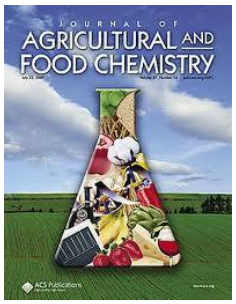

Genotype and Growing Environment Interaction Shows a Positive Correlation between Substrates of Raffinose Family Oligosaccharides (RFO) Biosynthesis and Their Accumulation in Chickpea (Cicer arietinumL.) Seeds

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ABSTRACT: To develop genetic improvement strategies to modulate raffinose family oligosaccharides (RFO) concentration in chickpea (*Cicer arietinum* L.) seeds, we analyzed RFO and their precursor concentration in 171 chickpea genotypes from diverse geographical origins. The genotypes were grown in replicated trials over two years in field (Patancheru, India) and Greenhouse (Saskatoon, Canada). Analysis of variance revealed significant impact of genotype, environment and their interaction on RFO concentration in chickpea seeds. Total RFO concentration ranged from 1.58 to 5.31 and 2.11 to 5.83 mmol/100 g in desi and kabuli genotypes, respectively. Sucrose (0.60-3.59 g/100 g) and stachyose (0.18-2.38 g/100 g) were distinguished as major soluble sugar and RFO, respectively. Correlation analysis revealed a significant positive correlation between substrate and product concentration in RFO biosynthesis. In chickpea seeds, raffinose, stachyose and verbascose showed a moderate broad sense heritability (0.25-0.56) suggesting use of multi-location trials based approach in chickpea seed quality improvement programs.

Keywords: Chickpea, *Cicer arietinum*, Raffinose Family Oligosaccharides (RFO), *Myo*-inositol, Galactinol, Raffinose, Stachyose, Verbascose and Genotype × Environment (G×E)

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the second most important pulse crop after dry beans cultivated over 11.98 million hectare area with a total production of 1.09 million tonnes around the world during 2010.[1,2] Chickpea is broadly classified into two clusters, (a) Kabuli type (white flower and large, cream-colored seeds) is usually grown in temperate regions, whereas (b) desi type (purple flower and small, dark, angular seeds) is mainly produced in semi-arid tropical regions of the world.[3,4] Chickpea seeds make an important nutritional contribution to the population of developing countries as they are excellent source of carbohydrate (40-59%), protein (13.5-31.7%), vitamins and minerals. In addition, chickpea seed constituents like PUFA (polyunsaturated fatty acid), saturated fatty acid (<1%) and dietary fibers (about 10%) have been associated with several beneficial health-promoting properties.[5] Hence, chickpea is considered as part of a health promoting diet.[6] However, presence of some anti-nutritional factors like raffinose family oligosaccharides (RFO) or α-galactosides reduce chickpea's acceptability in food products particularly in western countries.[7] In legume seeds, total α-galactosides vary from 0.4 to 16.1% of dry matter and in chickpea seeds range from 2.0 to 7.6%.[8] Raffinose is the first member of this family followed by stachyose and verbascose.[9] Some alternative RFO like lychnose and manninotriose have been

recently reported from Caryophyllacean[10] and Lamiaceae[11] plants, respectively but their presence in chickpea seeds has not yet been reported. RFO represent a class of soluble but non-reducing and non-structural oligosaccharides having α (1→6) linkage between sucrose and galactosyl subunit.[12] Therefore, these sugars are indigestible in human and monogastric animals as they lack α-galactosidase a hydrolyzing enzyme responsible for RFO breakdown.[13,14] Consequently, RFO escape digestion and absorption in small intestine but large intestinal microflora metabolize RFO and produce carbon dioxide, hydrogen, and small quantities of methane causing flatulence, diarrhea and stomach discomfort in humans.[15-17] As RFO act as substrate for intestinal bacteria they are also considered as prebiotics. These oligosaccharides also participate in important plant processes such as desiccation during seed maturation, carbon source in early stages of germination, translocation of photo-assimilates and abiotic stress tolerance.[8,18-20] Utilization of RFO may also support the growth of root nodulating bacteria (e.g. *Rhizobium meliloti*) in the rhizosphere of legume plants thus helping in nitrogen fixation.[21] Therefore, to increase the acceptability of chickpea in human and animal diet, RFO concentration needs to be reduced without affecting their physiological role in plants and beneficial effect on human health. Different treatments such as soaking, enzyme treatment and gamma radiation exposure can be used to reduce RFO in legume seeds.[22-24] Exposure to such mechanical and chemical treatments can reduce the nutritional quality of seeds. Therefore, it is desirable to develop genetic strategies to reduce RFO concentration in chickpea seeds. In this study we show that there is natural variation in RFO concentration in chickpea seeds. Both genotype and environment affect accumulation of RFO concentration in chickpea seeds.

