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Biochemical composition of pigeonpea genotypes in Kenya

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Abstract

Pigeonpea is an important crop in semi-arid tropics and sub-tropics. The improvement and utilization of this crop in East Africa can enhance food and nutrition security. A study was carried out to examine variation in biochemical composition (crude protein, total phenols, total flavonoid and total anti-oxidant activity) of 55 pigeonpea genotypes grown in Kabete field station during the long rains of April-September 2017. The experiment was set up in a randomized complete block design with three replications. After harvest, 100g of dry seed samples were collected for biochemical analyses. The biochemical analyses were performed at nutrition platform of Biosciences eastern and central Africa- International Livestock Research Institute (BecA-ILRI) Hub, Nairobi, Kenya. Treatment means were separated based on Tukey test using Genstat, SAS and R software. The genotypes varied significantly ($P \leq 0.05$) for all the parameters measured with a mean of 20.88 g/100g, 46.21 mg/100g, 23.98 mg/100g and 38.13 mg/100g for crude protein, total phenol, total flavonoid and total anti-oxidant activity, respectively. Advanced elite materials out performed for all parameters analyzed except for crude protein with a mean of 59.57 mg/100g, 26.64 mg/100g and 30.23 mg/100g for total phenols, total flavonoid and total anti-oxidant activity, respectively. The total antioxidant activity had significantly positive correlation ($P \leq 0.05$) with total phenol, total flavonoid and crude protein. Similarly, total phenol and total flavonoid had significantly positive correlation (≤ 0.05). The results revealed that the advanced elite materials contain high phenolics and antioxidant activity that contribute to lowering oxidation of free radicals due to their redox properties. Therefore, these germplasm are valuable genetic resources for improving pigeonpea varieties for nutritional qualities.

Keywords: Biochemicals; elite genotypes; nutritional quality; oxidation; pigeonpea; redox properties.
Abbreviations: ANOVA_ Analysis of variance; BSA_ Bovine serum albumin; OD_ Optical density; RPD_ Relative percent difference.

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a legume crop mainly grown in the semi-arid tropics (Saxena et al., 2002) where millions of people are living below the poverty line and their livelihood primarily depends on the rain fed agriculture (Choudhary et al., 2013). Pigeonpea is a drought tolerant crop and produces reasonable grain yield during the dry spell when other legumes have dried up. The ability of the pigeonpea to withstand severe drought better than many other legumes is attributed to its deep roots and osmotic adjustment in the leaves (Subbarao et al., 2000).
Pigeonpea is a favorite crop of small holder farmers in dry lands because it provides large amounts of proteins, calories, vitamins, and essential minerals for human nutrition (Saxena et al., 2002). It has been identified as a source of dietary protein mainly in vegetarian based diets (Chitra et al., 1996). Pigeonpea is among the best solution to protein-calorie malnutrition in the developing world where such malnutrition is persistently a serious challenge among the children leading to physical, mental and motor retardation.
Pigeonpea has the ability to fix atmospheric nitrogen in to the soil at about 40 kg/ha per season (Saxena et al., 2002). It can access bound phosphorus in the soil due to presence of piscidic acid in root exudates that solubilize phosphorus in the rhizosphere (Rao et al., 2001). Besides its nutritional value and nitrogen fixing capabilities, pigeonpea also possesses various medicinal properties due to the presence of polyphenols and flavonoids with health benefits. Phenolic compounds such as flavonoids are plant secondary metabolites that play an

important role in plant protection (Pal et al., 2011). Although plant phenolics have been classified as anti-nutrients, they are useful as natural antioxidants because of their positive correlation with antioxidant activity (Rani et al., 2014). Epidemiological studies have revealed that the consumption of phenolic, flavonoid-rich foods provide protection against human diseases associated with oxidative stress (Wang et al., 2009). Pigeonpea seeds are a good source of antioxidants (Al-Saeedi and Hossain, 2015).
As stated earlier, pigeonpea is climate smart legume that plays significant roles in food and nutrition security, human health, soil fertility improvement. Pigeonpea germplasm are genetically diverse and differs in the biochemical properties that determine the nutritive and medicinal values (Al-Saeedi and Hossain, 2015). However, there is little information available on biochemical composition of currently cultivated pigeonpea genotypes in Kenya. Therefore, this study offers first-hand information on biochemicals content in seeds of different pigeonpea genotypes grown in Kenya. This information can be utilized in selecting genotypes with elevated levels of protein and antioxidant potential for enhanced nutritional quality.

