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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 antioxidant activity, respectively. The total antioxidant activity had significantly positive correlation (\leq 0.05) with total phenol, total flavonoid and crude protein. Similarly, total phenol and total flavonoid had significantly positive correlation (\leq 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 \leq 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 \leq 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 \leq 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.

IckP 0005 Advanced 27.97" 60.17" 50.911 44.019 ICKAP 00054 Advanced 27.842" 50.047" 33.441" 38.877" ICKAP 00057 Advanced 27.75" 52.484" 31.478" 31.511" ICKAP 00057 Advanced 27.75" 52.484" 31.478" 31.417" ICKAP 00157 Advanced 22.57" 64.707" 25.555" 27.282" ICKAP 00150 Advanced 24.892" 59.555" 20.137" 26.205" ICKAP 00150 Advanced 24.892" 66.55" 20.137" 26.205" ICKAP 00151 Advanced 24.58" 64.10" 27.05" 19.221" ICKAP 00151 Advanced 23.86" 64.20" 27.05" 49.415" ICKAP 00151 Advanced 24.56" 64.20" 77.45" 79.25" ICKAP 00151 Advanced 24.80" 77.44" 77.45" 79.25" ICKAP 00152 Improved 29.61" 77.84" 79.64" 79.84"	Pigeonpea genotypes	Туре	Crude protein	Total phenols (mg/100g)	Total flavonoids	Total antioxidant activity
ICLAP 00058 Advanced 27.97 ⁺ 0.1173 ⁺ 30.91 ⁺ 4.010 ⁺ ICLAP 00057 Advanced 27.75 ⁺ 52.438 ⁺ 31.431 ⁺ 38.87 ⁺ ICLAP 00057 Advanced 23.92 ⁺ 53.711 ⁺ 25.61 ⁺ 61.744 ⁺ ICLAP 00147 Advanced 23.92 ⁺ 61.475 ⁺ 21.414 ⁺ 45.52 ⁺ 31.141 ⁺ ICLAP 00147 Advanced 23.98 ⁺ 64.09 ⁺ 25.52 ⁺ 31.131 ⁺ ICLAP 00159/1 Advanced 23.98 ⁺ 69.99 ⁺ 25.64 ⁺ 21.92 ⁺ ICLAP 00159/1 Advanced 26.32 ⁺ 64.210 ⁺ 27.92 ⁺ 19.52 ⁺ ICLAP 00159/1 Advanced 26.517 ⁺ 43.07 ⁺ 10.517 ⁺ 43.02 ⁺ ICLAP 00111 Advanced 23.94 ⁺ 82.79 ⁺ 22.40 ⁺ 19.82 ⁺ ICLAP 00111 Advanced 23.94 ⁺ 82.79 ⁺ 23.44 ⁺ 72.50 ⁺ 43.41 ⁺ ICLAP 00250 improved 24.89 ⁺ 72.44 ⁺ 72.50 ⁺ 72.50 ⁺ 72.54 ⁺ <			(g/100g)		(mg/100g)	(mg/100g)
ICAP 00551 Advanced 22.434' 50.02's 33.41' 38.77' ICAP 0057 Advanced 22.942' 53.71' 25.63' 61.74' ICAP 00502 Advanced 22.942' 63.71' 25.63' 61.74' ICAP 0117/1 Advanced 22.98' 64.70' 25.83' 27.245' ICAP 01150 Advanced 23.98' 63.63' 25.83' 27.245' ICAP 01150 Advanced 23.98' 93.95' 25.43' 27.245' ICAP 01150 Advanced 23.98' 63.05' 22.04'' 30.35'' ICAP 0151 Advanced 26.25'' 63.07'' 25.05'' 83.44'' ICAP 00179 Advanced 20.29'' 25.12'' 13.64'' 27.24'' ICAP 00179 Advanced 23.94'' 7.88'' 27.54'' 30.05'' ICAP 00179 Advanced 23.94'' 7.62.12'' 26.63'' 43.64'' ICAP 00179 Advanced 23.94'' 7.81.8''' 27.54''' 43.64''	ICEAP 00068	Advanced	27.977 ^b	60.173 ^g	30.911 [°]	44.019 ^h
ICAP 00957 Advanced 27.785' 52.438' 31.714' 25.613' 61.74' ICAP 01917 Advanced 22.507' 61.423' 71.41' 46.526' ICAP 01917/1 Advanced 22.587' 61.423' 71.41'' 46.526' ICAP 0150/1 Advanced 23.589' 63.684' 25.983' 27.282'' ICAP 0150/1 Advanced 23.510'' 67.655'' 21.17'' 26.032'' ICAP 0150/1 Advanced 23.810'' 67.655'' 21.31'' 25.232'' ICAP 0019/1 Advanced 23.81'' 67.655'' 21.31'' 23.51'' ICAP 0097/1 Advanced 23.81'' 67.657'' 18.13''' 57.66''' ICAP 0097/1 Advanced 23.81'' 67.46''' 23.46''' 23.46''' 23.46''' 23.46''' 23.46''' 23.46''' 23.46''' 23.46''' 23.46''' 23.46''' 23.46''' 23.46''' 23.46''' 23.46''' 23.46''' 23.46''' 23.46''' 23.46''' 23.46''' <t< td=""><td>ICEAP 00554</td><td>Advanced</td><td>28.434^a</td><td>55.026^g</td><td>33.481^ª</td><td>38.877^j</td></t<>	ICEAP 00554	Advanced	28.434 ^a	55.026 ^g	33.481 ^ª	38.877 ^j
ICAP 00902 Advanced 22.392' 57.71' 26.83' 61.741' ICAP 01147/1 Advanced 22.958' 64.709' 25.552' 31.415' ICAP 01150/1 Advanced 22.958' 64.709' 25.552' 31.415' ICAP 01150/1 Advanced 23.989' 59.959' 23.48' 22.387' ICAP 01150/1 Advanced 23.98' 59.959' 23.41'' 26.03'' ICAP 01514/1 Advanced 23.41'' 56.94'' 23.01'' 23.95'' ICAP 01514/1 Advanced 23.02'' 46.12'' 13.65'' 46.12'' ICAP 01514 Advanced 23.02'' 46.12'' 13.65'' 46.12'' ICAP 0050 Improved 23.40'' 79.88'' 23.40'' 49.85'' ICAP 00550 Improved 23.40'' 71.98'' 24.40'' 43.85'' ICAP 00550 Improved 23.40'' 71.31'' 23.40'' 43.65'' ICAP 00550 Improved 23.51''' 53.34'''' 25.51'	ICEAP 00557	Advanced	27.785 [°]	52.438 ^g	31.478 ^b	31.951 ⁿ