MATERIALS AND METHODS

Plant material and growing conditions. A set of 171 chickpea genotypes (116 desi and 55 kabuli type, supporting information Table 1 and 2) was selected from genebank of ICRISAT (International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India) based on geographic origin. These genotypes represented eight different geographic regions including chickpea's center of origin and center of diversity (Table 1). These genotypes were grown in field as well as in greenhouse conditions in two biological replications. The field trials were conducted at ICRISAT (17°53' N latitude, 78°27' E longitude and 545 m altitude, Patancheru, India) for two seasons: 2008-2009 and 2009-2010 (from October to mid-March). For 2008-2009, mean daily minimum and maximum temperature was 15.0 and 31.1 °C, respectively. The average bright sunshine hours were 8.9 with approximately 352.1 μM m^{-2} sec^{-1} of solar radiation. The daily mean minimum and maximum temperatures during 2009-2010 were 16.2 and 30.0 °C, respectively along with average 8.1 h of bright sunshine and approximately 333.4 μM m^{-2} sec^{-1} of solar radiation. These genotypes were also grown in controlled greenhouse (GH) conditions at the University of Saskatchewan (52°07' N latitude, 106°38' W longitude and 481.5 m altitude, Saskatoon, SK, Canada) from March to July, 2010. In greenhouse, the average daily minimum and maximum temperatures were 18 and 23 °C with 18 h photoperiod and 385 μM m^{-2} s^{-1} of photosynthetically active radiation.

Total RFO determination. Total RFO concentration in chickpea seed meal (500 ± 5 mg) was determined by stepwise enzymatic hydrolysis of complex RFO into D-

galactose, D-fructose and D-glucose molecules using α-galactosidase (from *Aspergillus niger*) and invertase (from yeast) using a commercial assay kit (Megazyme International Ireland Ltd, Wicklow, Ireland). The resulting D-glucose concentration was determined using glucose oxidase/peroxidase reagent (GOPOD) that produced a red colored quinoneimine whose concentration was determined at $A_{510\ nm}$ using spectrophotometer (DU® 800, Beckman Coulter Inc., Fullerton, CA, USA). This method determined all oligosaccharides including raffinose, stachyose and verbascose concentration as a group. Total RFO concentration was calculated on molar basis as one mole of each oligosaccharide contains one mole of D-glucose.

HPAEC-PAD analysis of chickpea seeds' soluble sugars. Soluble sugars from chickpea seed meal (500 ± 5 mg) were extracted using method described by Frias et al.[25], Sanchez-Mata et al.[26] with some modifications.[27] For quantification of each member of raffinose family, a recently optimized analytical method was followed using high performance anion exchange chromatography [Ion chromatography system (ICS 5000), Thermo Fisher Scientific, Stevens Point, WI, USA] coupled with disposable gold electrode, Ag/AgCl reference electrode and CarboPac PA100 (4 x 250 mm) analytical column (unpublished). Raffinose (16.1 min), stachyose (17.0 min) and verbascose (19.5 min) were determined along with *myo*-inositol (1.7 min), galactinol (2.0 min), glucose (7.4 min), fructose (8.8 min) and sucrose (10.8 min) within 20 min of run time.

Data and statistical analysis. Box plot analysis was employed to represent variation among geographical regions for selected seed constituents (Fig. 1 and 2). Shannon-Weaver diversity index (SDI) was calculated to analyze the diversity present in

each geographical region (Table 2 and 3). For both SDI and box plot analysis, pooled data from all three growing environments was used.

General linear model was applied to calculate analysis of variance (ANOVA) using MINITAB 14 statistical software (Minitab Inc., State College, PA, USA). MSS (mean sum of squares) from ANOVA were utilized to calculate heritability (h^2).[28] To determine Shannon-Weaver diversity index (SDI), following formula was used.[29]

$$\mathrm{SDI} = \left(-\sum_{i=1}^{n} P_i \times log_e P_i \right) / \log_e n$$

Where, n represents total number of phenotypic classes and P_i is the proportion of total number of entries in the i^{th} class. Phenotypic classes were prepared by using MINITAB 14 statistical software.

RESULTS AND DISCUSSION

Diversity pattern among geographical regions. On the basis of their origin, desi and kabuli genotypes were grouped into seven geographical regions. In desi genotypes, South Asian region showed highest diversity index (0.33-0.87) for all the selected seed constituents, as this region has maximum representation (68 genotypes contributing about 59% to total desi genotypes) in the germplasm collection (Fig. 1). Consequently, South Asian genotypes showed the highest variation in seed constituents and it ranged from 0.01-0.10, 0.03-0.31, 0.03-0.42, 0.01-0.05, 0.60-2.93, 0.09-1.19, 0.18-2.36 and 0.01-0.13 g/100 g for *myo*-inositol, galactinol, glucose, fructose, sucrose, raffinose, stachyose and verbascose with an average value of 0.05, 0.17, 0.22, 0.01, 1.72, 0.74, 1.33 and 0.06 g/100 g of chickpea seed meal, respectively

(Fig. 1). Southwest Asia is one of chickpea's primary centers of origin whereas Sub Saharan Africa contained genotypes from Ethiopia considered as secondary center of genetic diversity for chickpea. Therefore, second highest SDI for all the traits were expressed by genotypes either from Southwest Asia or Sub Saharan Africa. SDI ranged from 0.29-0.76, 0.13-0.68, 0.15-0.68, 0.27-0.68 and 0.23-0.51 for Southwest Asia, Sub Saharan Africa, North Africa, Europe and Meso America, respectively. This germplasm collection represented no desi genotype from South America whereas only one and four from North America.

