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ORIGINAL PAPER

POTENTIAL BIOAVAILABILITY OF CALCIUM, MAGNESIUM, IRON, MANGANESE AND ZINC FROM SEEDS OF DIFFERENT CHICKPEA AND PEANUT LANDRACES*

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Abstract

Although seeds of many leguminous crops are rich in minerals, their availability to humans is limited due to antinutrients, e.g. phytic acid, which forms stable complexes with metal ions. In this context, 19 chickpea and 13 peanut local landraces were tested in order to determine concentrations of main antinutrients and promoters that affect the availability of mineral nutrients: phytic acid, glutathione, free soluble phenolics and yellow pigment, as well as mineral elements: inorganic P, Ca, Mg, Fe, Mn and Zn. Chickpea and peanut seeds are rich sources of mineral elements as well as promoters that improve their availability. High variability among the examined landraces presents the opportunity for their exploitation in breeding for increased bio-availability. This was particularly supported by the relatively low phytic acid concentration found in seeds of both species. Chickpea is richer in Ca, Fe, Mn and Zn than peanut, which is richer in Mg. Positive correlations between phenolics, yellow pigment and Zn in chickpea seeds, as well as between phenolics and Ca in peanut seeds could contribute to the improved bio-availability of these minerals. Peanut exhibited higher variability than chickpea in terms of potential bio-availability of mineral elements. Among chickpea landraces, C15 could be considered as an Fe source and C12 – as an Mn and Zn source. Among peanut landraces, P5 could be considered as an Mn source, P2 and P3 as an Mg source, P11 as an Fe source and P2 as Zn source. These genotypes could be recommended and used in biofortification programs.

Keywords: chickpea, peanut, mineral elements concentration, antinutrients, promoters, nutritive value.

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INTRODUCTION

Legume seeds are rich sources of proteins, starch, dietary fibres and oligosaccharides. Due to a low lipid content as well as a balanced content of highly digestible proteins (JUKANTI et al. 2012), some legumes, like chickpea, have successfully replaced animal proteins in nutrition, having great importance for developing countries, where protein intake and deficiencies in mineral nutrients, especially of Mg, Fe and Zn, are predominant (WELCH, GRAHAM 2004). Moreover, chickpea seeds are poor in sulphur-containing amino acids and rich in some vitamins (riboflavin, niacin, thiamin, folate and β-carotene) and mineral elements, like Zn, Se, Fe, Ca, Mg, K, Cu, P (ZIA-UL-HAQ et al. 2007, JUKANTI et al. 2012), making it a valuable source of minerals in diet. It was reported that a single portion of chickpea could satisfy the majority of the recommended daily allowance (RDA) of minerals (THAVARAJAH, THAVARAJAH 2012).

Peanut is another important leguminous source of proteins and oils, the latter being the reason for its high caloric value. Peanut seeds are also rich in polyphenolics of high antifungal and antioxidative activity (NEPOTE et al. 2005). Its positive impact on the human health is manifested through an unchanged blood lipid level, with increased glutathione and high-density lipoprotein (HDL-C) concentrations in patients with hyperlipidemia (EMEKLI--ALTURFAN et al. 2007). Peanuts could also be successfully used to combat malnutrition in developing countries (ENSERINK 2008).

Although seeds of many leguminous crops are rich in minerals, their availability to humans are limited, since legume seeds are rich in antinutrients, like phytic acid, which forms stable complexes with metal ions (URBANO et al. 2000). Other antinutrients, like fibers or phenolics, could also supress the availability of mineral elements, while promoters, like ascorbic acid, S-containing amino acids, etc., could enhance nutrient availability (HURRELL 2003, WELCH, GRAHAM 2004). Compared to other legumes, including peanuts (URBANO et al. 2000), chickpea seeds are relatively low in phytic acid (THAVARAJAH, THAVARAJAH 2012). SHARMA et al. (1996) showed that chickpea genotypes with less phytic acid and phenolics have increased the Ca and Fe availability, irrespective of the Ca and Fe concentrations in seeds. What is more, germination increases the bioavailability of certain mineral nutrients, as well as levels of promoters in leguminous sprouts (DUHAN et al. 2002, BAINS et al. 2014). For sustained bioavailability, however, phytic acid and some other antinutrients could have favourable effects on the human health, reflected in the reduction of cancer development or lower risk of various metabolic and cardiovascular diseases (GRAHAM et al. 2001). Thus, the homeostasis between antinutrients and promoters in foodstuff could be beneficial to the human health.