ICAP 01147 Advanced 22.257 ² 61.42 ³⁴ 1.413 ¹⁰ 45.256 ¹ ICAP 01150/1 Advanced 22.492 ³ 63.681 ¹ 25.562 ³ 27.828 ¹ ICAP 01150/1 Advanced 23.993 ¹ 67.655 ¹ 20.73 ² 25.037 ³ ICAP 01151/2 Advanced 25.637 ³ 67.655 ¹ 20.173 ² 25.037 ³ ICAP 01151/2 Advanced 26.33 ² 64.20 ¹ 27.037 ³ 25.218 ² ICAP 01159 Advanced 20.23 ² 61.12 ¹⁰ 30.121 ⁰ 39.55 ¹ ICAP 01159 Advanced 20.23 ² 61.12 ¹⁰ 30.121 ⁰ 39.55 ¹ ICAP 0050 Improved 20.23 ² 26.12 ¹⁰ 27.84 ⁰ 27.84 ⁰ ICAP 0050 Improved 22.49 ⁰ 79.89 ⁵ 23.46 ⁰ 27.84 ⁰ ICAP 0050 Improved 23.46 ⁰ 72.131 ¹ 23.42 ⁰ 23.46 ⁰ 23.55 ¹ ICAP 0050 Improved 23.46 ⁰ 73.84 ⁰ 24.64 ⁰ 23.55 ¹ ICAP 0050	ICEAP 00902	Advanced	23.942 ¹	53.771 ^g	25.613 ^g	61.741 ^b
TEAP 01140/1 Advanced 2.2.958' 64.000' 2.5.952' 31.4.15'' ICLAP 01150/1 Advanced 2.3.989' 59.959' 2.5.848' 2.7.2.49'' ICLAP 01150/1 Advanced 2.3.989' 59.959' 2.5.438' 2.7.2.49'' ICLAP 01150/1 Advanced 2.3.281'' 56.040'' 2.2.3.1'' 2.5.13'' ICLAP 01159 Advanced 2.2.62'' 43.072' 2.0.120'' 3.4.956'' ICLAP 00159 Advanced 2.2.62'' 43.072' 2.0.50'' 44.8.18'' ICLAP 00159 Advanced 2.2.63'' 43.072'' 1.9.6.13''' 5.2.27''' ICLAP 00150 improved 1.9.04'' 6.5.178'' 1.7.47'' 3.0.09'' ICLAP 00350 improved 2.4.49'' 7.144'' 2.4.41'' 2.4.41'' 2.4.41'' 2.4.41'' 2.4.41'' 2.4.41'' 2.4.41'' 2.4.41'' 2.4.41'' 2.4.41'' 2.4.41'' 2.4.41'' 2.4.41'' 2.4.41'' 2.4.41'' 2.4.41'' 2.4.41''' 2.4.41''' 2.4.41	ICEAP 01147	Advanced	22 267°	61 423 ^g	21 431 ^m	46 526 ^g
TetAP 01150 ¹ Advanced 24.492 ¹ 63.83 ^k 25.98 ^k 27.83 ^k ICEAP 01150 ¹ /1 Advanced 23.98 ^k 67.65 ^k 20.17 ^k 26.03 ^k ICEAP 01154 ¹ /1 Advanced 26.33 ^k 67.65 ^k 20.17 ^k 26.30 ^k ICEAP 00151 Advanced 26.32 ^k 64.10 ^k 27.43 ^k 13.28 ^k ICEAP 01159 Advanced 20.22 ^k 26.11 ^k 19.51 ^k 57.27 ^k ICEAP 00179 Advanced 20.22 ^k 26.12 ^k 19.51 ^k 57.27 ^k ICEAP 00179 Advanced 20.22 ^k 26.12 ^k 17.81 ^k 85.25 ^k ICEAP 0050 improved 19.34 ^k 47.82 ^k 27.46 ^k 27.54 ^k ICEAP 0050 improved 22.40 ^k 72.13 ^k 23.42 ^k 23.38 ^k ICEAP 0051 improved 23.54 ^k 72.83 ^k 53.42 ^k 24.42 ^k 23.54 ^k ICEAP 00150 improved 23.54 ^k 73.84 ^k 25.42 ^k 43.65 ^k ICEAP 01150 <td< td=""><td>ICEAP 01147/1</td><td>Advanced</td><td>22.207 22.958ⁿ</td><td>64 709^f</td><td>25 562^g</td><td>31 415ⁿ</td></td<>	ICEAP 01147/1	Advanced	22.207 22.958 ⁿ	64 709 ^f	25 562 ^g	31 415 ⁿ
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TEAP 01134/2 Advanced 19 339' 07 555' 20.173' 20.393' ICEAP 0151 Advanced 26.38' 66.20' 20.62' 13.281' ICEAP 00597/1 Advanced 26.33' 66.20' 20.02' 13.281' ICEAP 01139 Advanced 20.23' 26.12'' 25.05'' 34.95' ICEAP 01139 Advanced 23.961' 82.76'' 17.812' 82.56'' ICEAP 0050 Improved 19.34' 47.82'' 17.812' 82.56'' ICEAP 00550 Improved 24.89'' 52.445'' 23.46'' 42.85'' ICEAP 00550 Improved 24.89'' 72.131' 23.422' 30.318'' ICEAP 0033 Improved 25.591'' 58.322'' 24.45'' 44.16'' ICEAP 01157 Improved 25.591'' 58.322'' 24.42'' 42.45'' ICEAP 01157 Improved 25.591'' 58.322'' 24.42'' 44.65'' ICEAP 01157 Improved 25.91'' 73.44''' 25.4	ICEAP 01150/1	Advanced	23.922	59.000 59.950 ^g	25.303	27.020 27.249 ^q
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ILAP UD911 Advanced 2.3 sb. 2.4 rbs 2.3 sb. 2.4 rbs ICEAP 0050 Improved 19.04* 65.978* 17.512* 22.569* ICEAP 0050 Improved 24.489* 52.446* 27.346* 27.540* ICEAP 00502 Improved 22.408* 72.134* 23.422* 30.318* ICEAP 00503 Improved 23.466* 84.445* 25.541* 20.303* ICEAP 01055 Improved 24.369* 59.439* 24.069* 39.405* ICEAP 01155 Improved 24.369* 59.439* 24.069* 39.405* ICEAP 01155 Improved 25.572* 42.427* 28.963* 33.05* ICEAP 01160 Improved 25.522* 44.95* 26.05* 44.06* ICEAP 01164 Improved 25.02* 34.612* 26.05* 43.10* ICEAP 01164 Improved 25.02* 34.612* 27.75* 34.612* 27.62* 27.81* ICEAP 01164 Improved 25.02*	ICEAP 01179	Advanced	20.229	20.212 82.700 ^b	19.013	37.227