Results

Variation in biochemical composition among 55 pigeonpea genotypes

The pigeonpea genotypes varied for all four parameters measured (Table 1). Crude protein ranged in between 16.7 and 28.43g/100g with a mean of 20.88 g/100g while, total phenols ranged in between 20.15 and84.44mg/100gwith a mean of 46.21 mg/100g. Similarly, total flavonoids content ranged in between 13.88 and 33.48mg/100g with a mean of 23.98 mg/100g. However, a range in between 20.95 and 86.84mg/100 with mean of 38.13 mg/100g were measured for total anti-oxidant activity.

Variations in biochemical composition among three groups of pigeonpea genotypes

Variation among the three groups of pigeonpea genotypes (landraces, advanced elite lines, improved cultivars) in crude protein, total phenols, total flavonoids and total anti-oxidant activity was also determined. Crude protein contents did not differ significantly between the groups, advanced genotypes recorded a mean of 20.7 g/100g while improved cultivars and landraces recorded 20.5 and 20.3 g/100g, respectively (Fig 1).
The three group of pigeonpea genotypes differed significantly in total phenols (P≤0.001) with the advanced elite lines recording the highest (59.57 mg/100g) and improved cultivars recording the lowest (39.72g/100mg; Fig.2). Similar trend was observed in total flavonoids where the three group of pigeonpea genotypes differed significantly (P≤0.001) with advanced elite genotypes recording the highest mean (26.1 g/100mg) and landraces recording the lowest mean (21.2 g/100mg; Fig 3**)**.The three group of pigeonpea genotypes exhibited significant variation (P≤ 0.001) in total antioxidant activity. The landraces recorded the highest values (40.26 mg/100g) followed by improved (32.35 mg/100g) and finally advanced genotypes (30.23 mg/100g; Fig 4**)**.

Correlation analysis

Significant correlation was observed in all parameters analyzed with some correlating negatively and others positively. Positive significant correlation was found in total phenols (r=0.141***), total flavonoids (r=0.436**), proteins (r=0.219*) with antioxidant activity and total flavonoids (r=0.125***) with total phenols. However, negative correlation was observed in total phenols (r=-0.528**), total flavonoids (r=0.436**) with proteins (Table 2).

Discussion

Crude protein

Significant variation among the 55 genotypes was observed with a range of 17.48 – 24.79 g/100g and a mean of 20.88 g/100g crude protein. The results of this study concur with previous studies that reported crude protein content of 20.5 g/100g (Saxena et al., 2010) and 21 g/100g (Mohammed et al., 2010). However the crude protein content for genotypes evaluated in this study was lower compared to those reported by another study in Côte d'Ivoire with mean of 25.6g/100g (Digbeu et al., 2018). This variation may be attributed to crop production environment, input used in crop production, seed storage, samples processing methods and presence of polyphenols which affect the activity of digestive enzymes which in turn affect the protein quality (Digbeu et al., 2018).

Total phenol

The 55 genotypes of pigeonpea differed significantly in total phenols ranging (20.15 – 84.44 mg/100g) with a mean of 46.21 mg/100g. The advanced elite genotypes recorded the highest (59.57 mg/100g) and improved varieties (39.72mg/100mg) recording the lowest. These results are similar to previous findings (Nneka, 2016) who reported total phenolics content of 20.62 mg/100g in pigeonpea seeds. However, the total phenolic content (74 mg/100g) of the genotypes evaluated in this study was found to be lower than that reported by (Al-Saeedi & Hossain, 2015). Total phenols are produced naturally during growth and development of plants to protect themselves from biotic and abiotic stresses. The variations in total phenolics content among studies might have been influenced by genotypes, differences in maturity period, storage conditions, processing methods, phytochemical quantification methods, and environmental factors (Panche et al.,2016).

Total flavonoid

Total flavonoids content was recorded in a range of 13.87 to33.48 mg/100g with a mean of 23.98 mg/100g among the 55 genotypes analyzed in this study. Other studies reported flavonoids content of 8.65 mg/g (Rani et al., 2014), 8.11 - 16mg/100g (Nneka, 2016) and 1.14 mg/100g (Al-Saeedi &

Table 1. Biochemical composition of 55 pigeonpea genotypes.