In kabuli genotypes, South Asian region showed highest SDI for most chickpea seed constituents, such as fructose (0.67), raffinose (0.86), stachyose (0.89), verbascose (0.89) and total RFO (0.92). In South Asian genotypes, concentrations of fructose, raffinose, stachyose, verbascose and total RFO varied from 0.01-0.05, 0.48-1.13, 0.80-2.28, 0.02-0.12 and 2.27-5.83 g/100 g with mean values of 0.01, 0.79, 1.46, 0.07 and 3.96 g/100 g (mmol/100 g for total RFO) of chickpea seed meal, respectively (Fig. 2). Highest SDI for *myo*-inositol (0.88) and sucrose (0.77) was observed for North African genotypes with concentrations ranging from 0.02-0.09 and 1.29-3.59 g/100 g with a mean value of 0.05 and 2.41 g/100 g of chickpea seed meal, respectively. Galactinol concentration ranged from 0.05-0.30 g/100 g in European genotypes with a mean concentration of 0.17 g/100 g of chickpea seed meal that resulted in highest SDI of 0.89 among all geographical regions. However, highest SDI for glucose (0.75) was calculated for Southwest Asian genotypes with concentrations ranging from 0.11-0.31 g/100 g with a mean value of 0.21 g/100 g of chickpea seed meal. South Asian genotypes had the highest representation in the germplasm collection sharing about

32.7% of total kabuli genotypes followed by genotypes from Southwest Asia (20%), North Africa (18.2%), Europe (14.5%) and Sub Saharan Africa (9%), respectively. On the basis of SDI, these genotypes were conjointly considered as a diverse collection and used further to study variation in chickpea seed constituents.

Impact of genotype and environment influencing seed constituents' concentration. Analysis of variance (ANOVA) showed significant effect ($P \leq 0.001$) of genotype (G) and growing environment (E) on concentration of *myo*-inositol, galactinol, glucose, fructose, sucrose, raffinose, stachyose, verbascose and total RFO in both desi and kabuli genotypes. The interaction between genotype and growing environment (G×E) also exhibited significant effect ($P \leq 0.001$) on these seed constituents (Table 4). These results concur with the conclusions of Kumar et al.[14] showing significant effect ($P \leq 0.05$) of genotype × location on sucrose, raffinose and stachyose concentration in seven soybean genotypes. Recently, Tahir et al.[27] reported significant ($P \leq 0.0001$) effect of cultivar, environment and their interaction on glucose, sucrose and RFO concentration in lentil seeds.

Variation for selected seed constituents in desi and kabuli genotypes. HPAEC-PAD analysis revealed the highest concentration of sucrose among soluble sugars in chickpea seeds. Stachyose was the predominant RFO found in chickpea seeds followed by raffinose whereas verbascose was present only as a small fraction. Previously, Frias et al.[30]; El-Adawy[31]; Aguilera et al.[32]; Berrios et al.[33] also reported stachyose as a major RFO in chickpea seeds. In desi type (Fig. 3), genotypes grown in GH showed significantly lower ($P \leq 0.001$) total RFO concentration (1.58-4.67 mmol/100 g) compared to genotypes grown in field conditions during 2009 (1.88-5.31 mmol/100 g)

and 2010 (2.80-4.95 mmol/100 g). GH grown genotypes had total RFO with a mean concentration of 3.32 mmol/100 g, whereas in field 2009 and 2010 it was 4.09 and 3.66 mmol/100 g, respectively. Similar pattern of total RFO was observed in kabuli type (Fig. 4) showing lower concentration (2.11-4.56 mmol/100 g) in GH grown genotypes than that in field-grown during 2009 (3.46-5.83 mmol/100 g) and 2010 (3.01-5.35 mmol/100 g).

Individual RFO members also accumulated at significantly lower concentration in GH grown genotypes than their field grown counterparts. In GH grown desi type, raffinose (0.27-0.95 g/100 g), stachyose (0.43-1.86 g/100 g) and verbascose (0.01-0.11 g/100 g) had a mean value of 0.68, 1.15 and 0.05 g/100 g, respectively (Fig. 3). Genotypes grown in field during 2009 had average value of 0.85, 1.57 and 0.07 g/100 g for raffinose, stachyose and verbascose with a range of 0.09-1.10, 0.18-2.36 and 0.02-0.11 g/100 g, respectively whereas, genotypes grown in field during 2010 showed variation from 0.40 to 1.19, 0.78 to 1.99 and 0.01 to 0.13 g/100 g for raffinose, stachyose and verbascose with mean value of 0.75, 1.35 and 0.06 g/100 g, respectively (Fig. 3). Kabuli type chickpea genotypes followed the same pattern for variation among RFO members. In GH grown kabuli type, raffinose (0.27-0.95 g/100 g), stachyose (0.40-1.65 g/100 g) and verbascose (0.01-0.11 g/100 g) showed a mean value of 0.66, 1.12, and 0.05 g/100 g, respectively (Fig. 4). Kabuli genotypes grown in field during 2009 contained raffinose, stachyose and verbascose with mean values of 0.94, 1.79 and 0.08 g/100 g that ranged from 0.69-1.17, 1.31-2.38 and 0.05-0.13 g/100 g, respectively. However, genotypes grown in field during 2010 ranged from 0.58 to 1.08, 1.06 to 2.17 and 0.04 to 0.12 g/100 g for raffinose, stachyose and verbascose with mean values of 0.84, 1.59 and 0.08

g/100 g, respectively (Fig. 4). Lower concentration of RFO in controlled growing environment (GH with less temperature variation, longer photoperiod and higher photosynthetically active radiation) supports physiological roles of these oligosaccharides in providing tolerance against abiotic stresses.[8,34] RFO act as reactive oxygen species scavengers, signaling molecules and osmo-protectants thus providing protection against oxidative, freezing, salinity and drought stress.[35-40]