Kn toya Improved 19.344 47.629 1.447 39.029 ICEAP (0050) Improved 24.897 52.446 ⁴ 27.346 ⁴ 27.540 ⁴ ICEAP (0050) Improved 22.490 ⁶ 78.988 ⁴ 23.466 ⁴ 20.661 ⁴ ICEAP (0053) Improved 22.408 ⁶ 72.131 ⁴ 23.422 ⁴ 30.318 ⁴ ICEAP (0053) Improved 22.468 ⁶ 84.445 ⁴ 23.421 ⁴ 20.359 ⁴ ICEAP (0114) Improved 25.591 ⁴ 58.322 ⁴ 26.427 ⁴ 42.186 ⁴ ICEAP (01157) Improved 28.116 ¹⁶ 53.647 ⁴ 29.614 ⁴ 46.415 ⁴ ICEAP (01164) Improved 28.572 ⁴ 44.954 ⁴ 26.08 ⁴ 41.068 ⁴ ICEAP (01164) Improved 25.572 ⁴ 44.954 ⁴ 26.078 ⁴ 48.191 ⁴ ICEAP (01167) Improved 25.577 ⁵ 34.61 ² 77.451 ⁴ 49.265 ¹ ICEAP (01167) Improved 25.097 ¹ 32.56 ⁶ 19.500 ⁴ 35.256 ¹ ICEAP (01177) <		Auvanced	23.901	82.769 47.020 ^h	25.057	48.418
ILLAY (0350) Improved 12,004 65.97.8 17.812 82.2509 ILCAP (0352) Improved 22,490° 78.988° 25.460° 40.861° ILCAP (0352) Improved 22,408° 72.131° 23.462° 23.367° ILCAP (0353) Improved 23.468° 84.445° 25.451° 42.186° ILCAP (0155) Improved 23.488° 59.439° 23.661° 46.415° ILCAP (0155) Improved 25.527° 42.427° 28.963° 33.056° ILCAP (0156) Improved 25.522° 44.954° 25.614° 46.415° ILCAP (0156) Improved 25.522° 44.954° 25.038° 43.056° ILCAP (0156) Improved 25.522° 44.954° 25.614° 49.265° ILCAP (0156) Improved 25.027° 34.612° 27.451° 49.265° ILCAP (0157) Improved 25.092° 37.847° 27.633° 21.081° ILCAP (0157) Improved 25.092° 37.847°	KAT 60/8	Improved	19.344	47.829	17.477	39.029
ILEAP (0050) Improved 24.497 52.440° 27.46° 27.440° ICEAP (0053) Improved 22.480° 72.131° 23.422' 30.318° ICEAP (0053) Improved 23.468° 72.131° 23.422' 30.318° ICEAP (0154) Improved 25.591° 58.322' 26.427' 42.186' ICEAP (0155) Improved 28.116° 53.647' 29.614' 46.415' ICEAP (0157) Improved 28.116° 53.647' 29.614' 46.415' ICEAP (0156) Improved 28.572' 42.427' 28.963' 33.056'' ICEAP (01164) Improved 25.572'' 44.427' 28.963' 43.068' ICEAP (01164) Improved 25.572'' 44.427' 28.963' 43.068'' ICEAP (01167) Improved 25.577'' 44.620'' 75.32'' 21.061'' ICEAP (01167) Improved 26.927' 37.847'' 26.528'' 21.91'' ICEAP (01177) Improved 26.927'' 23.8	ICEAP 00850	improved	19.004	65.978	17.812	82.569
ILAP (0932) improved 22.408 72.131 ⁴ 23.422 ⁴ 40.861 ICEAP (0033) improved 23.468 ^a R4.445 ^a 23.462 ^a 20.335 ^a ICEAP (0034) improved 23.591 ^a 58.322 ^a 26.427 ^a 42.186 ^c ICEAP 01145 improved 23.89 ^a 59.439 ^a 24.069 ^a 39.405 ^c ICEAP 01157 improved 28.116 ^b 53.647 ^a 29.514 ^a 46.415 ^a ICEAP 01150 improved 25.972 ^a 42.427 ^a 28.663 ^a 33.056 ^m ICEAP 01151 improved 25.527 ^a 43.954 ^a 26.075 ^a 48.191 ^a ICEAP 01162 improved 25.7075 ^a 34.612 ^m 27.451 ^a 49.265 ⁱ ICEAP 01167 improved 25.027 ^b 34.461 ^a 92.62 ^a 27.451 ^a 49.265 ⁱ ICEAP 01166 improved 25.02 ^a 37.471 ^a 27.62 ^a 38.103 ⁱ ICEAP 01172 improved 25.02 ^a 34.20 ^a 27.85 ^a 34.157 ⁱ ICEA	ICEAP 00550	improved	24.897	52.446°	27.346°	27.540
ICEAP 00933 improved 22.468" 72.131 23.422" 30.318" ICEAP 00936 improved 25.51 ⁴ 55.322" 26.427 ⁵ 42.186' ICEAP 01155 improved 25.51 ⁴ 55.322" 26.427 ⁵ 42.186' ICEAP 01157 improved 28.16 ⁵ 55.647" 29.614" 46.415" ICEAP 01160 improved 25.972" 42.4277" 28.663" 41.668" ICEAP 01161 improved 25.972" 47.713" 19.431" 22.191" ICEAP 01162 improved 25.522" 47.612" 27.607" 49.265" ICEAP 01164 improved 25.022" 37.847" 27.623" 21.081" ICEAP 01167 improved 26.392" 37.847" 29.512" 40.614" ICEAP 01170 improved 26.392" 37.847" 27.623" 21.081" ICEAP 01172 improved 19.939" 29.512" 46.214" ICEAP 01172 improved 29.939" 27.841" 18.842" <	ICEAP 00932	improved	22.490	78.988°	25.460°	40.861
