928X.2018.00184.9

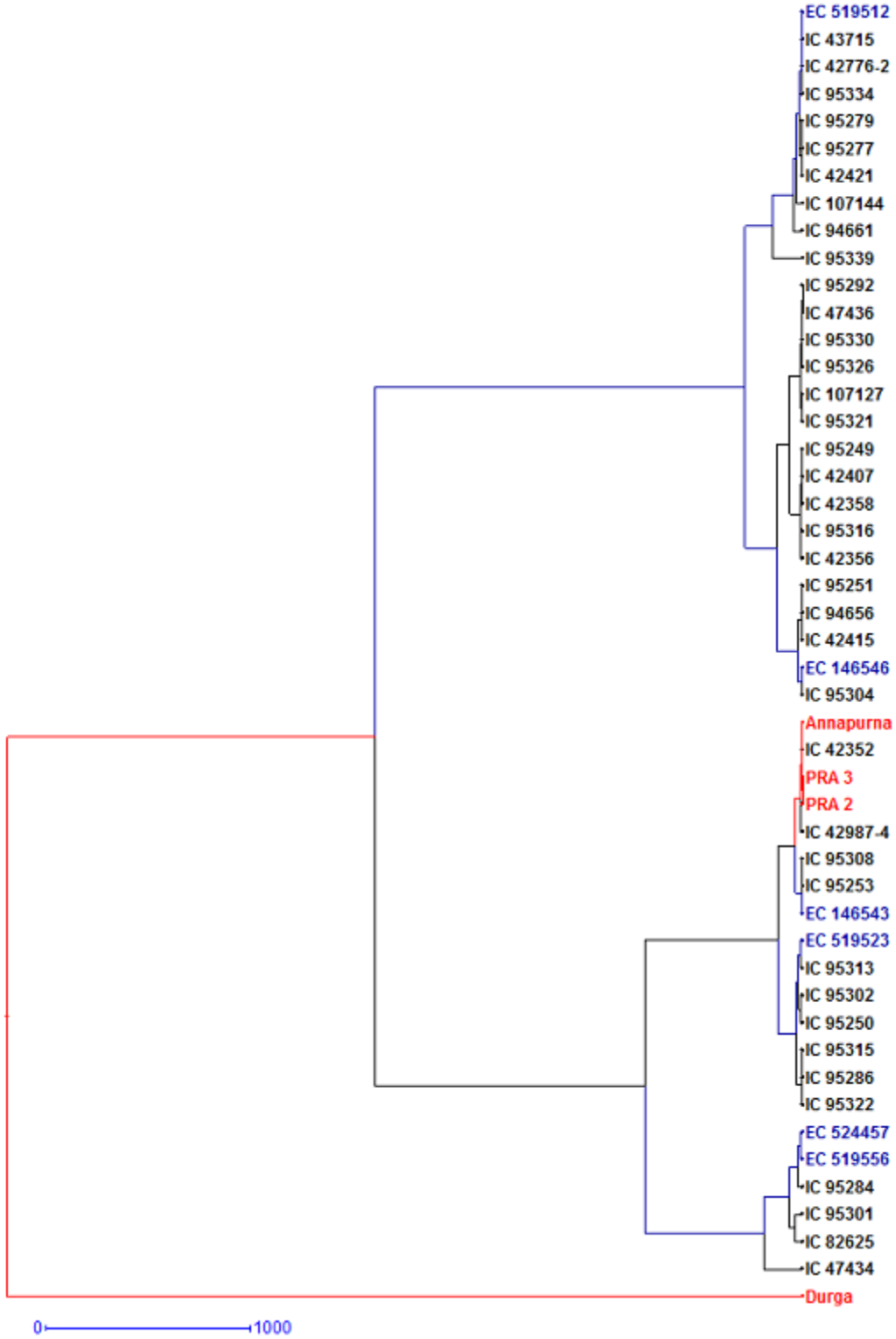

Fig. 1b. Cluster analysis of amaranth accessions based on agro-morphological data for the year 2014