In view of the above, 19 chickpea and 13 peanut local landraces were tested in order to determine concentrations of main antinutrients and promoters that affect the availability of mineral nutrients, phytic acid (expressed as phytic phosphorus), glutathione, free soluble phenolics and yellow pigment, as well as mineral elements, i.e. inorganic P, Ca, Mg, Fe, Mn and Zn.

MATERIAL AND METHODS

Plant material

Seeds of local chickpea and peanut landraces from the Macedonian Genebank were tested in this study.

Preparation of samples and chemical analysis

An average sample (containing 100 uniform seeds) for each landrace was milled in a Perten 120 mill, made in Sweden, to the particle size < 500 µm. Oil-defatted samples, aimed for phytic P (P_{phy}), inorganic P (P_i), glutathione (GSH) and free soluble phenolics determination, were obtained by the extraction with petroleum ether. After the extraction with 5% trichloroacetic acid, P_{phy} and P_i from the samples were determined spectrophotometrically (Biochrom Libra S22 UV/Vis Spectrophotometer – Biochrom, UK) by the method of Dragičević et al. (2011), while GSH was determined by the method of Sari Gorla et al. (1993). Free soluble phenolics were also determined spectrophotometrically, after the extraction with double distilled water (Simić et al. 2004) and expressed as µg of 3-hydroxy-4-methoxycinnamic acid g^{-1}. Yellow pigment (YP) was determined after the extraction with water-saturated butanol by the method described by Vancetovic et al. (2014) and expressed as µg of β-carotene equivalent (βCE) g^{-1}. After wet digestion with $HClO_4$ + HNO_3, the concentration of mineral elements (Ca, Mg, Fe, Mn and Zn) was determined with inductively coupled plasma-optical emission spectrometry (Spectroflame, 27.12 MHz and 2.5 kW, model P, Spectro Analytical Instruments, Kleve, Germany).

Statistical analysis

Analyses of the seed chemical composition were performed in four replicates (n = 4) and subjected to one-way ANOVA. Significant differences between landraces means were determined by the Fisher's least significant difference (LSD) test at the 0.05 probability level. Also, the coefficient of variation (CV) was determined for chickpea and peanut landraces, as well as for ratios between phytic and inorganic P (P_{phy}/P_i) and between phytic acid (PA) and the examined elements: PA/Mg, PA/Ca, PA/Fe, PA/Mn and PA/Zn. Correlation analyses were performed using the Pearson's correlation coefficient. Principal component analysis (PCA) was used for evaluation of interdependence between the analysed promoters (GSH and YP), as well as analysed antinutrients (P_{phy} and phenolics) and mineral elements. Statistical analysis was processed by SPSS 15.0 (IBM Corporation, Armonk, New York, USA) for Windows Evaluation version.

RESULTS

Chemical composition of the chickpea and peanut seeds

Significant variability in concentrations of the examined promoters, antinutrients and mineral elements is observed among chickpea and peanut landraces (Table 1). Compared to peanut, chickpea seeds are richer in GSH, YP, Ca, Fe, Mn and slightly richer in P_{phy} and Zn, but poorer in phenolics and Mg. The lowest variability among chickpea and peanut landraces was found for the GSH concentration (12.8% and 7.5%, respectively), while the highest variability was in the P_{phy} concentration in chickpea seeds (20.8%), as well as in the YP concentration in peanut seeds (39.7%). Among the minerals examined, the highest variability in the Fe and Zn concentration (41.9% and 46.2%, respectively) was observed for chickpea landraces, while the highest variability in the Ca and Mn concentration (41.9% and 39.4%, respectively) was recorded for peanut landraces (Table 1).