ICEAP 00936 improved 23.68° 84.445° 25.451° 20.359° ICEAP 01145 improved 23.389° 59.339° 24.667° 39.405' ICEAP 01155 improved 23.18° 53.647° 29.514° 64.215° ICEAP 01157 improved 25.972° 42.427° 28.963° 33.056° ICEAP 01161 improved 29.514° 47.713° 19.431° 22.191° ICEAP 01162 improved 25.527° 44.612° 26.058° 49.106° ICEAP 01164 improved 25.527° 44.612° 26.078° 49.54° ICEAP 01166 improved 25.7075° 34.612° 72.623° 21.081° ICEAP 01166 improved 25.027° 37.847° 27.623° 21.081° ICEAP 01167 improved 25.027° 37.847° 27.623° 34.157° ICEAP 01172 improved 19.393° 29.707° 20.193° 34.157° ICEAP 01172 improved 19.737° 23.392° 20.390	ICEAP 00933	improved	22.408	72.131	23.422	30.318
ICEAP 01145 improved 25.591* 58.322* 26.427* 42.186* ICEAP 01155 improved 24.389* 53.439* 20.605* 39.005* ICEAP 01157 improved 25.972* 42.427* 28.963* 33.056* ICEAP 01161 improved 25.972* 42.427* 28.963* 48.19* ICEAP 01162 improved 25.52* 44.954* 26.078* 48.19* ICEAP 01164 improved 25.52* 44.954* 26.078* 48.19* ICEAP 011667 improved 25.02* 37.847* 27.62* 21.08* ICEAP 011667 improved 20.717* 23.206* 29.50* 33.85* ICEAP 01170 improved 20.92* 37.84* 18.842* 38.976* ICEAP 01175 improved 19.50* 23.39* 20.30* 38.10* ICEAP 01172 improved 19.79* 23.39* 20.30* 38.10* ICEAP 01172 improved 29.97* 20.39* 20.80*	ICEAP 00936	improved	23.468	84.445	25.451°	20.359
ICEAP 01155 improved 23.89" 29.409" 29.609" 39.409" ICEAP 01157 improved 25.972" 42.427" 28.614" 46.415" ICEAP 01160 improved 25.972" 42.427" 28.963" 33.056" ICEAP 01162 improved 24.597" 42.427" 28.614" 46.015" ICEAP 01162 improved 24.597" 44.4954" 26.078" 41.066" ICEAP 01167 improved 25.7075" 34.612" 27.451" 49.265" ICEAP 01166/2 improved 25.027" 37.847" 27.623" 21.081" ICEAP 01167 improved 25.027" 37.847" 27.623" 21.081" ICEAP 01172 improved 25.027" 37.847" 27.623" 21.081" ICEAP 01175 improved 29.39" 29.70" 20.133" 20.825" ICEAP 01181 improved 29.39" 29.70" 20.139" 20.825" ICEAP 01525 improved 23.65" 27.844" 45.874	ICEAP 01145	improved	25.591 ^s	58.322 ^₅	26.427	42.186
ICEAP 01157 improved 28.16° 58.64° 29.614° 46.415° ICEAP 01160 improved 24.95° 30.18° 26.058° 41.068° ICEAP 01161 improved 19.951° 47.713° 19.431° 22.191° ICEAP 01164 improved 25.52° 44.954° 26.078° 44.191° ICEAP 01166/2 improved 25.7075° 36.612° 27.451° 49.265° ICEAP 01167 improved 25.092° 37.84° 27.633° 21.081° ICEAP 01170 improved 25.092° 37.84° 27.633° 21.081° ICEAP 01172/2 improved 19.685° 27.841° 18.842′ 38.976° ICEAP 01172/2 improved 19.397° 23.392° 20.399° 34.157° ICEAP 01174/15 improved 22.946° 23.392° 20.193° 20.825° ICEAP 01174/15 improved 22.365° 27.71° 20.133° 27.914° ICEAP 01526 improved 22.365° 27.33°	ICEAP 01155	improved	24.389	59.439 ^s	24.069	39.405
ICEAP 01160 improved 25.972* 4.4.27' 28.963' 33.05''' ICEAP 01161 improved 19.951' 47.713*' 19.431° 22.191*' ICEAP 01162 improved 19.551' 47.713*' 19.431° 22.191*' ICEAP 01164 improved 25.52*' 44.05*' 26.078*' 48.19' ICEAP 01166/2 improved 25.7075*' 34.612'''' 27.451*' 49.265'' ICEAP 01169 improved 26.092''' 37.847*''' 27.623*''' 23.081''''''''''''''''''''''''''''''''''''	ICEAP 01157	improved	28.116	53.647 ⁸	29.614 ^e	46.415 ^g
ICEAP 01161 improved 24.459' 30.318' 26.058' 41.068' ICEAP 01162 improved 25.521' 44.77.13'' 19.431'' 22.191'' ICEAP 01164 improved 25.522'' 44.954' 26.078'' 49.265' ICEAP 01167 improved 25.707.5'' 34.612''' 27.451'' 49.265' ICEAP 01169 improved 25.092''' 37.847'' 27.623'' 46.214'' ICEAP 01170 improved 25.092'' 34.203''' 29.512''' 46.214''' ICEAP 01172/2 improved 19.234'' 24.808''' 17.86'' 34.157'' ICEAP 01175 improved 19.797'' 23.392''' 20.390''' 38.103'' ICEAP 01525 improved 22.946'' 28.588'' 24.924''' 46.387'' ICEAP 01526 improved 24.171'' 23.939''' 27.714''' 43.871''' ICEAP 01530 improved 24.171'' 23.939''' 25.771'''' 43.871''' ICEAP 01536 improved <	ICEAP 01160	improved	25.972 ^g	42.427 ^J	28.963'	33.056
