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Determination of oligosaccharide fraction in a worldwide germplasm collection of chickpea (*Cicer arietinum* L.) using high performance liquid chromatography

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Abstract

Awareness on nutritive value and health benefits of legumes is of vital importance in order to increase the consumption of legumes in daily diet of the human beings. Despite of high nutritional value, it contains one of the most important antinutritional compounds such as the Raffinose Family Oligosaccharides (RFOs). The RFOs are predominantly found in the seeds of leguminous crops and are indigestible by humans and animals causing flatulence or diarrhea. In the present study, a total of 213 chickpea accessions were analysed for seed oligosaccharides (Sucrose, Raffinose, Ciceritol, and Stachyose) through high performance liquid chromatography with the aim of selecting promising accessions with low RFOs and high sucrose. Data with three replications was used to measure Pearson's simple correlation co-efficient in order to establish the relationship among various oligosaccharide fractions. The results showed that the sucrose content ranged from 3.57 mg g^{-1} to 54.12 mg g^{-1} with 17 accessions contained more than 35 mg g^{-1} and 10 accessions had less than 10 mg g^{-1}. The stachyose content ranged from 2.77 mg g^{-1} to 59.43 mg g^{-1} with 12 accessions had less than 11 mg g^{-1}. Ciceritol was the main sugar found in all the samples ranged from 4.36 mg g^{-1} to 90.65 mg g^{-1}. The identified germplasm accessions with unique sugar profiles especially those with low RFO will be valuable in breeding specialty chickpea for improved sugar content.

Keywords: Germplasm collection, Chick pea, Raffinose family oligosaccharides, Flatulence, Core collection.
Abbreviations: ICRISAT_International Crops Research Institute for the Semi-Arid-Tropics, RFOs_Raffinose Family Oligosaccharides, HPLC_High Performance liquid Chromatography

Introduction

Chickpea (*Cicer arietinum* L.) is a self-pollinating cool season food legume crop. It ranks second in area and third in production among the pulse crops worldwide (FAO, 1994; Zia Ul – Haq et al., 2007). Globally chickpea crop is cultivated in more than 50 different countries in an area of 11.9 million ha with a production of 10.9 million tons and an average yield of 911 kg ha^{-1} (FAOSTAT, 2012). Chickpea is rich in protein (20-22%) and serves an important source of fiber, minerals (phosphorus, calcium, magnesium, iron and zinc) and vitamins namely β-carotene, thiamin, riboflavin and niacin (Gaur et al., 2010). The overall nutritional status of chickpea shows that it posses 891 g kg^{-1} dry matter content, 217 g kg^{-1} crude protein, 78 g kg^{-1} crude fibre, 43 g kg^{-1} ether extract, 32 g kg^{-1} ash and 619 g kg^{-1} nitrogen-free extract (Dixon and Hosking, 1992). Chickpea is highly consumed by millions of people living in developing countries including India, Pakistan and Bangladesh. In fact, the developing countries contribute about 96% of the global chickpea production. In the recent years, chickpea is cultivated in several developed countries including Australia, Canada and USA for export purposes. For instance, area under chickpea cultivation has drastically increased from 3100 ha to 500,000 ha in the past three decades in Australia (FAOSTAT, 2012). It is mainly due to ever-growing demand for chickpea from various Asian and Middle East countries and also due to their valuable role in the cropping systems (Siddique and Sykes, 1997; Siddique et al., 2008 and 2012). Although endowed with many nutritionally important components, chickpea contain significant amounts of the oligosaccharides of the raffinose family. These oligosaccharides attract attention because of their known tendency to cause flatulence (Swennen et al. 2006; Kumar et al. 2010). Flatulence in humans after consumption of raffinose family oligosaccharides (RFOs) from grain legumes is caused by their inability to hydrolyze the β-1, 6-galactoside linkages in RFOs that end up in the lower gut (Calloway and Murphy, 1968). Bacterial fermentation of RFOs in the colon leads to the formation of methane, hydrogen, and carbon dioxide. Legume scientists and growers consider flatulence as a single most undesirable factor that may restrict the intake of more

Table 1. Correlations between individual sugars in 213 chickpea germplasm accessions.

Description	Sucrose	Raffinose	Ciceritol	Stachyose	Total sugars
Sucrose	**1**				
Raffinose	0.207*	**1**			
Ciceritol	0.577*	0.208*	**1**		
Stachyose	0.415*	0.291*	0.588*	**1**	
Total sugars	0.741*	0.382*	0.894*	0.822*	**1**

*Significant at the 0.05 probability level.