Interdependence between promoters, antinutrients and mineral elements

Significant and positive correlations between P_i and P_{phy} and P_i and Mg, respectively, were observed in chickpea and peanut seeds (Table 2). A similar trend is noticed for the Fe concentration, while the Mn concentration correlated positively only with P_{phy} in chickpea seeds. In peanut seeds, however, P_i positively correlated with YP and Mn and negatively with GSH, while P_{phy} correlated positively with phenolics and Ca. An increase in the GSH concentration was followed by a significant increase in phenolics in chickpea seeds and a Zn decrease in peanut seeds. In addition, an increase in the concentration of phenolics was followed by a YP and Zn increase in chickpea and a Ca increase in peanut seeds. The correlation trend was followed by a significant decrease in the Ca concentration in chickpea, and the Mg and Zn concentrations in peanut seeds, as well. YP correlated positively with Zn in chickpea and with Ca in peanut seeds. In chickpea seeds, however, a negative correlation between YP and Ca was found. Positive correlations between mineral elements were observed mainly in chickpea: between Mg and Fe, Ca, Fe and Mn, as well as Mn and Zn. In peanut seeds, positive correlations were found between Mg and Zn and Ca and Fe, while negative correlations were between Mg, Ca and Fe (Table 2).

The PC analysis indicated that first two axes for chickpea landraces explained 75.1% and 67.2% of total variability for examined promoters, antinutrients and mineral elements, respectively, as well as 63.6% and 67.6%

Table 1

Analysis of variance for the effect of genotype on phytic (P_{phy}) and inorganic P (P_i), yellow pigment (YP), glutathione (GSH), phenolics, Ca, Mg, Fe, Mn, and Zn contents in seed of 19 chickpea landraces and 13 peanut landraces

Specification			P_{phy}	P_i	GSH	YP	Phenol.	Ca	Mg	Fe	Mn	Zn
			(mg g^{-1})		(nmol g^{-1})	(µg g^{-1})						
Chickpea	Source of variation	df	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS
	replication	3										
	landrace	18	1.83*	25.58*	270930*	33.1325*	12792*	8102*	3966.7*	253.39*	16.67*	574.8*
	error	19	0.0043	0.0078	61119	0.0081	720	136	43.2	0.501	0.126	27.8
	CV (%)		20.8	26.4	12.8	15.9	20.7	10.3	8.6	41.9	21.2	46.2
	LSD		0.0678	0.082	247.2	0.0897	26.8	11.66	6.57	0.708	0.355	5.27
	min		2.39	7.06	1496.77	15.48	522.74	516.88	450.94	15.53	10.19	14.84
	max		4.46	14.83	2364.26	29.21	1075.11	737.19	603.12	53.12	21.97	52.97
	average		3.24	9.56	2026.97	20.96	858.73	616.22	515.63	26.89	14.81	26.58
Peanut	replication	3										
	landrace	12	1.32*	9.96*	23593*	6.9459*	162164*	33446*	8664.1*	242.68*	15.86*	9.7*
	error	13	0.0211	0.136	62.1	0.0136	24073	1740	84.8	0.493	0.0826	4.31
	CV (%)		24.4	23.1	7.5	39.7	20.4	41.9	8.2	21.9	39.4	9.52
	LSD		0.1453	0.369	7.88	0.1167	155.2	41.71	9.21	0.702	0.287	2.077
	min		1.44	4.74	907.54	1.76	727.33	144.37	658.44	0.31	4	20
	max		2.96	10.15	1232.45	6.69	1369.56	500.31	875	23.28	14.06	28.25
	average		2.35	6.83	1018.39	3.83	988.81	308.56	795.75	15.02	7.15	24.05

* Significant at 5% probability level, df – degrees of freedom, MS – mean squares.

Table 2

Correlation between examined promoters (GSH and YP), as well as antinutrients (P_{phy} and phenolics) and mineral elements for 19 chickpea and 13 peanut landraces

Specification		P_i	P_{phy}	GSH	Phenolics	Yellow pigment	Mg	Ca	Fe	Mn
Chickpea	P_{phy}	0.846*								
	GSH	0.058	0.090							
	phenolics	-0.105	0.107	0.723*						
	yellow pigment	0.020	0.111	0.121	0.415*					
	Mg	0.759*	0.777*	-0.133	-0.234	-0.040				
	Ca	0.022	0.016	-0.269	-0.419*	-0.427*	-0.031			
	Fe	0.521*	0.622*	0.070	0.150	0.191	0.507*	0.320*		
	Mn	0.205	0.303*	0.014	0.211	-0.197	0.277	0.410*	0.588*	
	Zn	-0.124	-0.153	0.257	0.460*	0.500*	-0.188	-0.056	0.021	0.324*
Peanut	P_{phy}	0.342*								
	GSH	-0.423*	0.106							
	phenolics	0.181	0.315*	-0.074						
	yellow pigment	0.490*	0.238	-0.057	0.248					
	Mg	0.346*	-0.138	-0.217	-0.371*	-0.021				
	Ca	-0.049	0.380*	-0.195	0.580*	0.511*	-0.328*			
	Fe	-0.061	0.227	0.062	-0.079	-0.009	-0.506*	0.340*		
	Mn	0.473*	0.036	0.037	0.221	0.125	0.279	-0.292	-0.292	
	Zn	-0.090	-0.199	-0.420*	-0.376*	0.167	0.518*	0.098	-0.201	0.015