In desi genotypes, sucrose concentration varied from 0.84 to 2.84 g/100 g in GH grown genotypes with a mean value of 1.79 g/100 g, whereas in field grown genotypes it ranged from 0.60-2.93 g/100 g and 0.81-2.64 g/100 g during 2009 and 2010 having average values of 1.87 and 1.52 g/100 g, respectively. However, sucrose varied from 1.05 to 3.33, 1.33 to 3.59 and 1.07 to 2.94 g/100 g in kabuli genotypes grown in GH and field conditions (2009 and 2010) with mean values of 2.11, 2.62 and 2.03 g/100 g, respectively. Higher sucrose concentration can be due to its role as universal molecule to transport carbon and a substrate for raffinose biosynthesis.[41-43] Sosulski et al.[44] estimated sucrose content in hull free chickpea seeds with mean value of 2.69 g/100 g that was about 32% of total sugars and highest among soluble sugars. Later, Xiaoli et al.[45] reported the amount of sucrose, raffinose, stachyose and verbascose in seeds of 19 chickpea cultivars varied from 1.80 to 5.22, 0.46 to 0.92, 1.60 to 3.10 and 0.27 to 0.70 g/100 g, respectively. The variations for important chickpea seeds' constituents described in the present study concur with the range reported in previous studies conducted by Sanchez-Mata et al.[46]; Frias et al.[30], Alajaji and El-Adawy[23]; Aguilera et al.[32]; Berrios et al.[33] concluding varying range of mean values for sucrose, raffinose and stachyose from 0.79 to 3.53, 0.32 to 1.45 and 0.74 to 2.56 g/100 g, respectively.

Other minor components of chickpea seeds, such as *myo*-inositol, galactinol, glucose and fructose were also determined. In desi type (Fig. 3), *myo*-inositol and galactinol ranged from 0.01 to 0.10 and 0.03 to 0.37 g/100 g with a mean value 0.05 and 0.17 g/100 g, respectively. Similarly, *myo*-inositol in kabuli type (Fig. 4) varied from 0.02 to 0.10 g/100 g but with relatively higher mean value of 0.03 g/100 g. Kabuli genotypes showed variation from 0.05 to 0.32 g/100 g for galactinol having a mean concentration of 0.1 g/100 g. Desi and kabuli genotypes showed variation from 0.03 to 0.42 and 0.11 to 0.34 g/100 g for glucose concentration with an average of 0.22 and 0.10 g/100 g, respectively. Whereas, fructose concentration varied from 0.001 to 0.03 and 0.003 to 0.07 g/100 g in desi and kabuli genotypes with a mean value of 0.01 and 0.006 g/100 g, respectively (Fig. 3 and 4). Sosulski et al.[44]; Jukanti et al.[4] also reported low concentration of galactinol in chickpea seeds with a mean value of 0.50 and 0.39% of chickpea seed dry matter, respectively. These results correspond to the concentrations of glucose (0.05-0.10% of dry matter) and fructose (0.1-0.3% of dry matter) in chickpea seeds reported earlier.[32,33]

Correlation among chickpea seed components. Total RFO showed a positive correlation with raffinose (r = 0.85/0.89), stachyose (r = 0.91/0.92) and verbascose (r = 0.60/0.69) in chickpea genotypes (desi/kabuli) significant at $P \leq 0.001$. Raffinose, stachyose and verbascose were collectively determined during total RFO assay; hence resulted correlation confirmed the accuracy and precision of HPAEC-PAD method for the concentration of RFO members with enzymatic assay for total RFO determination.

Myo-inositol was significantly ($P \leq 0.001$) and positively correlated with galactinol (r = 0.64/0.68), glucose (r = 0.39/0.47), sucrose (r = 0.36/0.68), raffinose (r = 0.40/0.42),

stachyose (r = 0.50/0.44) and verbascose (r = 0.49/0.47) in desi/kabuli genotypes. Galactinol also showed a significant ($P \leq 0.001$) positive correlation with raffinose (r = 0.39/0.55), stachyose (r = 0.53/0.64) and verbascose (r = 0.40/0.49) in chickpea genotypes (desi/kabuli). In desi genotypes, sucrose was positively correlated with raffinose (r = 0.15; $P \leq 0.001$), stachyose (r = 0.09; $P \leq 0.05$) and verbascose (r = 0.18; $P \leq 0.001$) whereas in kabuli types, sucrose showed positive correlation with raffinose (r = 0.41), stachyose (r = 0.35) and verbascose (r = 0.41) significant at $P \leq 0.001$. In previous studies also, sucrose showed a significant positive correlation with raffinose and stachyose concentration in soybean seeds.[47,48]

A significant positive correlation was observed between substrate and product concentrations in RFO biosynthetic pathway in chickpea seeds. The first committed step in RFO biosynthesis is galactinol formation in which *myo*-inositol and UDP-galactose act as substrates. Further, galactinol in conjunction with sucrose, raffinose and stachyose participates in the biosynthesis of raffinose, stachyose and verbascose, respectively. Correlation analysis suggested substrate concentration as one of the main regulating factors for varying RFO concentration in different chickpea genotypes. The other regulatory factors might be expression of genes encoding RFO biosynthetic enzymes and/or their activities that still need to be studied. Such studies would be utilized to identify the key step of RFO biosynthesis. Like in case of *Brassica napus*[49], antisense technology was used to down-regulate galactinol synthase that resulted into substantial reduction in galactinol and stachyose concentration in mature transgenic seeds. Such transgenic approaches can also be followed in chickpea to develop varieties with reduced RFO concentration.