ICEAP 01162 improved 19.951 ¹ 47.13 ¹ 19.431 ⁿ 22.191 ¹ ICEAP 01164 improved 25.7075 ⁶ 34.612 ^m 27.451 ⁶ 49.265 ¹ ICEAP 01169 improved 25.7075 ⁶ 34.612 ^m 27.451 ⁶ 49.265 ¹ ICEAP 01169 improved 25.092 ¹ 37.847 ^k 27.623 ^k 21.081 ^k ICEAP 01170 improved 26.292 ¹ 34.203 ^m 29.512 ⁿ 46.214 ^k ICEAP 01172/2 improved 19.234 ^k 24.808 ^k 17.286 ^k 34.157 ^k ICEAP 01175 improved 19.797 ^m 23.392 ⁿ 20.300 ⁿ 38.03 ^k ICEAP 01175 improved 22.946 ^k 28.88 ^k 24.924 ^k 46.387 ^k ICEAP 01525 improved 22.365 ⁿ 25.035 ⁿ 20.212 ⁿ 35.371 ^k ICEAP 01520 improved 24.171 ^k 23.393 ⁿ 25.771 ^k 46.387 ^k ICEAP 01531 improved 25.163 ⁿ 25.131 ^k 27.286 ^k 46.946 ^k ICEAP 01534 improved 25.163 ^k 26.591 ^k 26.311 ^k 37.081 ^k	ICEAP 01161	improved	24.459 ^J	30.318 ⁿ	26.058 ^g	41.068
ICEAP 01164 improved 25.522* 9.4954' 26.078* 48.191' ICEAP 01166/2 improved 25.7075* 34.612" 27.451* 49.265' ICEAP 01167 improved 20.717* 32.506" 19.500° 35.256' ICEAP 01169 improved 26.922' 34.203" 29.512* 46.214* ICEAP 01170 improved 19.685* 27.441* 18.842' 38.976' ICEAP 01175 improved 19.734* 24.808* 17.286* 34.157' ICEAP 01151 improved 20.939* 20.399* 20.193* 20.212* ICEAP 01525 improved 20.339* 20.399* 20.193* 20.514/15 ICEAP 01526 improved 20.335* 25.036* 17.831* 27.914* ICEAP 01520 improved 24.94* 26.337* 23.393* 25.714* 36.33** ICEAP 01530 improved 24.171* 23.628* 26.121* 35.371 ICEAP 01530 improved 25.714* 3	ICEAP 01162	improved	19.951 ^t	47.713 ^h	19.431 ^p	22.191 ^s
ICEAP 01166/2 improved 25.7075 ⁶ 3.6.12 ^m 27.631 ⁶ 4.9.265 ⁶ ICEAP 01167 improved 25.092 ^h 37.847 ^h 19.500 ^a 35.256 ^c ICEAP 01170 improved 25.092 ^h 37.847 ^h 27.623 ^s 4.6.214 ^s ICEAP 01172/2 improved 19.685 ^h 27.841 ^h 18.842 ^c 38.976 ^c ICEAP 01175 improved 19.739 ^h 23.392 ^h 20.390 ^h 38.103 ^l ICEAP 01175 improved 20.393 ^h 29.707 ^h 20.139 ^a 20.825 ^s ICEAP 01152 improved 22.946 ^h 28.88 ^h 24.92 ^h 46.387 ^s ICEAP 01528 improved 22.365 ^c 25.036 ^h 20.121 ^h 35.371 ^l ICEAP 01529 improved 24.807 ^l 33.623 ^m 27.286 ^k 46.946 ^s ICEAP 01531 improved 25.771 ^k 36.155 ^l 25.677 ^k 41.651 ^l ICEAP 01535 improved 25.834 ^k 26.917 ^h 26.311 ^k 37.08 ^l ICEAP 01536	ICEAP 01164	improved	25.522 ^g	44.954 ⁱ	26.078 ^g	48.191 ^f
ICEAP 01167 improved 20.717' 32.806" 19.500" 35.256' ICEAP 01169 improved 25.092' 34.203" 29.512' 46.214' ICEAP 01170 improved 19.685'' 27.841'' 18.842' 38.976' ICEAP 01177 improved 19.785'' 24.808'' 17.286'' 34.157' ICEAP 01154 improved 19.797'' 23.392'' 20.390'' 38.103' ICEAP 01151 improved 29.394'' 28.588'' 24.924'' 46.337'' ICEAP 01525 improved 23.65'' 25.036'' 20.939'' 29.14'' ICEAP 01528 improved 24.807'' 33.623''' 25.771'' 43.871'' ICEAP 01530 improved 24.807'' 36.155' 25.627'' 41.651' ICEAP 01534 improved 25.169'' 23.112'' 26.789'' 46.671'' ICEAP 01537 improved 25.834'' 25.504'' 21.908'' 21.908'' ICEAP 01538 improved 26.729'' <td< td=""><td>ICEAP 01166/2</td><td>improved</td><td>25.7075^g</td><td>34.612^m</td><td>27.451^g</td><td>49.265^f</td></td<>	ICEAP 01166/2	improved	25.7075 ^g	34.612 ^m	27.451 ^g	49.265 ^f
ICEAP 01169 improved 25.092 ^h 37.847 ^h 77.623 ^f 21.081 ⁱ ICEAP 01170/ improved 26.292 ⁱ 34.203 ^m 29.512 ^s 46.214 ^f ICEAP 01172/2 improved 19.234 ⁱ 27.841 ⁿ 18.842 ⁱ 38.976 ⁱ ICEAP 01175 improved 19.737 ⁱ 23.392 ⁿ 20.390 ⁿ 38.103 ⁱ ICEAP 01175 improved 29.46 ^h 28.588 ⁱ 24.924 ⁱ 46.387 ⁱ ICEAP 01525 improved 28.56 ^s 25.036 ⁱⁿ 20.212 ⁿ 35.371 ⁱ ICEAP 01528 improved 24.174 ⁱ 26.265 ⁿ 17.831 ⁱ 27.914 ^q ICEAP 01530 improved 24.174 ⁱ 23.393 ⁿ 25.771 ⁱ 43.871 ^h ICEAP 01531 improved 25.771 ⁱ 36.62 ⁱⁿ 27.86 ⁱⁿ 46.64 ^{6ⁱⁿ} ICEAP 01536 improved 25.16 ⁱⁿ 23.112 ⁿ 26.789 ⁱⁿ 37.08 ⁱⁿ ICEAP 01536 improved 27.28 ⁱⁿ 27.82 ⁱⁿ 27.98 ⁱⁿ 37.08 ⁱⁿ ICEAP 01537 <td>ICEAP 01167</td> <td>improved</td> <td>20.717^r</td> <td>32.506^m</td> <td>19.500^p</td> <td>35.256¹</td>	ICEAP 01167	improved	20.717 ^r	32.506 ^m	19.500 ^p	35.256 ¹