* Correlation is significant at 0.05 probability level.

for peanut landraces, respectively. For chickpea landraces, projection of the variables pointed that the GSH and phenolics concentrations contributed to PCA1 mainly (0.833 and 0.943; respectively, Figure 1), while P_i and P_{phy} to PCA2 (0.965 and 0.954, respectively). Also, Fe and Mn contributed mostly to PCA1 (0.792 and 0.901, respectively), while PCA2 was defined by Mg and Zn (0.756 and -0.758, respectively). The landraces C6, C7, C11, C13, C14 and C17 accumulate higher P_i and P_{phy} content, while C15 is important for the accumulation of GSH (and to a lesser extent, landraces C11, C17 and C18), yellow pigments and phenolics. Also, the genotypes C12 and C15 are the main Ca and Mn accumulators, while C6, C7, C13 and C14 are the main Mg accumulators. Moreover, C10 and C12 are important for Zn accumulation (Figure 1).

For peanut landraces, PCA1 was defined mainly by P_{phy} (0.798; Figure 2) and Ca (0.808), while PCA2 was determined by P_i and GSH (0.738 and -0.884, respectively), as well as by Mg and Zn (0.783 and 0.905, respectively).

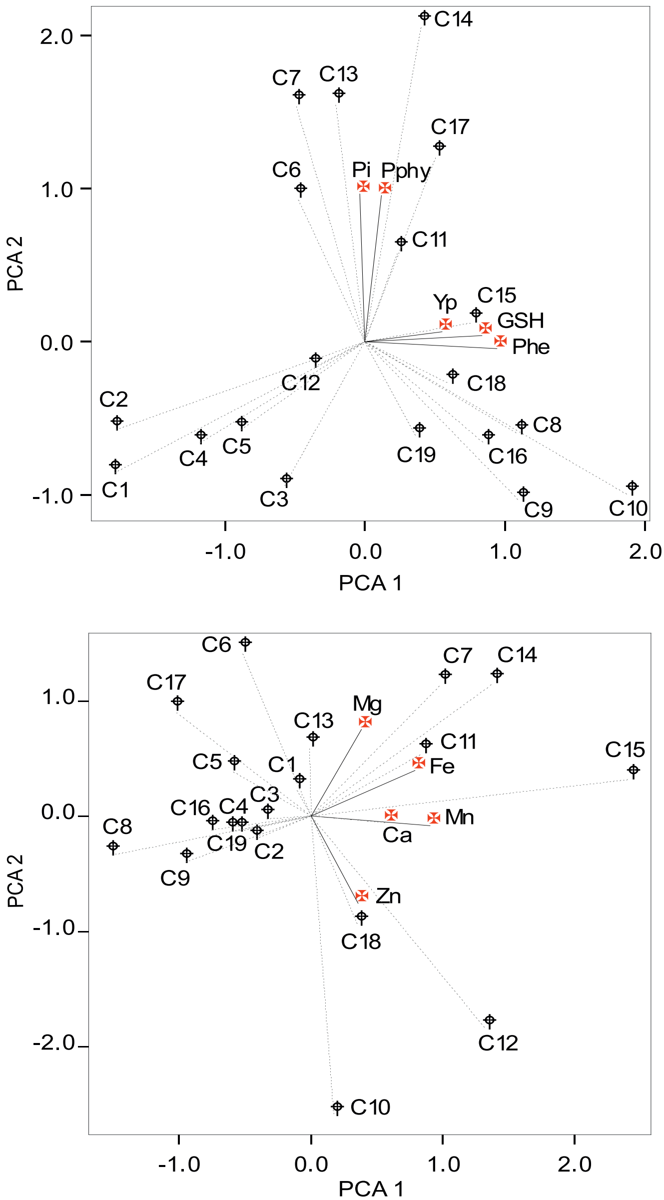

Fig. 1. Principal Component Analysis for phytic P(P_{phy}), inorganic P(P_i), yellow pigment (YP), glutathione (GSH), phenolics (Phe), Mg, Ca, Fe, Mn and Zn concentration in examined chickpea landraces