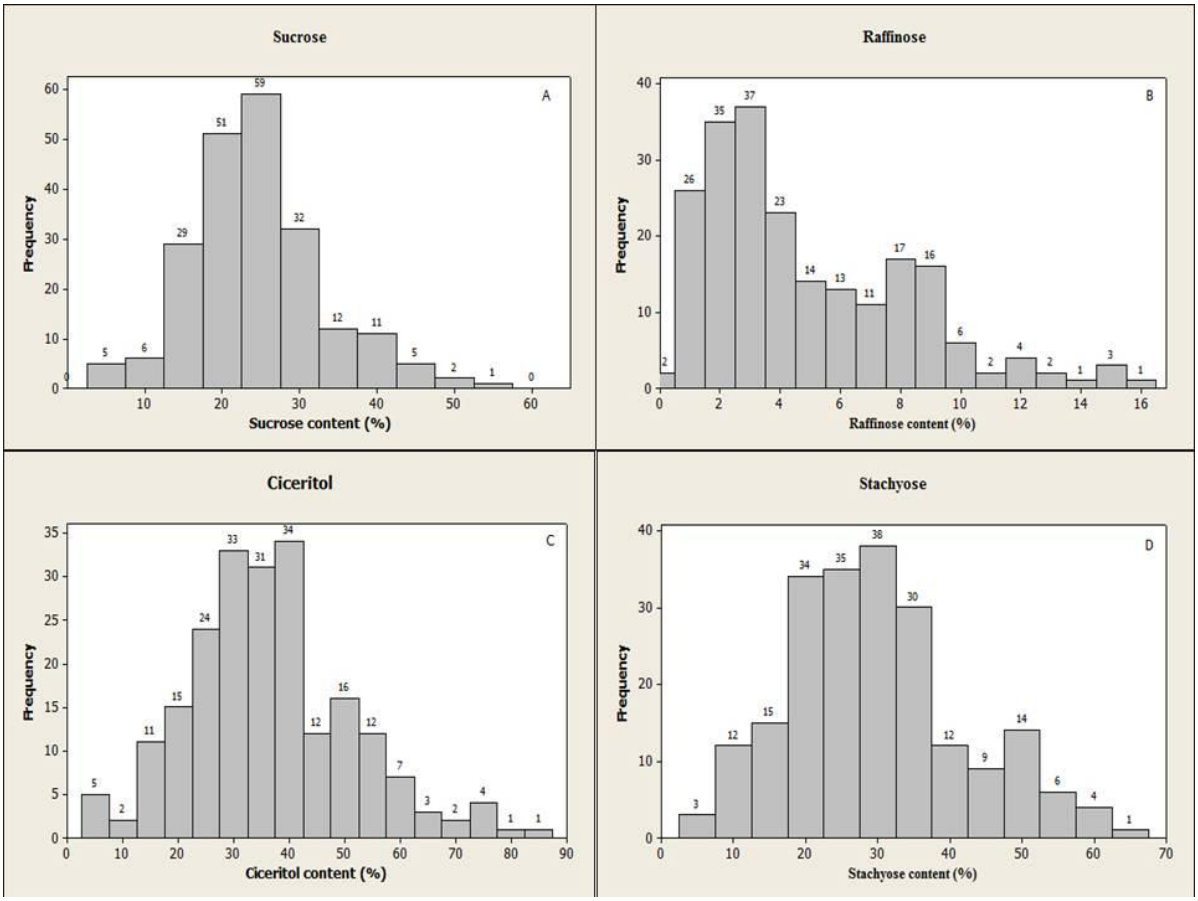

Fig 1. Distribution of the sugar profiles in 213 chickpea minicore collection as determined by HPLC system (a) Sucrose, (b) Raffinose (c) Ciceritol (d) Stachyose. The x-axis represents the range of sugar content, and the Y-axis, the number of accessions.

chickpea products. Therefore, to convert chickpea as a more acceptable source of inexpensive protein, it is pertinent to reduce the flatulence production. Oligosaccharides are functional food ingredients having great potential to improve Variability for sugar composition among cultivars has been reported in chickpea. The soluble sugars routinely detectable include glucose, fructose, sucrose, raffinose, ciceritol and stachyose. The amount of sucrose in chickpea seed ranges from 1.80 to 5.22 % of total dry matter (Xiaoli et al., 2008). The other three major sugar components of chickpea seed are ciceritol, stachyose and raffinose in their order of predominance. Ciceritol varies from 1.2 to 3.1 % among cultivars (Xiaoli et al., 2008; Bernabe et al., 1993; Quemener and Brillouet, 1983). Ciceritol is not correlated with flatulence owing to its rapid hydrolysis resulting from its varied structure from other α-galactosides (Quemener and Brillouet 1983). High total sugars in chickpea seeds especially sucrose is essential to achieve proper fermentation the quality of many foods. Experiments with human subjects have demonstrated that legume species and cultivars exhibit wide variations in flatus production (Sanchez and Tucker, 1966; Liener, 1980; Arora, 1983; Hill-Cottingham, 1983). levels for its usage in bread making (Hatzikamari et al., 2007). However, α-galactosides, such as stachyose and raffinose are undesirable in chickpeas as they are not readily digestible and cause flatulence and diarrhea (Price et al., 1988; Swennen et al., 2006; Kumar et al., 2010).