Heritability of important chickpea seed constituents. Significant impact of environment and genotype × environment on the performance of a particular genotype suggests complex genetic regulation of traits.[48,50] Broad sense heritability (h^2) was estimated on the basis of the pooled ANOVA of genotypes grown in field and greenhouse environments (Table 4). Ayele[51] described high, medium and low heritability as ≥0.6, 0.3-0.6 and <0.3, respectively. The h^2 of important chickpea seed constituent was estimated with a maximum of 0.61 for total RFO and a minimum of 0.05 for fructose in desi genotypes whereas h^2 in kabuli genotypes showed a minimum of 0.02 for glucose and a maximum of 0.53 for sucrose. The results for h^2 are in agreement with the heritability range reported for sucrose (0.43 - 0.87), raffinose (0.42-0.56) and stachyose (0.30-0.74) in soybean seeds.[48,52,53] McPhee et al.[50] also estimated narrow sense heritability for sucrose, raffinose and stachyose in common bean seeds with a value of 0.22, 0.54 and 0.44, respectively.

Present study revealed significant impact of genotype (G), environment (E) and G × E on concentration of raffinose family oligosaccharides suggesting their complex genetic regulation in chickpea seeds. Sucrose and stachyose were identified as predominant soluble sugar and RFO in chickpea seeds. A significant positive correlation was observed between substrate and product concentration in RFO biosynthetic pathway. Among all the genotypes screened, some were identified having low RFO concentration. Desi genotypes ICCV 07115, ICCV 07116 and ICCV 07117 showed the lowest total RFO (1.58-2.46 mmol/100 g), raffinose (0.27 – 0.52 g/100 g) and stachyose (0.43-1.05 g/100 g) in field as well as GH growing environments. Accession ICC 16528 performed stably in different environmental conditions and it is one of the kabuli

genotypes with low total RFO (2.11-3.84 mmol/100 g), raffinose (0.39-0.74 g/100 g), stachyose (0.90-1.46 g/100 g) and verbascose (0.02-0.06 g/100 g). These genotypes can be utilized in chickpea improvement programs to develop cultivars with reduced RFO concentration. Moderate heritability of RFO trait suggested the use of multi-location trials based approach while using germplasms for chickpea improvement programs.

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Supporting Information

Details of desi and kabuli type chickpeas used in the study. This information is available free of charge via the Internet at http: //pubs.acs.org

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Table 1. Geographical origin of chickpea genotypes used in the study

Region	No. of Genotypes	
	Desi	Kabuli
1. Europe	10	8
2. Meso America	4	1
3. North Africa	9	10
4. North America	1	0
5. South America	0	2
6. South Asia	68	18
7. Southwest Asia	13	11
8. Sub Saharan Africa	11	5

Table 2. Shannon-Weaver diversity index (SDI) of selected chickpea seed constituents in different geographical regions for desi genotypes

Seed constituents	SDI as per geographical region					
	Europe	Meso America	North Africa	South Asia	Southwest Asia	Sub Saharan Africa
Myo-inositol	0.59	0.29	0.61	0.76	0.62	0.38
Galactinol	0.58	0.26	0.43	0.75	0.46	0.67
Glucose	0.51	0.50	0.68	0.85	0.76	0.68
Fructose	0.27	0.23	0.15	0.33	0.29	0.13
Sucrose	0.68	0.51	0.64	0.80	0.56	0.68
Raffinose	0.54	0.21	0.48	0.74	0.68	0.62
Stachyose	0.56	0.38	0.64	0.68	0.67	0.46
Verbascose	0.57	0.39	0.64	0.87	0.56	0.62
Total RFO	0.61	0.42	0.67	0.74	0.69	0.66

Table 3. Shannon-Weaver diversity index (SDI) of selected chickpea seed constituents in different geographical regions for kabuli genotypes

Seed constituents	SDI as per geographical region					
	Europe	South America	North Africa	South Asia	Southwest Asia	Sub Saharan Africa
Myo-inositol	0.64	0.33	0.88	0.68	0.80	0.46
Galactinol	0.89	0.36	0.87	0.75	0.86	0.35
Glucose	0.63	0.32	0.54	0.65	0.75	0.43
Fructose	0.62	0.36	0.33	0.67	0.58	0.00
Sucrose	0.71	0.32	0.77	0.66	0.73	0.61
Raffinose	0.60	0.32	0.71	0.86	0.82	0.61
Stachyose	0.60	0.33	0.65	0.89	0.80	0.51
Verbascose	0.62	0.36	0.73	0.89	0.78	0.35
Total RFO	0.65	0.30	0.70	0.92	0.56	0.41