ICEAP 01170 improved 26.292 ¹ 34.203 ^m 29.512 ⁶ 46.214 ⁴ ICEAP 01172 improved 19.685 ^m 27.841 ⁿ 18.842 ¹ 38.976 ¹ ICEAP 01175 improved 19.797 ⁿ 23.392 ⁿ 20.390 ⁿ 38.103 ¹ ICEAP 01151 improved 19.797 ⁿ 23.92 ⁿ 20.193 ⁿ 20.825 ¹ ICEAP 01525 improved 22.946 ⁿ 28.588 ⁿ 24.924 ^h 46.387 ^s ICEAP 01526 improved 22.365 ^o 25.06 ⁿ 20.193 ⁿ 25.371 ¹ ICEAP 01529 improved 24.807 ¹ 33.62 ^s 25.06 ⁿ 27.286 ^s 46.946 ⁶ ICEAP 01530 improved 24.807 ¹ 36.155 ¹ 25.627 ^s 41.651 ¹ ICEAP 01533 improved 25.834 ⁸ 26.917 ⁿ 26.318 ⁸ 36.671 ^k ICEAP 01536 improved 25.834 ⁸ 26.917 ⁿ 26.818 ^s 37.68 ^s ICEAP 01537 improved 25.834 ⁸ 26.917 ⁿ 26.318 ^s 21.908 ^s <td< td=""><td>ICEAP 01169</td><td>improved</td><td>25.092^h</td><td>37.847^k</td><td>27.623^g</td><td>21.081^s</td></td<>	ICEAP 01169	improved	25.092 ^h	37.847 ^k	27.623 ^g	21.081 ^s
ICEAP 01172/2 improved 19.685 ^u 27.841 ⁿ 18.842 ^r 38.103 ^r ICEAP 01175 improved 19.23 ^v 24.808 ⁿ 17.286 ^t 34.157 ^r ICEAP 01514/15 improved 20.939 ⁿ 20.330 ⁿ 20.133 ⁿ 20.825 ^s ICEAP 01525 improved 22.946 ⁿ 25.58 ⁿ 24.924 ^h 46.387 ^s ICEAP 01528 improved 23.55 ^s 25.036 ⁿ 20.212 ⁿ 35.371 ^l ICEAP 01529 improved 24.171 ^k 23.939 ⁿ 27.71 ^g 43.81 ⁿ ICEAP 01530 improved 24.171 ^k 23.939 ⁿ 27.286 ^g 46.946 ^g ICEAP 01531 improved 25.771 ^g 36.155 ^l 25.627 ^g 41.651 ^l ICEAP 01536 improved 25.83 ^d 26.917 ⁿ 26.311 ^g 37.08 ^l ICEAP 01536 improved 27.85 ^d 25.264 ^s 29.49 ^s 36.671 ^k ICEAP 01537 improved 27.85 ^d 27.91 ^s 36.81 ^s 37.08 ^l ICEAP 01536 improved 26.37 ^g 55.256 ^s 29.739 ^g 23.103 ^s	ICEAP 01170	improved	26.292 ^f	34.203 ^m	29.512 ^e	46.214 ^g
ICEAP 01175 improved 19.234 [°] 24.808 ⁿ 17.286 ^t 34.157 ^t ICEAP 01181 improved 19.79 [°] 23.392 ⁿ 20.300 ⁿ 38.103 ^t ICEAP 01525 improved 20.939 [°] 29.70 ^{rn} 20.193 ⁿ 20.825 ^t ICEAP 01525 improved 22.946 ⁿ 28.588 ⁿ 24.924 ^h 46.387 ^g ICEAP 01528 improved 18.174 [*] 26.265 ⁿ 17.81 ^t 27.914 ^a ICEAP 01530 improved 24.171 ^k 23.939 ⁿ 25.771 ^g 43.871 ^h ICEAP 01531 improved 24.171 ^k 23.939 ⁿ 27.786 ^s 49.94 ^g ICEAP 01536 improved 25.771 ^s 36.655 ^t 25.627 ^g 41.651 ^t ICEAP 01536 improved 25.169 ^h 23.112 ⁿ 26.789 ^g 36.671 ^k ICEAP 01536 improved 27.88 ^d 26.917 ⁿ 26.314 ^g 37.08 ^t ICEAP 01538 improved 26.72 ^g 25.26 ^d 29.739 ^s 23.103 ^s ICEAP 01538 improved 26.72 ^g 55.26 ^f 27.82 ^s 24.99 ^f <	ICEAP 01172/2	improved	19.685 ^u	27.841 ⁿ	18.842 ^r	38.976 ⁱ
ICEAP 01181 improved 19.797 ^u 23.392 ⁿ 20.390 ⁿ 38.103 ⁱ 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 ^k 46.387 ^k ICEAP 01529 improved 22.365 ⁿ 25.036 ⁿ 20.212 ⁿ 35.371 ⁱ ICEAP 01530 improved 24.171 ^k 23.939 ⁿ 27.286 ^k 46.946 ^k ICEAP 01531 improved 24.171 ^k 23.939 ⁿ 27.286 ^k 46.946 ^k ICEAP 01534 improved 25.171 ^k 36.155 ⁱ 25.62 ⁿ 41.651 ⁱ ICEAP 01536 improved 25.834 ^k 26.917 ⁿ 26.311 ^k 37.081 ^k ICEAP 01537 improved 27.85 ^d 22.504 ⁿ 29.88 ^s 57.69 ^d ICEAP 01537 improved 26.73 ^s 63.65 ^s 27.39 ^s 23.10 ^s ICEAP 0154/2 improved 26.33 ^s 63.65 ^s 27.89 ^s 24.91 ^s ICEAP 01538 improved 18.84 ^s 29.82 ^s 24.91 ^s 24.91 ^s <t< td=""><td>ICEAP 01175</td><td>improved</td><td>19.234^v</td><td>24.808ⁿ</td><td>17.286^t</td><td>34.157¹</td></t<>	ICEAP 01175	improved	19.234 ^v	24.808 ⁿ	17.286 ^t	34.157 ¹
ICEAP 01514/15 improved 20.939 ⁹ 29.707 ⁿ 20.193 ⁿ 20.825 ^s ICEAP 01525 improved 22.946 ⁿ 28.58 ⁿ 24.924 ⁿ 46.387 ^s ICEAP 01528 improved 22.365 ^o 25.036 ⁿ 7.831 ^s 27.914 ^q ICEAP 01530 improved 24.171 ^s 23.939 ⁿ 25.771 ^s 43.871 ⁿ ICEAP 01530 improved 24.071 33.623 ^m 27.286 ^s 46.946 ^s ICEAP 01534 improved 25.71 ^s 36.155 ⁱ 25.627 ^s 41.651 ⁱ ICEAP 01536 improved 25.834 ^s 26.97 ⁿ 26.789 ^s 36.671 ^k ICEAP 01537 improved 27.285 ^s 29.917 ⁿ 29.883 ⁿ 57.693 ^d ICEAP 01536 improved 26.729 ^s 25.56 ^k 29.739 ^o 23.103 ^s ICEAP 0154/2 improved 26.03 ^s 23.982 ⁿ 17.225 ^s 24.919 ^s ICEAP 87015 improved 18.846 ^w 23.982 ⁿ 17.225 ^s 28.971 ⁿ ICEAP 87015 <	ICEAP 01181	improved	19.797 ^u	23.392 ⁿ	20.390 ⁿ	38.103 ^j