Even though some information on variability of the sugar composition of chickpea germplasm is available (Mulimani and Ramalingam, 1997; Alajaji and El-Adawy, 2006), but all these studies have dealt with few accessions from narrow genetic and geographical backgrounds. Hence, it is imperative to explore huge collections of chickpea germplasm from a wider geographical origin to identify promising types with more desirable sugar composition so that they can be employed in breeding programs aiming at

Table 2. List of chickpea accessions with high sucrose content.

ICC number	Origin	Mean±SE				
		Sucrose (mg g^{-1})	Raffinose (mg g^{-1})	Ciceritol (mg g^{-1})	Stachyose (mg g^{-1})	Total sugars (mg g^{-1})
12654	Malawi	54.12±0.356^{a}	14.03±0.356^{a}	50.40±0.475^{abcde}	30.35±0.356^{abcd}	148.89±1.543^{abc}
9137	Morocco	47.78±2.019^{ab}	04.83±0.118^{a}	71.79±2.163^{a}	52.93±1.900^{ab}	177.34±6.20^{a}
11198	Ethiopia	45.40±1.306^{ab}	05.39±0.237^{a}	58.56±1.781^{abcd}	49.21±3.445^{abc}	158.56±6.769^{abc}
12328	Ethiopia	44.29±1.306^{ab}	11.81±0.118^{a}	58.64±2.613^{abcd}	33.91±0.475^{abcd}	148.65±4.512^{abc}
12307	Ethiopia	43.74±1.663^{ab}	14.10±3.620^{a}	56.58±1.887^{abcde}	25.99±0.712^{bcd}	140.41±7.882^{bcd}
12155	Cyprus	43.66±2.969^{ab}	10.46±1.187^{a}	39.78±0.000^{cde}	18.23±0.475^{d}	112.12±4.631^{de}
14098	Myanmar	41.20±1.425^{ab}	05.86±0.237^{a}	48.89±0.356^{abcde}	57.61±4.157^{a}	153.57±6.175^{abc}
11944	Bangladesh	40.49±1.781^{ab}	05.39± 0.237^{a}	70.44±1.069^{ab}	51.27±3.207^{abc}	167.59±6.294^{ab}
9402	Nepal	40.02±0.831^{ab}	02.85±0.950^{a}	64.26±2.257^{abc}	26.15±0.000^{bcd}	133.28±4.038^{cd}
15406	India	39.62±1.187^{ab}	01.74±0.000^{a}	32.01±0.237^{de}	24.09±2.138^{bcd}	097.46±3.562^{e}
10341	Turkey	39.22±0.593^{ab}	03.41±0.118^{a}	45.72±1.781^{abcde}	48.49±2.138^{abcd}	136.85±4.630^{cd}
1356	Iran	38.35±0.237^{b}	04.83±0.118^{a}	50.48±2.257^{abcde}	47.70±0.712^{abcd}	141.36±3.324^{bcd}
4593	Iran	38.03±0.150^{b}	02.30±0.118^{a}	41.05±0.000^{bcde}	59.43±0.950^{a}	140.81±1.218^{bcd}
8058	Iran	37.40±1.663^{b}	00.87±0.118^{a}	56.10±0.000^{abcde}	47.46±0.356^{abcd}	141.84±2.137^{bcd}
12851	India	36.85±1.069^{b}	05.63±0.118^{a}	63.00±2.732^{abc}	45.96±4.303^{abcd}	151.43±8.223^{abc}
1392	India	36.69±1.782^{b}	02.85±1.425^{a}	44.77±1.306^{abcde}	21.55±0.475^{cd}	105.86±4.988^{e}
16374	India	36.53± 1.069^{b}	01.58±0.000^{a}	26.23±1.306^{e}	26.07±0.831^{bcd}	090.41±3.206^{e}
	Mean±SE	41.38±1.260^{ab}	05.76±0.540^{a}	51.69±1.30^{abcde}	39.2±1.560^{abcd}	138.03±4.66^{bcd}

*Genotypes with high sucrose exhibiting moderate glycemic index can serve as an alternative to artificial sweeteners in confectionery food items, while sucrose with high glycemic index (e.g white sugar) may not be suitable form of the healthy diet.
Mean values within a column followed by different letters are significantly different ($p < 0.05$)