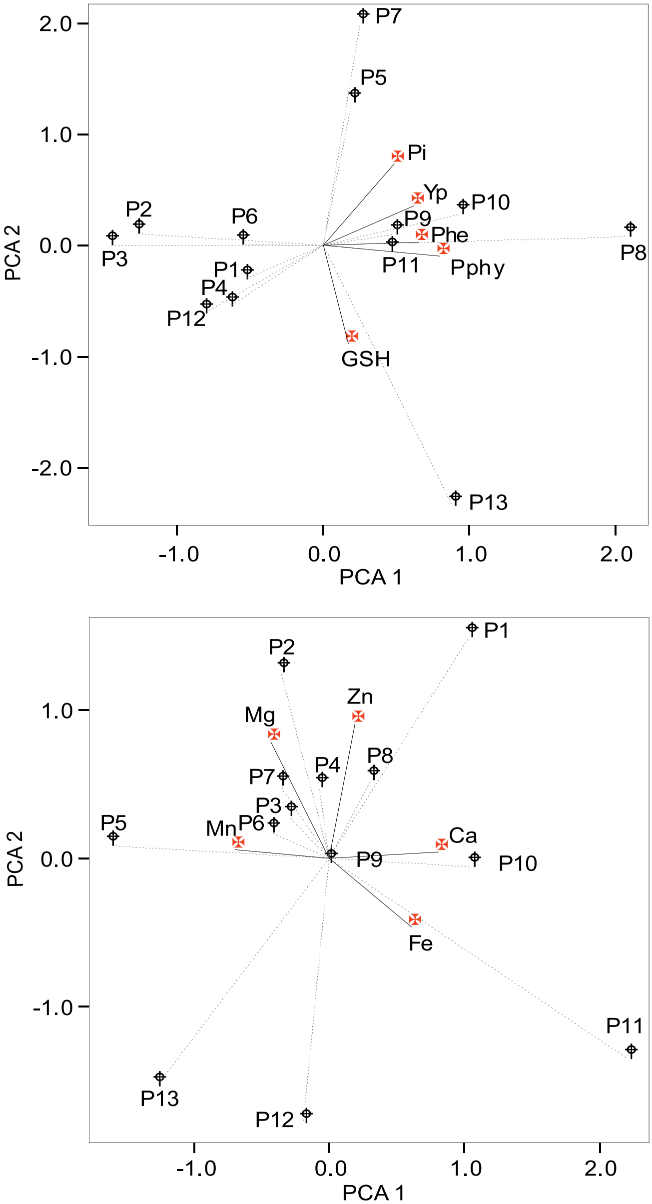

Fig. 2. Principal Component Analysis for phytic P(P_{phy}), inorganic P(P_i), yellow pigment (YP), glutathione (GSH), phenolics (Phe), Mg, Ca, Fe, Mn and Zn concentration in examined peanut landraces

The landraces P8, P9, P10 and P11 accumulate higher amounts of P_{phy} and phenolics, P13 is high in GSH, while P5, P7 and P10 are high in yellow pigment. Important landraces for Mg accumulation are P2, P3, P4, P6 and P7; P9 and P10 are important landraces for Ca accumulation. The main Fe accumulators are P9, P10 and P11; genotypes P3, P5, P6 and P7 are Mn accumulators, while landraces P1, P2, P4 and P8 are the main Zn accumulators (Figure 2).

Potential availability of mineral elements

High variability was observed for the ratio between phytic acid and mineral elements, ranging from 0.35 (P_{phy}/P_i) to 29.4 (PA/Mn) for chickpea seeds and from 0.36 (P_{phy}/P_i) to 49.3 (PA/Mn) for peanut seeds, on average (Table 3). The highest variability among landraces was found for PA/Zn (37.2%) in chickpea seeds and for PA/Ca (53.2%) in peanut seeds. For chickpea, landraces with the lowest ratio values were: C2 for P_{phy}/P_i, C1, C2, C3 and C9 for PA/Mg, C3 for PA/Ca, C15 for PA/Fe, C12 for PA/Mn and PA/Zn, respectively. For peanut genotypes, landraces P2, P3 and P4 are with the lowest ratio values: P2 for PA/Mg, PA/Mn and PA/Zn, P3 for PA/Mg and PA/Fe and P4 for P_{phy}/P_i and PA/Ca, respectively.