Pigeonpea genotypes	Type	Crude protein (g/100g)	Total phenols (mg/100g)	Total flavonoids (mg/100g)	Total antioxidant activity (mg/100g)
ICEAP 00068	Advanced	27.977^{b}	60.173^{g}	30.911^{c}	44.019^{h}
ICEAP 00554	Advanced	28.434^{a}	55.026^{g}	33.481^{a}	38.877^{j}
ICEAP 00557	Advanced	27.785^{c}	52.438^{g}	31.478^{b}	31.951^{n}
ICEAP 00902	Advanced	23.942^{l}	53.771^{g}	25.613^{g}	61.741^{b}
ICEAP 01147	Advanced	22.267^{o}	61.423^{g}	21.431^{m}	46.526^{g}
ICEAP 01147/1	Advanced	22.958^{n}	64.709^{f}	25.562^{g}	31.415^{n}
ICEAP 01150	Advanced	24.492^{j}	63.638^{f}	25.983^{g}	27.828^{q}
ICEAP 01150/1	Advanced	23.989^{l}	59.959^{g}	25.438^{g}	27.249^{q}
ICEAP 01154/2	Advanced	19.539^{u}	67.655^{e}	20.173^{n}	26.203^{q}
ICEAP 01541	Advanced	23.410^{m}	56.904^{g}	22.431^{k}	22.182^{s}
ICEAP 00979/1	Advanced	26.238^{f}	64.210^{f}	27.062^{g}	19.281^{t}
ICEAP 01159	Advanced	26.637^{e}	43.072^{j}	30.210^{d}	34.956^{l}
ICEAP 01179	Advanced	20.229^{s}	26.212^{n}	19.613^{p}	57.227^{d}
ICEAP 00911	Advanced	23.961^{l}	82.769^{b}	25.057^{h}	48.418^{f}
KAT 60/8	Improved	19.344^{v}	47.829^{h}	17.477^{t}	39.029^{j}
ICEAP 00850	improved	19.004^{v}	65.978^{f}	17.812^{s}	82.569^{a}
ICEAP 00550	improved	24.897^{i}	52.446^{g}	27.346^{g}	27.540^{q}
ICEAP 00932	improved	22.490^{o}	78.988^{c}	25.460^{g}	40.861^{i}
ICEAP 00933	improved	22.408^{o}	72.131^{d}	23.422^{j}	30.318^{o}
ICEAP 00936	improved	23.468^{m}	84.445^{a}	25.451^{g}	20.359^{s}
ICEAP 01145	improved	25.591^{g}	58.322^{g}	26.427^{g}	42.186^{i}
ICEAP 01155	improved	24.389^{j}	59.439^{g}	24.069^{i}	39.405^{j}
ICEAP 01157	improved	28.116^{b}	53.647^{g}	29.614^{e}	46.415^{g}
ICEAP 01160	improved	25.972^{g}	42.427^{j}	28.963^{f}	33.056^{m}
ICEAP 01161	improved	24.459^{j}	30.318^{n}	26.058^{g}	41.068^{i}
ICEAP 01162	improved	19.951^{t}	47.713^{h}	19.431^{p}	22.191^{s}
ICEAP 01164	improved	25.522^{g}	44.954^{i}	26.078^{g}	48.191^{f}
ICEAP 01166/2	improved	25.7075^{g}	34.612^{m}	27.451^{g}	49.265^{f}
ICEAP 01167	improved	20.717^{r}	32.506^{m}	19.500^{p}	35.256^{l}
ICEAP 01169	improved	25.092^{h}	37.847^{k}	27.623^{g}	21.081^{s}
ICEAP 01170	improved	26.292^{f}	34.203^{m}	29.512^{e}	46.214^{g}
ICEAP 01172/2	improved	19.685^{u}	27.841^{n}	18.842^{r}	38.976^{j}
ICEAP 01175	improved	19.234^{v}	24.808^{n}	17.286^{t}	34.157^{l}
ICEAP 01181	improved	19.797^{u}	23.392^{n}	20.390^{n}	38.103^{j}
ICEAP 01514/15	improved	20.939^{q}	29.707^{n}	20.193^{n}	20.825^{s}
ICEAP 01525	improved	22.946^{n}	28.588^{n}	24.924^{h}	46.387^{g}
ICEAP 01528	improved	18.174^{x}	26.265^{n}	17.831^{s}	27.914^{q}
ICEAP 01529	improved	22.365^{o}	25.036^{n}	20.212^{n}	35.371^{l}
ICEAP 01530	improved	24.171^{k}	23.939^{n}	25.771^{g}	43.871^{h}
ICEAP 01531	improved	24.807^{i}	33.623^{m}	27.286^{g}	46.946^{g}
ICEAP 01534	improved	25.771^{g}	36.155^{l}	25.627^{g}	41.651^{i}
ICEAP 01535	improved	25.169^{h}	23.112^{n}	26.789^{g}	36.671^{k}
ICEAP 01536	improved	25.834^{g}	26.917^{n}	26.311^{g}	37.081^{k}
ICEAP 01537	improved	27.285^{d}	22.504^{n}	29.883^{e}	57.693^{d}
ICEAP 01538	improved	21.340^{p}	23.541^{n}	22.141^{l}	21.908^{s}
ICEAP 01544/2	improved	26.729^{e}	55.256^{g}	29.739^{e}	23.103^{s}
ICEAP 87105	improved	26.037^{g}	63.655^{f}	27.825^{g}	24.919^{r}
ICPL 7035W	improved	18.846^{w}	23.982^{n}	17.225^{t}	28.971^{p}
ICPL 86012	improved	16.703^{z}	21.983^{n}	13.883^{v}	27.030^{q}
ICPL 87091	improved	19.341^{v}	20.151^{o}	17.866^{s}	51.518^{e}
UGACC 22	Landrace	20.709^{r}	56.608^{g}	19.894^{o}	34.908^{l}
ICEAP 00540	Landrace	23.396^{m}	53.322^{g}	25.445^{g}	44.334^{h}
ICEAP 00777	Landrace	21.202^{p}	52.431^{g}	21.647^{m}	43.732^{h}
Mthawajuni	Landrace	20.071^{t}	68.317^{e}	19.118^{q}	50.780^{e}
MZ 2/9	Landrace	17.765^{y}	60.721^{g}	16.423^{u}	59.388^{c}
Mean		20.883	46.21	23.980	38.131
CV%		2.790	3.170	3.530	3.4300
Tukey's HSD		0.150	2.270	0.250	1.0500