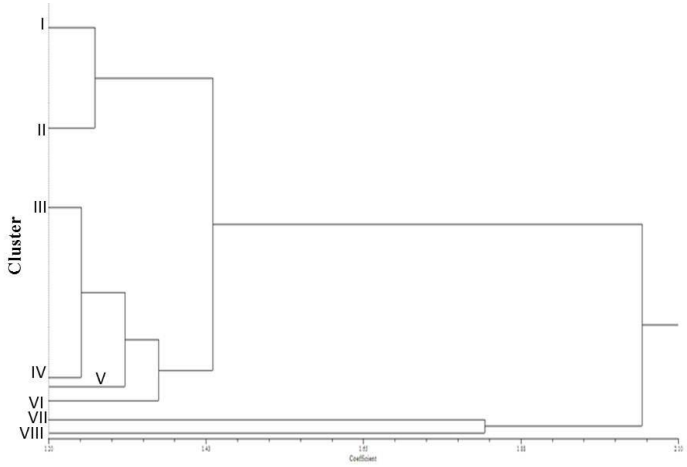

Fig 2. Dentrogram showing grouping of genotypes in different clusters.

Table 3. Descriptive statistics for the sugar profiles of 213 genotypes of chick pea mini-core collection.

S.No.	Description	Mean±SE (mg g^{-1})	Minimum (mg g^{-1})	Maximum (mg g^{-1})
1.	Sucrose	23.07±0.936	3.57	54.12
2.	Raffinose	04.65±0.447	0.16	15.13
3.	Ciceritol	34.47±1.067	4.36	90.65
4.	Stachyose	28.08±2.905	2.77	59.43
5.	Total sugars	90.26±5.355	15.53	177.34

Table 4. List of chickpea accessions with low stachyose content lower than 11 mg g^{-1}.

ICC number	Origin	Mean±SE				
		Sucrose (mg g^{-1})	Raffinose (mg g^{-1})	Ciceritol (mg g^{-1})	Stachyose (mg g^{-1})	Total sugars (mg g^{-1})
7323	Russia & CISs	21.71±0.475^{ab}	6.66±0.950^{a}	24.17±0.831^{ab}	10.62±0.237^{a}	63.15±2.493^{abc}
2507	India	25.52±0.950^{ab}	2.69±1.663^{a}	35.5±1.187^{a}	10.54±0.118^{a}	74.25±3.918^{a}
2919	Afghanistan	19.65±0.712^{ab}	2.69±1.425^{a}	19.57±0.356^{ab}	10.38±0.356^{a}	52.3±2.849^{abcde}
2242	Russia & CISs	13.00±0.712^{ab}	1.27±0.237^{a}	36.29±1.425^{a}	09.98±0.475^{a}	60.54±2.849^{abcd}
6306	Iran	9.03±0.475^{ab}	2.46±0.118^{a}	11.81±0.118^{ab}	08.24±0.475^{a}	31.54±1.186^{def}
3761	Russia & CISs	05.94±0.356^{ab}	0.79±0.000^{a}	06.42±0.118^{ab}	07.84±0.356^{a}	21.00±0.830^{f}
6811	Russia & CISs	08.16±0.593^{ab}	2.54±0.475^{a}	17.43±0.237^{ab}	07.84±0.118^{a}	35.97±1.423^{cdef}
6263	Iran	5.71±0.237^{ab}	0.16±0.000^{a}	10.06±0.118^{ab}	07.45±0.237^{a}	23.38±0.592^{ef}
9895	Iran	14.50±0.118^{ab}	3.01±0.475^{a}	14.90±0.237^{ab}	07.37±0.118^{a}	39.78±0.948^{bcdef}
12968	Iran	05.15±2.019^{ab}	3.41±0.118^{a}	04.36±0.118^{b}	03.17±0.237^{a}	16.09±2.492^{f}
2072	India	34.31±1.069^{a}	2.30±1.069^{a}	27.34±0.118^{ab}	02.93±1.306^{a}	66.88±3.562^{ab}
13816	India	03.57±0.831^{b}	0.16±0.000^{a}	09.03±0.237^{ab}	02.77±1.306^{a}	15.53±2.374^{f}
	Mean±SE	13.85±0.712^{ab}	2.35±0.544^{a}	18.07±0.425a	7.43±0.445^{a}	41.70±2.126^{bcdef}

Mean values within a column followed by different letters are significantly different ($p < 0.05$).

quality improvement. Hence, the present investigation was taken up to evaluate sugar profiles through HPLC analysis in 213 accessions of the international minicore collection of chickpea.