ICEAP 01525 improved 22.946 ⁿ 28.588 ⁿ 24.924 ^h 46.387 ^g ICEAP 01528 improved 18.174 ^s 26.265 ⁿ 17.831 ^s 27.914 ^a ICEAP 01529 improved 22.365 ^o 25.036 ⁿ 20.212 ⁿ 35.371 ⁱ ICEAP 01530 improved 24.171 ^s 23.939 ⁿ 27.286 ^g 46.946 ^g ICEAP 01531 improved 25.771 ^g 33.623 ^m 27.286 ^g 41.651 ⁱ ICEAP 01534 improved 25.169 ^b 23.112 ⁿ 26.789 ^g 36.671 ^k ICEAP 01536 improved 25.169 ^b 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 ^g 25.526 ^g 29.739 ^g 23.103 ^s ICEAP 0154/2 improved 26.037 ^g 53.5256 ^g 27.325 ^g 28.971 ^g ICEAP 0154/2 improved 16.703 ^s 21.983 ⁿ 17.825 ^s 24.919 ^s ICPL 8031	ICEAP 01514/15	improved	20.939 ^q	29.707 ⁿ	20.193 ⁿ	20.825 ^s
ICEAP 01528 improved 18.174* 26.265" 17.831* 27.914° ICEAP 01529 improved 22.365° 25.036° 20.212° 35.371 ICEAP 01530 improved 24.171* 23.939° 25.771* 43.871* ICEAP 01531 improved 24.807' 33.623" 27.286* 46.946* ICEAP 01534 improved 25.771* 35.621" 25.627* 41.651* ICEAP 01535 improved 25.169* 26.789* 36.671* 37.08* ICEAP 01536 improved 25.834* 26.917" 26.314* 37.08* 37.09* ICEAP 01537 improved 27.285* 22.504" 29.883* 57.693* 37.09* ICEAP 01538 improved 26.729* 55.256* 29.739* 23.90* 24.91* 21.908* ICEAP 8705 improved 26.037* 63.655' 27.825* 28.974* 24.91* ICEAP 8705 improved 18.846" 23.982" 17.225* 28.974* 24.91* ICEAP 8705 improved 19.73* 17.866* 19.894* <td>ICEAP 01525</td> <td>improved</td> <td>22.946ⁿ</td> <td>28.588ⁿ</td> <td>24.924^h</td> <td>46.387^g</td>	ICEAP 01525	improved	22.946 ⁿ	28.588 ⁿ	24.924 ^h	46.387 ^g
ICEAP 01529 improved 22.365° 25.036° 20.212° 35.371 ICEAP 01530 improved 24.174 ^k 23.939° 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 36.615 ^l 25.627 ^g 41.651 ^l 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 26.729 ^e 55.256 ^g 29.739 ^e 23.103 ^s ICEAP 01544/2 improved 26.037 ^g 63.655 ^l 27.825 ^g 24.919 ^d ICPL 80512 improved 18.846 ^m 23.982 ⁿ 17.225 ^s 28.971 ^p ICPL 87091 improved 19.341 ^v 20.151 ^o 17.866 ^s 51.518 ^e UGACC 22 Landra	ICEAP 01528	improved	18.174 [×]	26.265 ⁿ	17.831 ^s	27.914 ^q
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ICEAP 01531 improved 24.807 ⁱ 33.623 ^m 27.286 ^g 46.946 ^g ICEAP 01534 improved 25.771 ^g 36.155 ⁱ 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 26.729 ^e 55.256 ^g 29.739 ^e 23.103 ^s ICEAP 01544/2 improved 26.037 ^g 63.655 ^f 27.825 ^g 24.909 ^f ICEAP 01544/2 improved 18.846 ^w 23.982 ⁿ 17.225 ^f 24.909 ^f ICPL 7035W improved 18.846 ^w 23.982 ⁿ 17.225 ^f 28.971 ^p ICPL 80012 improved 19.341 ^v 20.151 ^o 17.866 ^s 51.518 ^e UGAC 22 Landrace 20.709 ^r 56.608 ^g 19.894 ^o 34.908 ⁱ ICEAP 00540 Landrace 20.071 ^t 68.317 ^e 19.118 ^q 50.780 ^e </td <td>ICEAP 01530</td> <td>improved</td> <td>24.171^k</td> <td>23.939ⁿ</td> <td>25.771^g</td> <td>43.871^h</td>	ICEAP 01530	improved	24.171 ^k	23.939 ⁿ	25.771 ^g	43.871 ^h
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ICEAP 01536 improved 25.834 ^g 26.917 ⁿ 26.311 ^g 37.081 ^k ICEAP 01536 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 01544/2 improved 26.037 ^g 63.655 ^f 27.825 ^g 24.919 ^f ICEAP 87105 improved 16.703 ^z 21.983 ⁿ 13.883 ^v 27.030 ^q 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 ^f 56.608 ^g 19.894 ^o 34.908 ^l ICEAP 00540 Landrace 20.071 ^s 53.322 ^g 25.445 ^g 44.334 ^h ICEAP 00777 Landrace 20.071 ^s 68.317 ^e 19.118 ^q 50.780 ^e Mtawajuni Landrace 20.071 ^s 60.721 ^g 16.423 ^u 59.388 ^c <td>ICEAP 01535</td> <td>improved</td> <td>25 169^h</td> <td>23 112ⁿ</td> <td>26 789^g</td> <td>36 671^k</td>	ICEAP 01535	improved	25 169 ^h	23 112 ⁿ	26 789 ^g	36 671 ^k
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ICPL 7035W improved 18.846 ^w 23.982 ⁿ 17.225 ^t 28.971 ^o ICPL 8012 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 ^s 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 ^o 52.431 ^g 21.647 ^m 43.732 ^h Mthawajuni Landrace 20.701 ^s 68.317 ^e 19.118 ^q 50.788 ^e MZ 2/9 Landrace 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	ICEAP 01344/2	improved	20.729 26.027 ^g	62 655 ^f	23.733	23.103