Note: Values are mean of three replicates and Tukey-test (p<0.05) was used to separate the treatment means.

Table 2. Correlation analysis for biochemical composition

Parameters	Phenols	Flavonoids	Proteins	Antioxidant
Phenols	1			
Flavonoids	0.125***	1		
Proteins	-0.528**	-0.408**	1	
Antioxidant	0.141**	0.436**	0.219*	1

* = significant at P ≤ 0.05, **= (P ≤ 0.01) & ***= (P ≤ 0.001).

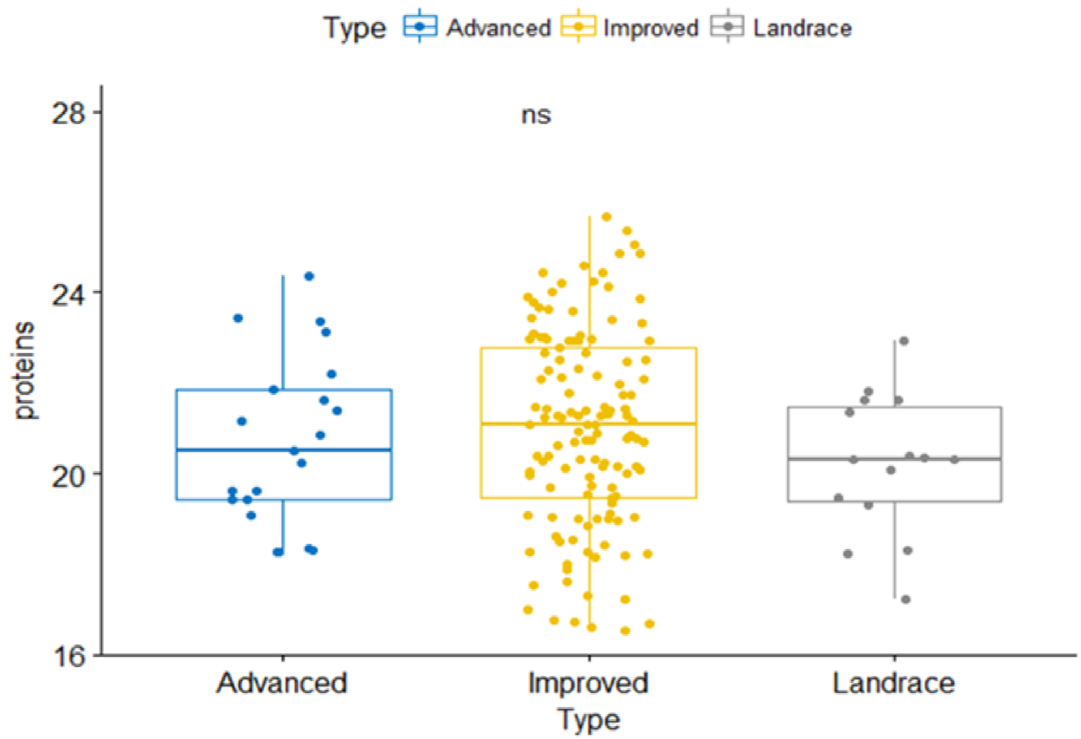

Fig 1. Box plots for crude proteins means (g/100g), ns=not significant (P>0.05).