DISCUSSION

High variability in the examined mineral elements and factors that affect their bio-availability in chickpea and peanut landraces present great potential for improved nutritional quality breeding programs. Opposite to the finding that chickpea is low in sulphur-containing amino acids (Zia-Ul-Haq et al. 2007, Jukanti et al. 2012), a relatively high average GSH concentration with slight but significant variability gives a great opportunity for the examined landraces to be used as a GSH source in human nutrition. In parallel, high concentration and variability of yellow pigment found in chickpea landraces support its potential to be used as an important vitamin source in nutrition (Jukanti et al. 2012). Irrespective of the high antioxidant activity of peanut seeds (Blomhoff et al. 2006), they are low in yellow pigment and GSH (when compared to chickpea). Nevertheless, peanut consumption increases the GSH level in hyperlipidemic patients, expressing beneficial effects on health (Emekli-Alturfan et al. (2007). Antinutrients, such as phytic acid and phenolics, varied in a wider range among chickpea landraces, indicating also differences in digestibility and availability of mineral elements. Besides, chickpea is considered as a legume with a relatively low content of phytic acid (Thavarajah, Thavarajah 2012), making it a necessary part in the human diet. Significant and positive correlations between phenolics and GSH and yellow pigment, respectively, could stimulate the promoting role of GSH and yellow pigment in the absorption of mineral elements (Welch,

Table 3

Molar ratios between phytic P (P_{phy})/inorganic P (P_i), phytic acid (PA)/Mg, PA/Ca, PA/Fe, PA/Mn and PA/Zn

Landrace		P_{phy}/P_i	PA/Mg	PA/Ca	PA/Fe	PA/Mn	PA/Zn
Chickpea	C1	0.36	0.64	0.82	14.99	24.26	17.45
	C2	0.26	0.65	0.79	16.22	23.72	15.94
	C3	0.33	0.65	0.76	15.74	24.85	20.28
	C4	0.36	0.76	0.90	19.05	28.12	24.39
	C5	0.38	0.73	1.05	17.14	27.13	22.88
	C6	0.34	0.84	1.45	22.11	40.40	24.17
	C7	0.30	0.92	1.36	11.24	34.02	20.28
	C8	0.32	0.79	1.00	22.90	34.91	23.32
	C9	0.31	0.65	1.00	15.00	23.85	10.68
	C10	0.33	0.69	0.91	13.54	23.95	6.05
	C11	0.30	0.77	1.34	15.22	23.57	12.23
	C12	0.32	0.82	0.96	19.71	18.26	7.54
	C13	0.30	0.98	1.30	22.27	39.90	27.80
	C14	0.30	1.03	1.51	10.99	34.03	23.35
	C15	0.43	0.91	1.08	9.12	21.89	19.24
	C16	0.42	0.82	1.21	16.81	27.66	18.70
	C17	0.35	0.99	1.73	26.25	43.19	31.76
	C18	0.45	0.88	1.14	18.14	29.00	9.40
	C19	0.44	0.83	1.12	16.27	30.36	17.65
	average	0.35	0.82	1.14	17.09	29.38	18.65
	CV (%)	15.14	15.01	23.23	25.59	23.50	37.25
Peanut	P1	0.52	0.44	1.78	20.93	89.95	12.74
	P2	0.24	0.22	1.29	17.15	22.72	7.10
	P3	0.24	0.23	1.40	12.30	28.77	7.64
	P4	0.17	0.25	1.16	14.93	31.39	8.63
	P5	0.38	0.39	3.66	20.62	22.81	12.69
	P6	0.26	0.41	3.28	23.43	71.78	14.87
	P7	0.29	0.35	1.78	23.89	45.53	12.79
	P8	0.44	0.48	1.27	37.36	40.96	15.57
	P9	0.41	0.45	1.36	24.30	39.73	16.00
	P10	0.40	0.48	1.24	27.72	81.17	14.55
	P11	0.58	0.52	1.18	15.34	79.89	15.57
	P12	0.29	0.35	1.57	15.24	41.40	10.92
	P13	0.43	0.50	4.05	21.75	44.59	19.37
	average	0.36	0.39	1.92	21.15	49.28	12.96
	CV (%)	33.53	26.41	53.18	31.46	47.29	27.81