Results and discussion

Separation and quantification of sugar profiles

Four individual sugars were detected with the HPLC system derived from 213 accessions of chickpea. Ciceritol, stachyose and sucrose were the major sugars and present in large amounts whereas Raffinose was the minor sugar and present in relatively small amounts. Previous studies quantified similar sugar profiles in the seeds of chickpea and soybean (Xiaoli et al., 2008, Singh et al., 1982, Harpal and Edward, 1984 and Hou et al., 2009). Based on their retention time, chickpea seed sugars were eluted in the HPLC system in the sequence of sucrose, raffinose, ciceritol and stachyose. Significant difference was detected for all the soluble sugars among 216 accessions of chickpea. The distribution frequencies of sucrose, raffinose, ciceritol and stachyose in 216 accessions of chickpea were presented in Fig 1. The majority of chickpea accessions exhibited a regular sugar profile in which sucrose, ciceritol and stachyose were the major components. The high sucrose type chickpea accessions can be used as culinary purposes in the preparation of sweets like Thandoori dishes, Jalebi or Imarti, Laddu and Motichoor. The low stachyose type is fairly rare and highly desirable because it can minimize or eliminate the indigestibility problem of chickpea that causes unpleasant odors and flatulence or diarrhea for humans and animals. So far only one genotype with 16 mg of stachyose content has been reported by Xiaoli et al. (2008).

In the present investigation, we have identified 12 germplasm accessions with less than 10 mg stachyose content and they will serve as new source for seed quality improvement in chickpea breeding program. Similar results were also reported in soybean by Hartwig et al. (1997), Hymowitz and Collins (1974), Hymowitz et al. (1972) and in lentil by Tahir et al. (2011).

Correlation analysis

Significant correlation was detected among the four individual sugars and the total sugar (Table 1). Correlation analysis revealed that total sugar content was positively and significantly correlated with sucrose (r=0.741), raffinose (r= 0.382), ciceritol (r= 0.894) and stachyose (r= 0.822). Positive and significant correlation existed between raffinose and stachyose (r=0.291), raffinose and sucrose (0.207) and stachyose and sucrose (0.415). The positive correlations suggested concurrent accumulation of sugars in chickpea seed. This suggests that the breeders may take advantage of these positive correlations in changing multiple traits simultaneously or using one as an indirect selection indicator for another. Similar results were reported in soybean by Hou et al. (2009) and Hymowitz et al. (1972). Very low level of sucrose was reported in Vicia by Lattanzio et al. (1986). In the previous study of Xiaoli et al. (2008) with 19 cultivars of chickpea, the sucrose content was ranged from 18 to 52 mg g-1. Several accessions had low amount of raffinose and stachyose content, all of which are valuable for the development of chickpea types with reduced oligosaccharide content. The positive correlations existing between the sugars will be beneficially exploited by the breeders to improve sugar profiles. Further investigation is needed to improve the

Table 5. Comparison of individual sugars in diverse chickpea germplasm accessions according to morphotypes.

Morphotypes	No. of accessions	Mean±SE				
		Sucrose (mg g^{-1})	Raffinose (mg g^{-1})	Ciceritol (mg g^{-1})	Stachyose (mg g^{-1})	Total Sugars (mg g^{-1})
Desi	91	21.88±0.856	4.76±0.683	35.25±0.983	28.89±1.57	90.78±4.092
Kabuli	51	25.28±0.592	4.70±0.317	33.86±0.104	27.24±1.234	91.09±2.247
Introgressed	71	23.01±0.754	4.47±0.482	33.92±0.271	27.65±0.628	89.05±2.135