Table 4. Analysis of variance and heritability of chickpea selected seed constituents

	Seed constituents	Mean sum of squares				Heritability (h^2)
		Genotype (G)	Environment (E)	Replication	G × E	
Desi	*Myo*-inositol	3.3×10^{-4}***	7.5×10^{-2}***	5.7×10^{-6} ns	2.4×10^{-4}***	0.10
	Galactinol	5.8×10^{-3}***	0.5***	1.8×10^{-3} ns	1.5×10^{-3}***	0.55
	Glucose	5.2×10^{-3}***	0.2***	4.4×10^{-5} ns	3.2×10^{-3}***	0.16
	Fructose	1.5×10^{-4}***	1.8×10^{-3}***	2.8×10^{-5} ns	1.2×10^{-4}***	0.05
	Sucrose	0.4***	7.2***	2.8×10^{-4} ns	0.1***	0.37
	Raffinose	0.1***	1.3***	6.0×10^{-4} ns	1.0×10^{-2}***	0.56
	Stachyose	0.2***	10.3***	7.1×10^{-4} ns	4.6×10^{-2}	0.52
	Verbascose	8.0×10^{-4}***	3.7×10^{-2}***	1.4×10^{-4} ns	3.7×10^{-4}***	0.25
	Total RFO	1.3***	35.4***	4.2×10^{-2} ns	0.2***	0.61
Kabuli	*Myo*-inositol	3.8×10^{-4}***	4.0×10^{-2}***	7.0×10^{-7} ns	2.7×10^{-4}***	0.10
	Galactinol	6.2×10^{-3}***	0.3***	1.2×10^{-3} ns	2.5×10^{-3}***	0.31
	Glucose	3.5×10^{-3}***	0.1***	1.6×10^{-4} ns	3.3×10^{-3}***	0.02
	Fructose	5.4×10^{-5}***	1.1×10^{-4}***	1.5×10^{-5} ns	4.1×10^{-5}***	0.07
	Sucrose	0.8	10.1***	7.9×10^{-3} ns	0.2***	0.53
	Raffinose	5.5×10^{-2}***	2.2***	2.4×10^{-3} ns	1.8×10^{-2}***	0.39
	Stachyose	0.2***	13.2***	3.2×10^{-3} ns	6.0×10^{-2}***	0.39
	Verbascose	9.5×10^{-4}***	4.1×10^{-2}***	3.1×10^{-5} ns	2.9×10^{-4}***	0.39
	Total RFO	1.1***	47.1***	0.4×10^{-3} ns	0.3***	0.45

*** significant at $P \leq 0.001$; ns = non-significant

Table 5. Correlation among chickpea selected seed constituents in desi and kabuli genotypes

	***Myo*-inositol**	**Galactinol**	**Glucose**	**Fructose**	**Sucrose**	**Raffinose**	**Stachyose**	**Verbascose**
Desi								
Galactinol	0.64***							
Glucose	0.39***	0.00 ns						
Fructose	-0.03 ns	0.07 ns	0.01 ns					
Sucrose	0.36***	0.03 ns	0.56***	-0.07 ns				
Raffinose	0.40***	0.39***	0.12**	0.07 ns	0.15***			
Stachyose	0.50***	0.53***	-0.01ns	0.07 ns	0.09*	0.78***		
Verbascose	0.49***	0.40***	-0.03ns	0.08 ns	0.18***	0.50***	0.64***	
Total RFO	0.46***	0.47***	-0.01ns	0.04 ns	0.08*	0.85***	0.91***	0.60***
Kabuli								
Galactinol	0.68***							
Glucose	0.47***	0.12*						
Fructose	0.04 ns	0.15**	-0.01 ns					
Sucrose	0.33***	0.23***	0.39***	-0.08 ns				
Raffinose	0.42***	0.55***	0.11 ns	0.05 ns	0.41***			
Stachyose	0.44***	0.64***	0.01 ns	0.07 ns	0.35***	0.89***		
Verbascose	0.47***	0.49***	0.09 ns	0.05 ns	0.41***	0.66***	0.72***	
Total RFO	0.44***	0.62***	0.01 ns	0.06 ns	0.33***	0.89***	0.92***	0.69***

***, ** and * are significant at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$, respectively; ns = non-significant