ICPL 7053W Imploved 16.846 23.382 17.225 28.971 ICPL 86012 improved 16.703 ² 21.983 ⁿ 13.883 ^v 27.030 ^e ICPL 87091 improved 19.341 ^v 20.151 ^o 17.866 ⁵ 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 21.020 ^p 52.431 ^g 21.647 ^m 43.732 ^h Mthawajuni Landrace 20.071 ^t 68.317 ^e 19.118 ^q 50.788 ^e MZ 2/9 Landrace 20.071 ^t 68.317 ^e 19.118 ^q 50.788 ^e 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	ICEAP 8/103	improved	20.037	03.035 22.082 ⁿ	27.825 17.225 ^t	24.919 28.071 ^p
Intproved 16.05 21.985 15.885 27.050 ICPL 87091 improved 19.341 ^v 20.151 ^o 17.866 ⁵ 51.518 ^e UGACC 22 Landrace 20.70 ^g 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 21.020 ^p 52.431 ^g 21.647 ^m 43.732 ^h Mthawajuni Landrace 20.071 ^t 68.317 ^e 19.118 ^q 50.7808 ^e MZ 2/9 Landrace 20.0883 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		improved	16.040	23.962 21.082 ⁿ	17.225 12.992 ^V	28.971 27.020 ^q
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ICEAP 00540 Landrace 23.396 53.322 25.445° 44.334 ICEAP 00777 Landrace 21.00 ^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 20.071 ^t 68.317 ^e 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		Landrace	20.709	50.0U8"	19.894	34.908
Icanorace 21.202 52.431° 21.647 43.732° Mthawajuni Landrace 20.071 to 68.317° 19.118° 50.780° MZ 2/9 Landrace 17.765 V 60.721 to 23.980 59.388° 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		Landrace	23.396	53.322°	25.445°	44.334
Mitawajuni Landrace 20.0/1' 68.31/' 19.118' 50.780' MZ 2/9 Landrace 17.765' 60.721 ⁸ 16.423'' 59.386' 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'		Landrace	21.202 ⁻	52.431°	21.647	43./32
M2 2/9 Landrace 17.765' 60.721° 16.423' 59.388' 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	ivitnawajuni	Landrace	20.0/1	68.31/ ⁻	19.118"	50.780
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	MZ 2/9	Landrace	1/.765'	60.721°	16.423	59.388
LV% 2.790 3.170 3.530 3.4300 Tukey's HSD 0.150 2.270 0.250 1.0500	Mean		20.883	46.21	23.980	38.131
Tukey's HSD 0.150 2.270 0.250 1.0500	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
1				

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



Type 🖨 Advanced 🚔 Improved 🚔 Landrace





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





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



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

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 ungerminated 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.

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 $\mu l.$ 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 dH₂O) 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 ($r^2 \ge 0.98$) 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 μ l of 7 % Na₂CO₃ 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 ($r^2 \ge 0.98$). 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 ddH₂O was added followed by addition of 10 μ l 5% NaNO₂ with gentle priming. After 5 minutes, 10 µl of 10 % AlCl₃ 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 μ g/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 ($r^2 \ge 0.98$). 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 μ l of test samples, Trolox standards (10-100 μ g/ml) and blank were pippeted followed by addition of 50 μ l 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 μ g/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 ($r^2 \ge 0.98$). 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. Oneway 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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