Table 6. Grouping of the chick pea genotypes in different clusters

S. No.	Cluster	No. of Genotypes	Name of Genotypes
1	I	16	ICC67, ICC95, ICC118, ICC506, ICC867, ICC8740,ICC1164,ICC7554,ICC1194, ICC10341, ICC637, ICC5845,ICC6874, ICC3776, ICC7441, ICC5613
2	II	49	ICC4872,ICC8318,ICC1915,ICC7323,ICC1098,ICC4463,ICC3946,ICC5337,ICC15110,ICC2263,ICC708,ICC1710,ICC9402,ICC2619, ICC791,ICC14815,ICC13892,ICC4652,ICC4918,ICC8950,ICC15996,ICC13628,ICC16261,ICC8350,ICC12928,ICC8195,ICC1052,IC C8261,ICC1882,ICC13124,ICC6263,ICC12492,ICC13591,ICC14199,ICC14098,ICC13524,ICC16487,ICC13816,ICC8621,ICC12328,I CC13357,ICC14402,ICC13077,ICC13219,ICC13523,ICC1083,ICC8058,ICC12968,ICC12028
3	III	37	ICC13187,ICC14595,ICC2969,ICC5878,ICC7255,ICC8522,ICC2990,ICC12155,ICC8151,ICC283,ICC440,ICC456,ICC1205,ICC4841, ICC13441,ICC7668,ICC5639,ICC6811,ICC6279,ICC7315,ICC762,ICC2210,ICC2919,ICC1431,ICC10945,ICC14778,ICC4814,ICC98 95,ICC11627,ICC4973,ICC5518,ICC5618,ICC1715,ICC5504,ICC2580,ICC3362,ICC5383
4	IV	83	ICC9586,ICC11378,ICC13461,ICC11498,ICC6306,ICC9643,ICC16796,ICC10654,ICC12866,ICC2884,ICC3767,ICC8855,ICC15294,I CC11198,ICC16374,ICC6877,ICC9755,ICC13863,ICC1161,ICC15697,ICC1392,ICC11284,ICC4533,ICC6816,ICC10885,ICC16903,I CC9848,ICC13283,ICC10393,ICC14051,ICC1397,ICC2720,ICC5434,ICC1923,ICC4948,ICC8607,ICC15264,ICC6579,ICC5612,ICC1 5802,ICC10399,ICC15567,ICC3218,ICC15888,ICC8384,ICC4418,ICC11664,ICC11944,ICC11764,ICC12726,ICC12851,ICC1398,IC C4495,ICC5135,ICC7571,ICC10755,ICC15510,ICC15868,ICC7308,ICC1422,ICC2072,ICC11584,ICC2242,ICC3512,ICC16207,ICC1 4831,ICC15606,ICC2277,ICC12916,ICC15435,ICC15618,ICC2507,ICC3235,ICC4182,ICC1356,ICC3421,ICC9137,ICC7184,ICC165 24,ICC15333,ICC16269
5	V	4	ICC15406,ICC2065,ICC4593,ICC6571
6	VI	7	ICC7819,ICC4669,ICC3230,ICC9862,ICC6293,ICC6802,ICC3631
7	VII	8	ICC5879,ICC13764,ICC12947,ICC1230,ICC9942,ICC14077,ICC4567,ICC7867
8	VIII	9	ICC4639,ICC9002,ICC11121,ICC11879,ICC12824,ICC12037,ICC12299,ICC12307,ICC12537

digestible sugars, while reducing the oligosaccharides using unique germplasm lines.

Sucrose distribution

Sucrose was the major sugar detected in sugar profile of 213 chickpea germplasm accessions studied. Wide variation was detected among the accessions with the majority of germplasm containing more than 36 mg g^{-1} (Table 2 and Fig 1A). The sucrose concentration ranged from 3.57 mg g^{-1} to 54.12 mg g^{-1} with a mean of 23.07 mg g^{-1} (Table 3). Seventeen accessions had more than 36 mg g^{-1} sucrose content, whereas ten accessions from India, Iran and Russia and the Commonwealth of Independent States (CISs) had sucrose content below 10 mg g^{-1}. The average percentage of sucrose in total sugars among these 17 accessions was 30% as compared to the overall mean of 10.68%.

The highest sucrose content was detected in ICC 12564 (54.12 mg g^{-1}) and ICC 9137 (47.78 mg g^{-1}) collected from Malawi and Morocco, respectively. The lowest sucrose accessions were ICC 13816 (3.57 mg g^{-1}) and ICC 1230 (5.07 mg g^{-1}). ICC 13816 had extremely low concentration of raffinose (0.16 mg g^{-1}), stachyose (2.77 mg g^{-1}) and total soluble sugar (15.53 mg g^{-1}). Therefore, lower raffinose and stachyose content might have resulted in lower sucrose content in this accession. Hymowitz et al. (1972) reported that the sucrose content ranged from 30 to 102 mg g^{-1} with an average sucrose content of 61 mg g^{-1} in 195 diverse germplasms of soybean genotypes. Similar results were found in Soybean by Kuo et al. (1988) and Kennedy et al. (1985). Very low level of sucrose was reported in *Vicia* by Lattanzio et al. (1986) and more recently, Xiaoli et al. (2008) had reported the sucrose content of 18 mg g^{-1} to 52 mg g^{-1} in chickpea. The high sucrose accessions identified in this study will serve as excellent source for the breeding programme and also useful in the preparation of special dishes like chickpea sweets, besan burfi and puran poli without artificial sweetening agent.