Graham 2004, Germano, Canniatti-Brazaca 2011). Irrespective of the significant correlation between P_i and P_{phy}, seeds of the examined chickpea landraces have significant variability and higher P_i concentration in respect to P_{phy}, indicating also better P absorption. On the other hand, a higher level of P_{phy} and phenolics as well as a positive correlation between them observed in the examined peanut seeds contribute to its high antioxidative properties (Blomhoff et al. 2006). It is well known that peanut is rich in phytate and phenolics (Erdman 1979, Nepote et al. 2005), as a consequence having a potentially reduced bio-availability of mineral elements like Zn, Mg, Ca, Fe and P. In this research, peanut landraces have relatively lower P_{phy} concentrations in seeds.

Chickpea and peanut are rich sources of mineral elements, as well. The examined landraces demonstrate significant variability in concentrations of essential mineral elements: Ca, Mg, Fe, Mn and Zn, similarly to the results obtained by Asibuo et al. (2008), Phan-Thien et al. (2010) and Thavarajah, Thavarajah (2012). Chickpea is higher in Ca, Fe, Mn and Zn than peanut, with the variability ranging from 8.6% in Mg up to 46.2% in Zn concentrations. For peanut landraces, the variability ranges from 8.2% also for Mg, to 41.9% for Ca. This means that the examined landraces could be favourable genetic sources in breeding for increased concentrations of mineral elements in seeds. Nevertheless, their potential bio-availability could be restrained by antinutrients, since the increased Mg and Fe accumulation in chickpea seeds and Ca accumulation in peanut seeds are followed by an increased P_{phy} concentration. Also, the positive correlation between phenolics and Zn in chickpea seeds as well as between phenolics and Ca in peanut seeds could restrain their bio-availability (Welch, Graham 2004). However, the positive correlation between yellow pigment and Zn in chickpea seeds could contribute to its better bio-availability.

Molar ratios between phytic acid and mineral elements are the best way to describe their potential bio-availability to humans (Ma et al. 2007, Queiroz et al. 2011, Dragičević et al. 2015). In general, the potential bio-availability of the examined mineral elements ranges from 15.01% for PA/Mg to 37.25% for PA/Zn ratio for chickpea landraces, as well as from 26.41% also for PA/Mg to 53.18% for PA/Ca ratio for peanut landraces. This means that peanut has higher variability in the potential bio-availability of mineral elements than chickpea. According to the PC analysis, C14 and C17 chickpea landraces accumulate higher P_{phy} content, while C15 accumulates mainly GSH, yellow pigment and phenolics. The landrace C15 is also rich in Fe, with the lowest PA/Fe ratio, indicating higher Fe bio-availability. It is well known that chickpea varieties with low phytate and phenolics have better Fe and Ca bio-avaialbility, irrespective of their concentrations in seeds (Sharma et al. 1996). The landrace C12, as the main Mn accumulator and having the lowest PA/Mn ratio, could be considered as a good Mn source. Genotypes C6, C7, C13 and C14, as the main Mg accumulators, have high PA/Mg ratios, indicating its reduced bio-availability. Moreover, C10 and C12 are important for Zn accumulation, with the lowest PA/Zn ratio, pointing to its improved bio-availability. Among peanut accessions, P8, P9, P10 and P11 landraces accumulate higher amounts of antinutrients, P_{phy} and phenolics. Genotype P5 is a landrace with high yellow pigment and Mn content and low PA/Mn ratio that contributes to its better bio-availability. Genotypes important for improved Mg bio-availability are P2 and P3, while for Fe and Zn bio-availability they are P11, i.e. P2 peanut landraces.

CONCLUSIONS

Chickpea and peanut seeds are rich sources of mineral elements, as well as promoters that elevate the availability of mineral elements. Chickpea is richer in Ca, Fe, Mn and Zn than peanut, which is richer in Mg. Positive correlations between phenolics, yellow pigment and Zn in chickpea seeds as well as between phenolics and Ca in peanut seeds could stimulate bio-availability of these mineral elements. In addition, more pronounced variation in the potential bio-availability of mineral elements was found in peanut than in chickpea landraces. Among chickpea landraces, C15 could be considered as an Fe source, C12 as an Mn and Zn source, while among peanut landraces, P5 could be considered as an Mn source, P2 and P3 as an Mg source, P11 as an Fe source and P2 as a Zn source. These genotypes could be recommended and used in biofortification programs.

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