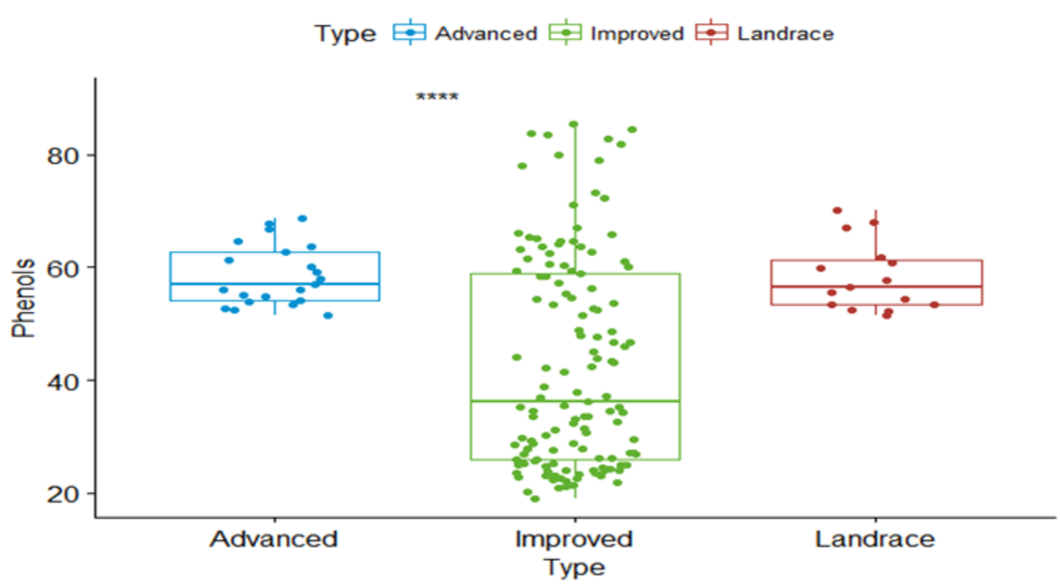

Fig 2. Box plots for total phenols means (mg/100g as gallic acid equivalent), **** = significant at $P \leq 0.001$.

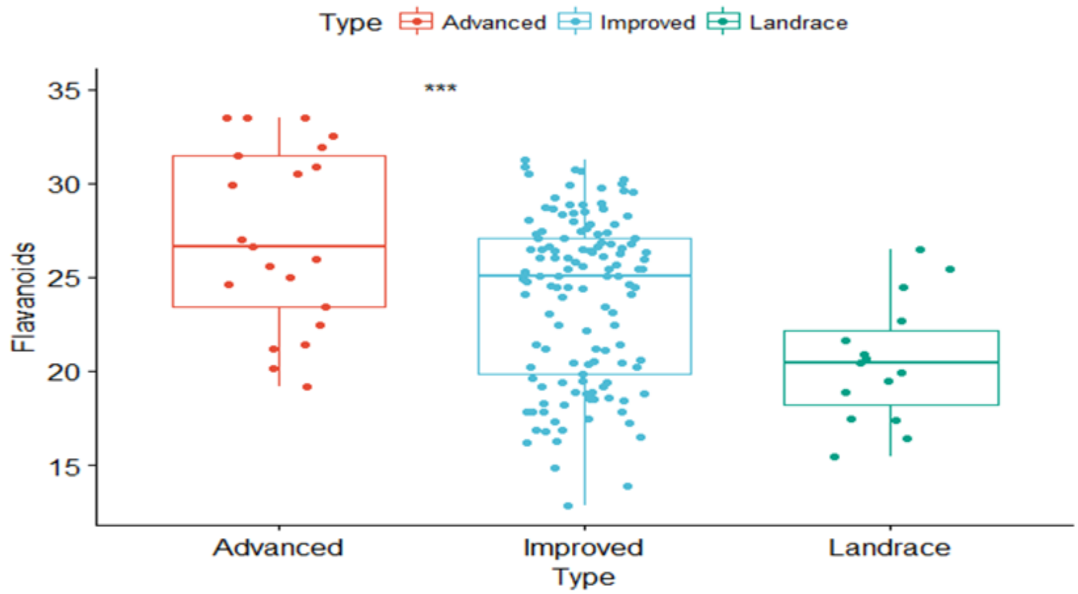

Fig 3. Box plots for total flavonoids means (mg/100g as catechin equivalent), *** = significant at P ⩽ 0.001.