Stachyose distribution

Stachyose is the second richest soluble sugar observed in the 213 accessions of chickpea. All the accessions exhibited highest level of variation for individual α-galactosides**.** Stachyose content in 216 accessions was ranged from 2.77 mg g^{-1} to 59.43 mg g^{-1} with a mean of 28.08 mg g^{-1} (Table 3). A total of 32 germplasm accessions were identified with stachyose content of above 40 mg g^{-1}, whereas 12 accessions exhibited stachyose content of below 10 mg g^{-1} (Table 4). The highest stachyose content was detected in ICC 4593 (59.43 mg g^{-1}) followed by ICC 1205 (57.69 mg g^{-1}) accessions which were originated from Ethiopia. On the other hand, the lowest stachyose content was found in ICC 13816 (2.77 mg g^{-1}) and ICC 2072 (2.93 mg g^{-1}), both originated from India. The low stachyose accession ICC 13816 also had low sucrose and low raffinose. The accession ICC 2072 had low oligosaccharides, but relatively higher amount of sucrose content (34.3 mg g^{-1}). Xiaoli et al. (2008) reported that the stachyose content in 19 cultivars of chickpea was ranged from 16.4 mg g^{-1} to 30.9 mg g^{-1}. The mean stachyose content observed in this study was similar to those in previous reports in soybean seeds (Giannoccaro et al., 2006; Hartwig et al., 1997; Hymowitz et al., 1972). Stachyose is considered to be the most undesirable oligosaccharide in chickpea seeds. Hence, the low stachyose lines identified in this study will aid chickpea breeding as unique germplasm resources and improve the food quality for human and other animals by increasing digestibility and metabolizing energy.

Raffinose distribution

The raffinose content of 213 accessions was ranged from 0.16 mg g^{-1} to 15.13 mg g^{-1} with a mean of 4.65 mg g^{-1} (Table 3, Fig 1B), accounting for 5.15% of the total sugars. The lowest raffinose content (0.16 mg g^{-1}) was detected in ICC 13816. Similar results were reported by Villaluenga et al. (2005) in lupins and Gulewicz (2000) in pea and lentil. Though ciceritol is present in larger quantity in chickpea, there has not been any report of its usefulness (Quemener and Brillouet, 1983). It is believed that these compounds play an important role in protecting plants and seeds against drought stress (Keller and Ludlow, 1993). Increasing the level of this compound may have an additional advantage of reducing the nutritional problems associated with the RFOs. There is evidence that ciceritol is more slowly hydrolysed than the RFO by α-galactosidase, which might result in a relative decrease in flatus potential of seeds with decreased levels of the RFO and an increased level of ciceritol (Quemener and Brillouet, 1983). Further detailed investigation is required to study the biochemistry and functionality of ciceritol in chickpea seeds.

Sugar profile by morphotypes

Significant variation in individual sugars was detected among the morphotypes. Data on the concentration of total soluble sugars and oligosaccharides in chickpea are given in Table 5. The content of soluble sugars in these morphotypes did not differ considerably, but minimum variations were observed in individual sugars. When the results of desi, kabuli and introgressed lines were compared, the sucrose and total sugar content was high in kabuli morphotypes (25.28 mg g^{-1} and 91.09 mg g^{-1}), with low raffinose (4.70 mg g^{-1}) and stachyose (27.42 mg g^{-1}) content as compared to two other morphotypes. These results were in complete agreement with the previous studies in chickpea (Lineback and Ke, 1975; Singh et al., 1982). Ciceritol, a new trisaccharide not correlated with the flatulence, was found high in chickpea accessions reported by Quemener and Brillouet (1983) and Xiaoli et al. (2008). This indicates that more sugar profiling studies are needed with these morphotypes to further verify the results as done in the case of soybean by Hou et al. (2009) and in lentil by Tahir et al., (2011). This kind of sugar profiling on the diverse collection of chickpea has not been reported earlier. This is the first report on the evaluation of sugar profiling in 213 chickpea germplasm accessions. Such an effort of detailed sugar profiling and precise identification of suitable accessions in a large chickpea germplasm will expand the scope for quality breeding in chickpea.

Cluster analysis

In the present study, 213 genotypes were subjected to cluster analysis for assessing the diversity based on sucrose, raffinose, ciceritol and stachyose. Based on dentrogram results, 213 genotypes were grouped into 8 clusters. The details of the genotypes that fall in different clusters are given in the Table 6 and Fig. 2. Among the 8 clusters, cluster IV was the largest with 83 genotypes followed by cluster II (49 genotypes). The highest sucrose accessions such as ICC11198, ICC16374, ICC11944, ICC2072 and ICC12851 were present in the cluster IV. Low stachyose accessions

ICC7323 and 6263 were included in the cluster II. The two lines ICC13816 and ICC2072 originated from India had relatively low stachyose content. The genotypes grouped in the diverse clusters may act as promising parents for obtaining potential hybrids. Similar results have been reported by Kumar *et al*. (1997) and Dwevedi and Lal (2009).