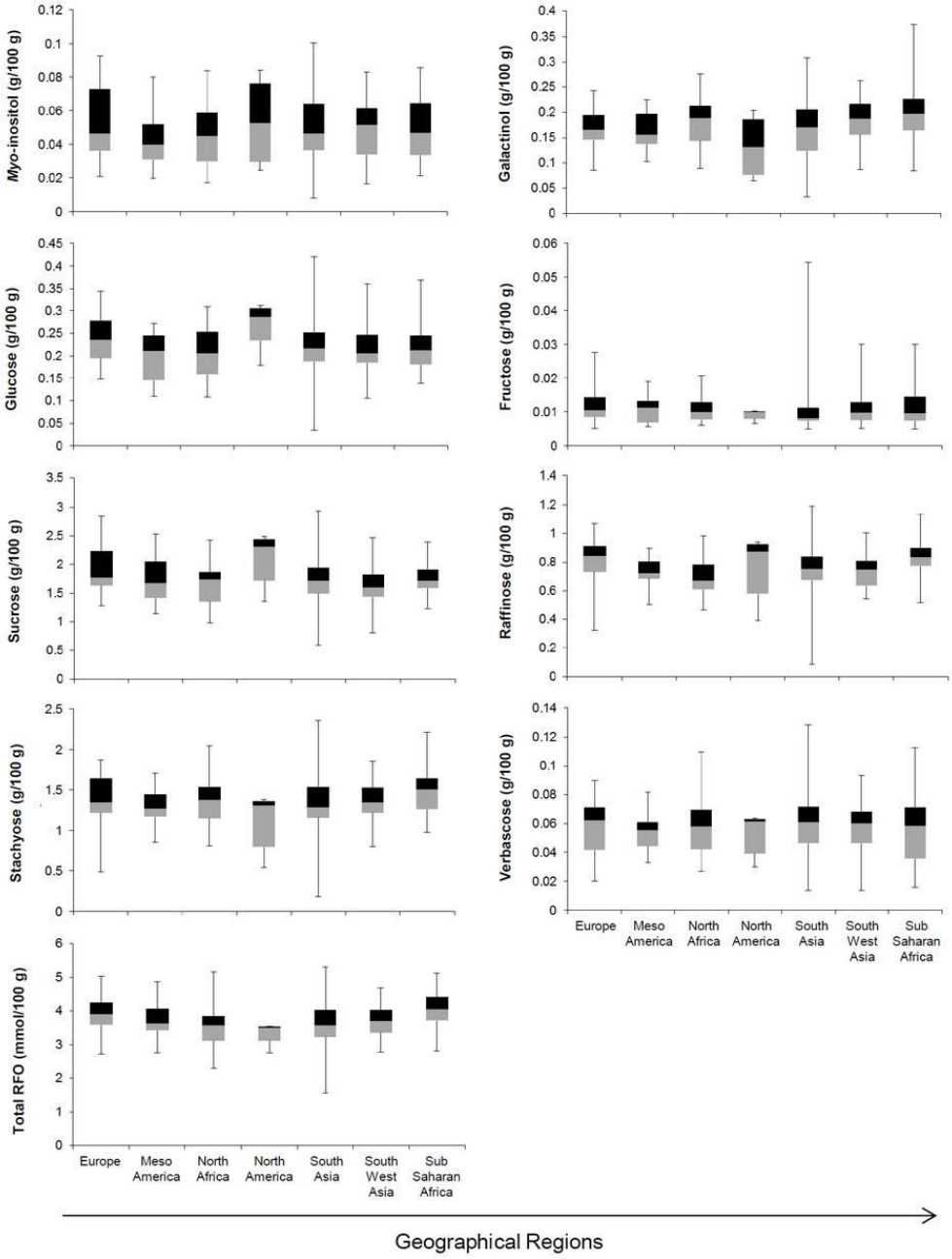

Figure 1. Box plot analysis for desi genotypes showing variation for selected chickpea seed constituents in different geographical regions using pooled data from different growing environments. Upper and lower error bars represent the lowest and highest concentration. Black and grey boxes indicate third and second quartile whereas middle line shows the median of the dataset.