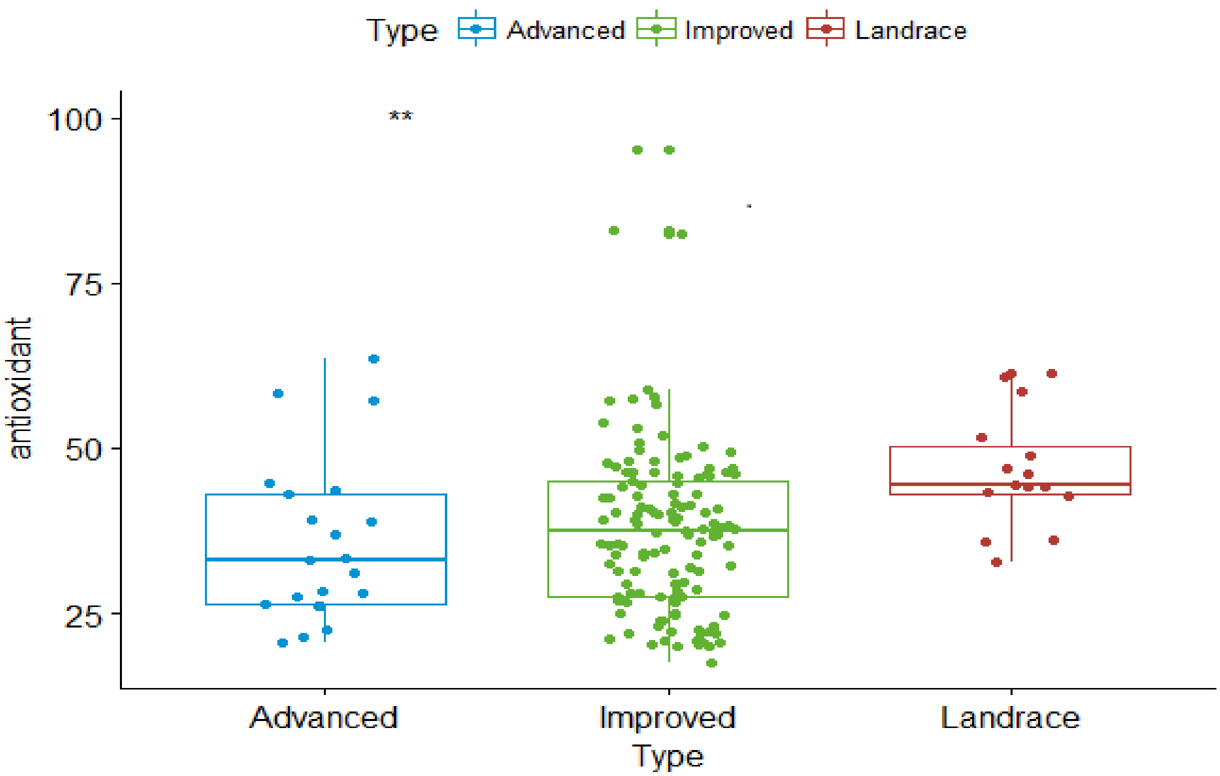

Fig 4. Box plots for total antioxidant activity (mg/100g as Trolox equivalent), ** = significant at P ≤ 0.01.

Hossain, 2015). Variations in total flavonoids content may be due to varied levels of flavonols, flavones, anthocyanidins, catechins, flavanones, and isoflavones in the tested genotypes and sample processing methods (Panche et al. , 2016).

Total anti-oxidant activity

Antioxidant activity of 55 pigeonpea genotypes ranged in between 20.95 to 86.84 mg/100g and with a mean of 38.13 mg/100g. Similar trend was observed with three types of pigeonpea genotypes: the landraces recorded the highest values (40.26mg/100g) followed by improved varieties (32.35 mg/100g) and advanced elite genotypes (30.23 mg/100g). These findings are in agreement with previous studies (Al-Saeedi and Hossain, 2015; Rani et al., 2014). However, a study that analyzed the biscuits prepared from germinated and un-germinated pigeonpea seeds reported higher mean total antioxidant activity (Nneka, 2016). Therefore, antioxidant property results from contribution of phenolic compounds which is mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Tapiero et al., 2002). Similarly, phenolic structures play an important role in bioactive activities. The number and location of hydroxyl groups in phenolic structures are linked to antioxidant activity. Levels of antioxidant activity depend on both concentration and types of phenolics present.