Materials and methods

Plant materials

A total of 213 chickpea accessions of the ICRISAT mini core collection (Name and origin of the core collections can be assessed in ICRISAT web link http://www.icrisat.org/what-we-do/crops/ChickPea/minicore.htm) were received from the Genetic Resources division of ICRISAT (Upadhyaya, 2001). The geographical origin of the above accessions covers about 24 countries from Asia, Africa, Europe, North and South American regions.

The majority of the germplasm were from India (93) and Iran (53). Among the 213 accessions evaluated, 91 were Desi types (black seeded), 51 were Kabuli (White seeded) and 71 were introgressed types. Each accession was grown in a single row in a plot of 3 m during 2009-10 winter season at the Department of Pulses, Centre for Plant breeding and Genetics, Tamil Nadu Agricultural university, Coimbatore, India and harvested at natural maturity.

Preparation of the seed extracts for HPLC analysis

Seeds of each accession were finely grounded using Glen coffee bean grinder and about 1 g of finely ground defatted flour in duplicates was weighed for each accession to the one-thousandth g accuracy and placed into a screw cap vial. The samples were thoroughly mixed in a solution of 10 ml of 50% ethanol and HPLC-grade water using a vortex. The samples were placed horizontally on a water bath shaker maintained at 50^{o} C and shaken at 100 strokes *per* min for 30 min. The extracts were centrifuged at 4000 rpm for 5 min. About 5 ml of the clear supernatant was transferred into a new vial. A total of 7 ml of acetonitrile (HPLC-grade) were added to precipitate soluble proteins and incubated at room temperature for 2 h. The mixture was then centrifuged at 3670 g for 5 min and the supernatant was collected. About 1 ml aliquot of the supernatant was evaporated to dryness in a dry bath incubator unit set at 50°C. The residue was redissolved with 500 µl of 65% HPLC grade acetonitrile and filtered through a 0.2 µm membrane filter and transferred to HPLC vials.

Calibration standards

Standard stocks solutions for sucrose, raffinose, stachyose and ciceritol were prepared separately at a concentration of 50 mg ml^{-1}. The sugars were weighed, dissolved in water and then acetonitrile was added to each solution to obtain a composition similar to that of the mobile phase. The calibration standards were prepared in three different concentrations; 1.25 mg ml^{-1}, 2.5 mg ml^{-1}, and 5.0 mg ml^{-1}. The standards were included with each group of samples loaded to the HPLC at the same time as a control on detector response.

Chromatographic Separation

The separation and quantification of oligosaccharides from the chickpea seed extracts were carried out by a Shimadzu Prominence HPLC system (Shimadzu, Kyoto, Japan), which consisted of a LC20AD pump and a RID-10A refraction index detector. The elution solvent was acetonitrile: water (65:35, v/v) with a pump rate of 1.0 ml min^{-1}. A 10 µl sample of the extract was injected with a SIL20AH auto sampler. The retention times for oligosaccharides and standards were identified by comparing the retention times with those of standard sugars.

Quantification

Quantification of each sugar was accomplished by comparing the peak areas of the samples with those of the standard solutions. A standard curve was plotted for each sugar and adjusted by least squares. The regression coefficients of the curves were always greater than 0.99.

Statistical analysis

Data with three replicates and two samples for each cultivar was analysed to calculate Pearson's simple correlation co-efficient and significance at a probability of 0.01% was tested to establish the relationship among various oligosaccharide fractions.

All statistical analysis was done with computer software Statistica version 5.0. Multiple means comparisons were determined with the Duncan's multiple range tests at $p < 0.05$ confidence levels. Cluster analysis for grouping of different genotypes was carried out by using NTSYS-PC software version 2.0.

Conclusion

Significant variation was observed for individual and total sugar content in 213 diverse chickpea germplasm accessions tested in this study from different origins and morphotypes. The accession from Russia and CISs contained less sucrose and α-galactosides content while the majority of chickpea accessions contained sucrose and stachyose as major sugars in relatively large quantities.

A total of 17 accessions from different origins were identified to have high sucrose with mean of 41.38 mg g^{-1}. The accession ICC 12654 (54.12 mg g^{-1}) was identified as high sucrose accession. Two accessions namely ICC 13816 and ICC 2072 were identified as low stachyose accession, while 32 accessions were identified with low oligosaccharides. The accession with unique sugar profiles is particularly valuable in breeding specialty chickpea for food purposes. Total sugar content was positively correlated with sucrose, raffinose, ciceritol and stachyose. Furthermore, positive correlation was found between all the sugars. These results will provide great scope for breeding of chickpea for high digestibility. Further investigation is needed to understand the genetic and environmental effects on seed soluble sugars in the promising accessions identified in the present study.

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