DOI:10.5958/0975-928X.2018.00184.9

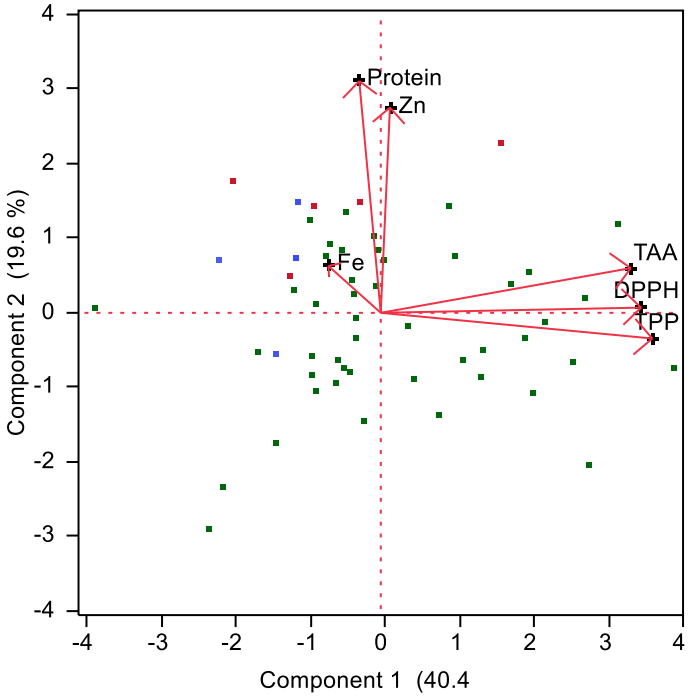

Fig.2. Score plot of PC1-PC2 for amaranth genotypes based on nutritional parameters

DOI:10.5958/0975-928X.2018.00184.9

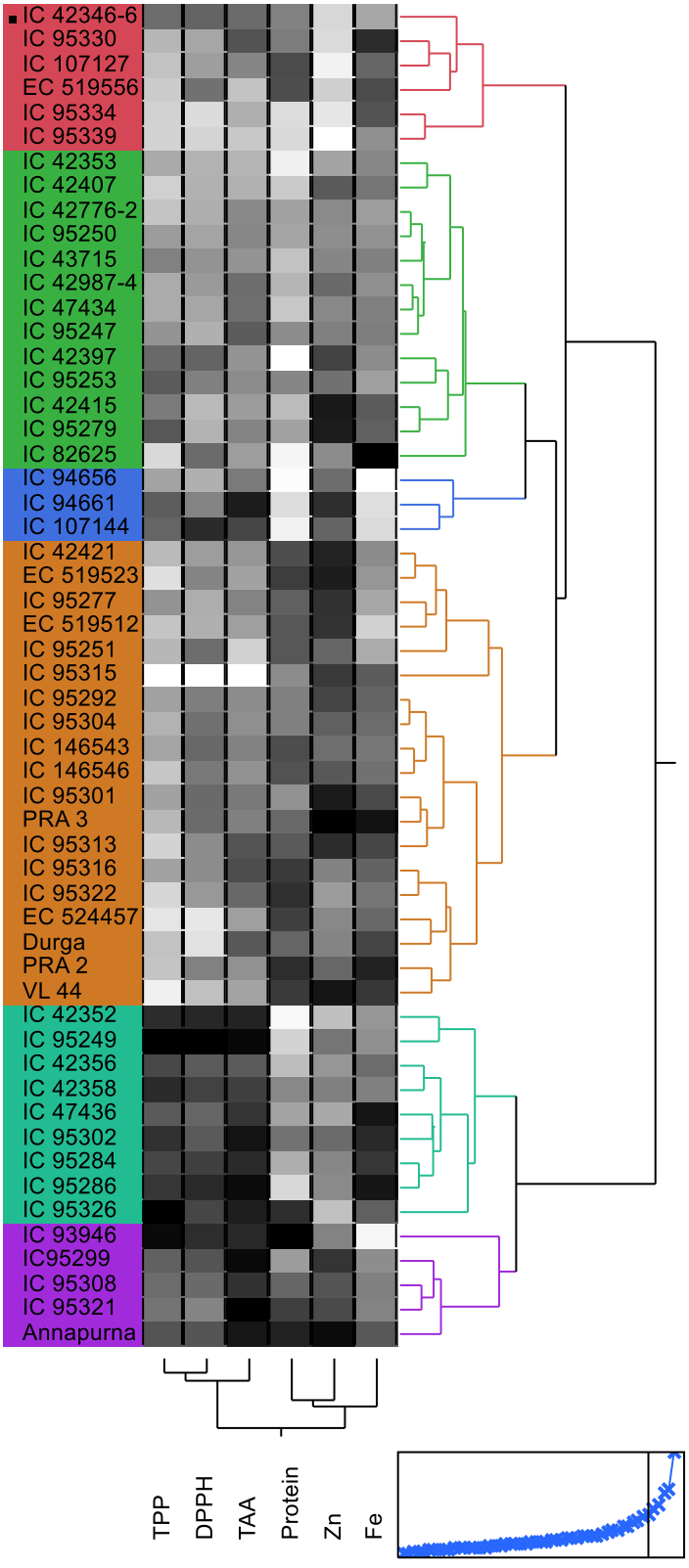

Fig. 3. Hierarchical clustering of Amaranth genotypes based on nutritional data