Correlation analysis for biochemical components

Correlation between traits is of great importance for the success of selection practiced in the breeding programs. In this study, the positive correlation between phenolic composition and antioxidant activity shows that total phenol and total flavonoid contents may be important contributors to the antioxidant activity (Stratil et al., 2006). A study on germinated and cooked pulses also reported a positive correlation between total phenols and antioxidant activity (Gujral, 2011). This explains that although plant phenolics have been classified as anti-nutrients, they are useful as natural antioxidants (Bouaziz et al., 2005). Human health is affected by free radicals which play an important role to human health by triggering different chronic diseases like hypertension, diabetes, cancer and heart diseases. Epidemiological studies have shown an inverse association between the risk of chronic human diseases and the consumption of phenolic rich diet (Pandey and Rizvi, 2009). The phenolic groups can accept an electron from relatively stable phenoxyl radicals, thereby distrupting chain oxidation reactions in cellular components by delaying or inhibiting oxidation process (Khanum et al., 2015). Total phenolics are naturally produced during the growth and development of plants to protect them from biotic stresses (Khang et al., 2016). Availability of genotypes with elevated levels of polyphenols and antioxidant activity help to reduce the occurrences of diseases.

Materials and methods

Plant materials

Fifty-five pigeonpea genotypes were received from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Nairobi. The field trials were carried out in Kabete Field Station of the University of Nairobi, Kenya during the long rains (April-September) of 2017. These 55 genotypes were composed of 5 landraces, 14 advanced elite lines and 36 improved cultivars. After harvest, 100g of dry seed samples were collected for crude proteins, total phenols, total flavonoids, and total antioxidant activity analyses. The biochemical analyses were performed at nutrition platform of Biosciences eastern and central Africa- International Livestock Research Institute (BecA-ILRI) Hub, Nairobi, Kenya.

Sample preparation

Pigeonpea seed samples were milled into fine homogenous state using CT 193 Cyclotec™ Sample Mill. Milled samples were analyzed for crude protein, total phenol, total flavonoid, and total antioxidant activity using standard operating procedures described below.

Determination of crude protein

Crude protein analysis was based on Folin-Lowry method (Lowry et al.,1951) with minor modifications. Approximately 100 mg of dried seed samples was weighed in triplicate into 15 ml Falcon tubes, 5 ml of 5% Sodium Dodecyl Sulfate (SDS) was added, vortexed and incubated for 2 hours at room temperature and centrifuged at 2000 rpm for 10 minutes. One hundred micro-litre supernatant was aliquoted into 2ml Eppendorf tube and added with 1900 µl of distilled water to a final volume of 2000 µl. Twenty micro-litre of the diluted extract and bovine serum albumin standard (20-100µg/ml) was aliquoted into respective wells in a 96 well micro-titre plate in duplicates. To each of the sample and standard, 100µl of reagent A (Copper-tartrate-carbonate reagent, 5% SDS, 0.8M NaOH and dH_2O) and 50µl of reagent B (0.4N Folin-Ciocalteu phenol) was added to each well after 20 seconds with gentle priming. The solution was incubated at room temperature for 30 minutes for colour development. Absorbance/optical density (OD) readings were obtained at 630 nm using a BioTek Synergy-HT (Vermont, USA) micro-titer plate reader. The average OD for the two readings of the standards were calculated and used for linear regression analysis. The OD standards and their corresponding protein concentrations were plotted to obtain a linear calibration curve ($r^2 \geq 0.98$) and determine the protein concentration of the test samples.
For quality control purposes BCR 708, a certified reference sample from the Institute for Reference Materials and Measurement, Joint Research Center of the European Commission was included in the analysis. The test samples falling outside the expected range were retested. The relative percent difference (RPD) of each sample was calculated from the duplicate OD readings and samples with RPD values > 10% was retested.

Determination of total phenol

Total phenols were determined following Folin-Ciocalteu method with minor modifications (Kujala et al., 2000). A total of 0.4 g of the milled samples was weighed in a 50ml Falcon tube and added with10 ml of the 80 % methanol. The samples were incubated for 24 hours on a mechanical shaker at 25 °C. The mixture was then centrifuged at 4,000 rpm for 10 minutes; the supernatant was aliquoted for determination of the total phenolic contents in a 96 well micro-titer plate. Upon adding 20 µl of the samples/blank/standards and 100 µl of Folin-Ciocalteu phenol reagent in duplicates at the respective wells, the solution was mixed gently by priming and after 5 minutes, 80 µl of 7 % Na_2CO_3 was added with gentle priming. The plate was covered with an aluminum foil and the reaction was incubated at room temperature for 90 minutes for colour development. The resulting blue colour was measured using BioTek Synergy-HT (Vermont, USA) at 725 nm. External calibration was used for quantification of total phenolics as their corresponding gallic acid equivalent.

The average OD for the two readings of the gallic acid standards (10-100 µg/ml) were calculated and used for linear regression analysis. The obtained OD standards versus their corresponding gallic acid concentrations were plotted to prepare a linear calibration curve ($r^2 \geq 0.98$). The RPD between two readings was calculated as described for total phenolics. The total phenolic content was determined after dilution factor correction and expressed as mg gallic acid equivalent per 100 grams of dry sample.

Determination of total flavonoids

The total flavonoid content was determined using Aluminum chloride colorimetric procedure (Kujala et al., 2000; Zhishen et al.,1999). A total of 0.4 g of the milled samples was weighed into clean 50ml Falcon tubes and added with 10 ml of the 80 % methanol. The samples were incubated on a mechanical shaker at 25 °C for 24 hours. The mixture was then centrifuged at 4,000 rpm for 10 minutes then the supernatant was aliquoted for determination of the total flavonoid contents. Then 20 µl of sample extracts or standard solution of catechin (10-100µg/ml) was aliquoted in duplicate into respective wells of the micro-titer plate, 80 µl of ddH_2O was added followed by addition of 10 µl 5% $NaNO_2$ with gentle priming. After 5 minutes, 10 µl of 10 % $AlCl_3$ was added and gently mixed by priming. After another 5 minutes, 80 µl of 2 M NaOH was added and gently mixed by priming. The reaction was incubated at room temperature for 30 minutes and the absorbance of the samples and standards was measured using a BioTek Synergy-HT (Vermont, USA) microplate reader at a wavelength of 510 nm.

The average OD for the two readings of the catechin standards (10-100 µg/ml) were calculated and used for linear regression analysis. The obtained standards OD versus their corresponding catechin acid concentrations were plotted to prepare a linear calibration curve ($r^2 \geq 0.98$). The relative percent difference (RPD) for each sample was calculated from two OD readings. Sample with RPD value greater than 10 % were retested. The total flavonoid content was determined after dilution factor correction and the results expressed as mg of catechin equivalent per 100 g of dry sample.

Determination of total antioxidant activity

Antioxidant activity was determined using DPPH procedure using Trolox as the standards (Shalaby & Shanab, 2013). The extract prepared for analysis of phenols was used for this assay in a 96 well micro-titre plate. Into the plate, 50 µl of test samples, Trolox standards (10-100µg/ml) and blank were pippeted followed by addition of 50 µl of 60mM DPPH in duplicates. The plates were shaken gently using plate shaker and incubated for 20 minutes. The absorbance was measured in a BioTek Synergy-HT (Vermont, USA) micro-titer plate reader at wave length of 515 nm.

The average OD for the two readings of the Trolox standards (10-100 µg/ml) were calculated and used for linear regression analysis. The obtained standards OD versus their corresponding Trolox concentrations were plotted to prepare a linear calibration curve ($r^2 \geq 0.98$). The RPD for each sample was calculated from two OD readings, and samples where the RPD was greater than 10 % were retested. The total antioxidant activity was determined by adjusting dilution factor and results were expressed in mg of Trolox equivalent per 100g of dry sample.

Correlation analysis

Correlation analysis was undertaken to determine the association effect among crude protein, total phenols, total flavonoids and total antioxidant activity.

Statistical analysis

Three replicates of each sample were used for statistical analysis and resulting values were expressed as mean. One-way analysis of variance (ANOVA) and F-test were carried out using both Genstat version 14 and R soft wares and Tukey test was used to assess the differences between the means at 95% confidence level ($P \leq 0.05$). Correlation analyses of biochemical components were carried out using Pearson correlation programme in SAS.

Conclusion

This study shows variations among test genotypes for total phenolic, total flavonoid contents and total antioxidant activity exhibiting utility of these genetic resources for improving nutritional qualities of pigeonpea. As in other crop species, the environment may have significant role in biochemical composition of pigeonpea, suggesting needs for multilocation evaluations of these genotypes to examine effect of genotype x environment interaction on nutritional qualities. We suggest further research with large number of pigeonpea genotypes for yield and nutritional qualities. The information generated through this research would be useful in developing pigeonpea varieties in Kenya.

Conflicts of interest

The authors declare that they have no competing interests

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