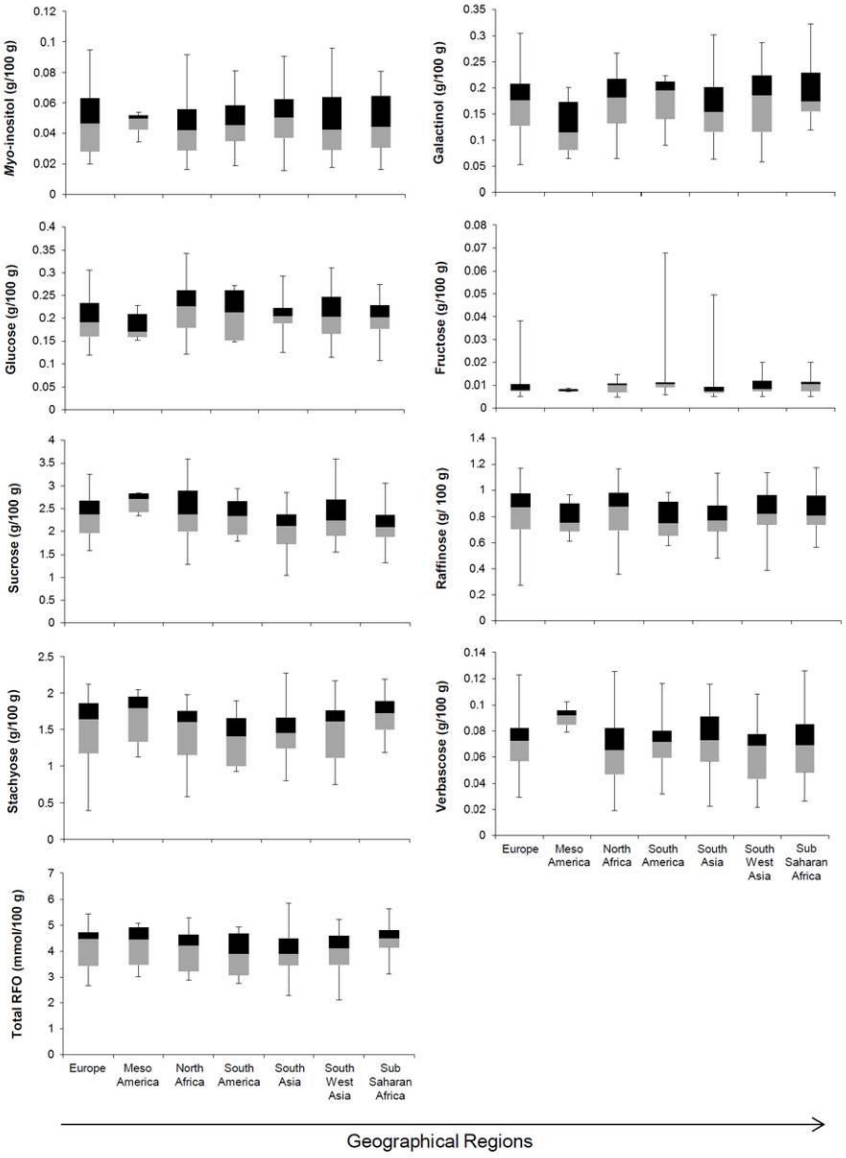

Figure 2. Box plot analysis for kabuli genotypes showing variation for selected chickpea seed constituents in different geographical regions using pooled data from different growing environments. Upper and lower error bars represent the lowest and highest concentration. Black and grey boxes indicate third and second quartile whereas middle line shows the median of the dataset.

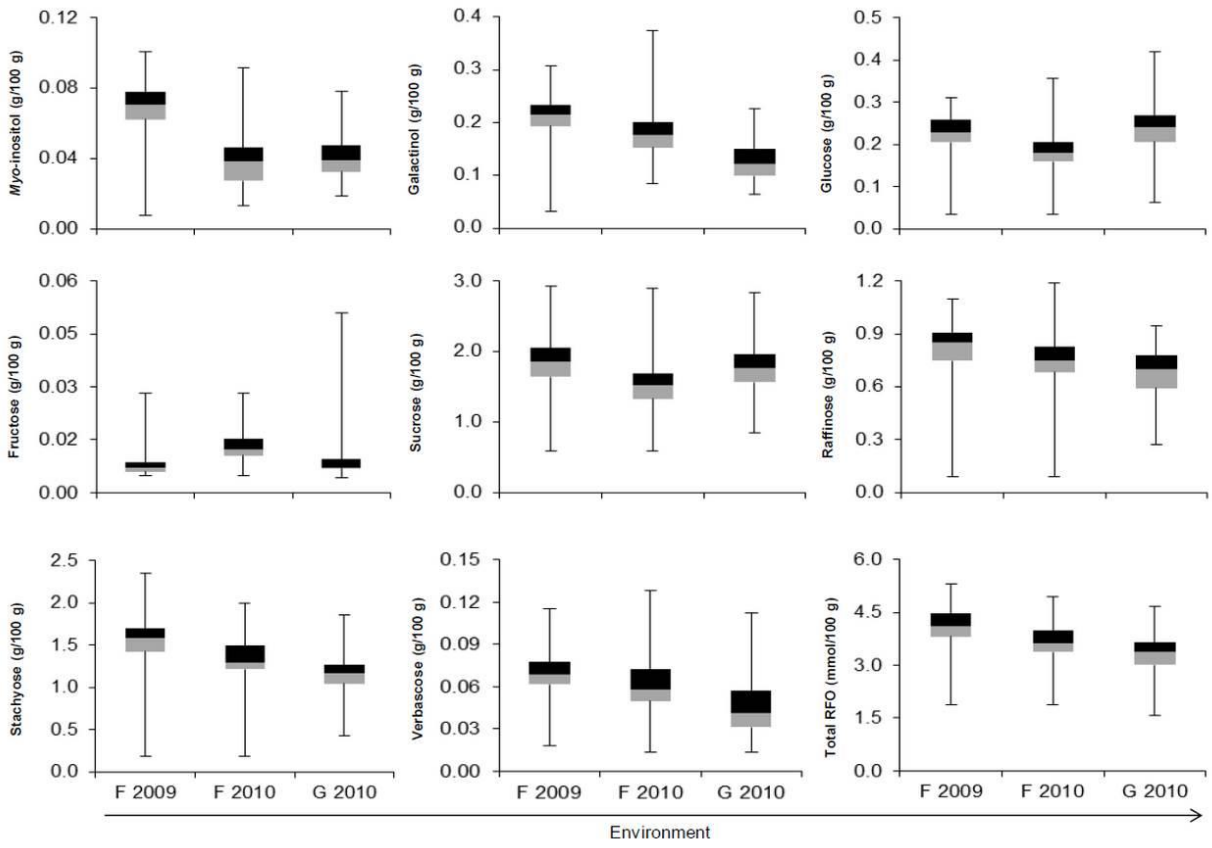

Figure 3. Box plot analysis for selected chickpea seed constituents of desi genotypes in different growing environments. Genotypes grown in field during 2008-2009 and 2009-2010 are represented as F 2009 and F2010, respectively whereas G 2010 represents greenhouse genotypes grown in 2010. Upper and lower error bars represent the lowest and highest concentration. Black and grey boxes indicate third and second quartile whereas middle line shows the median of the dataset.

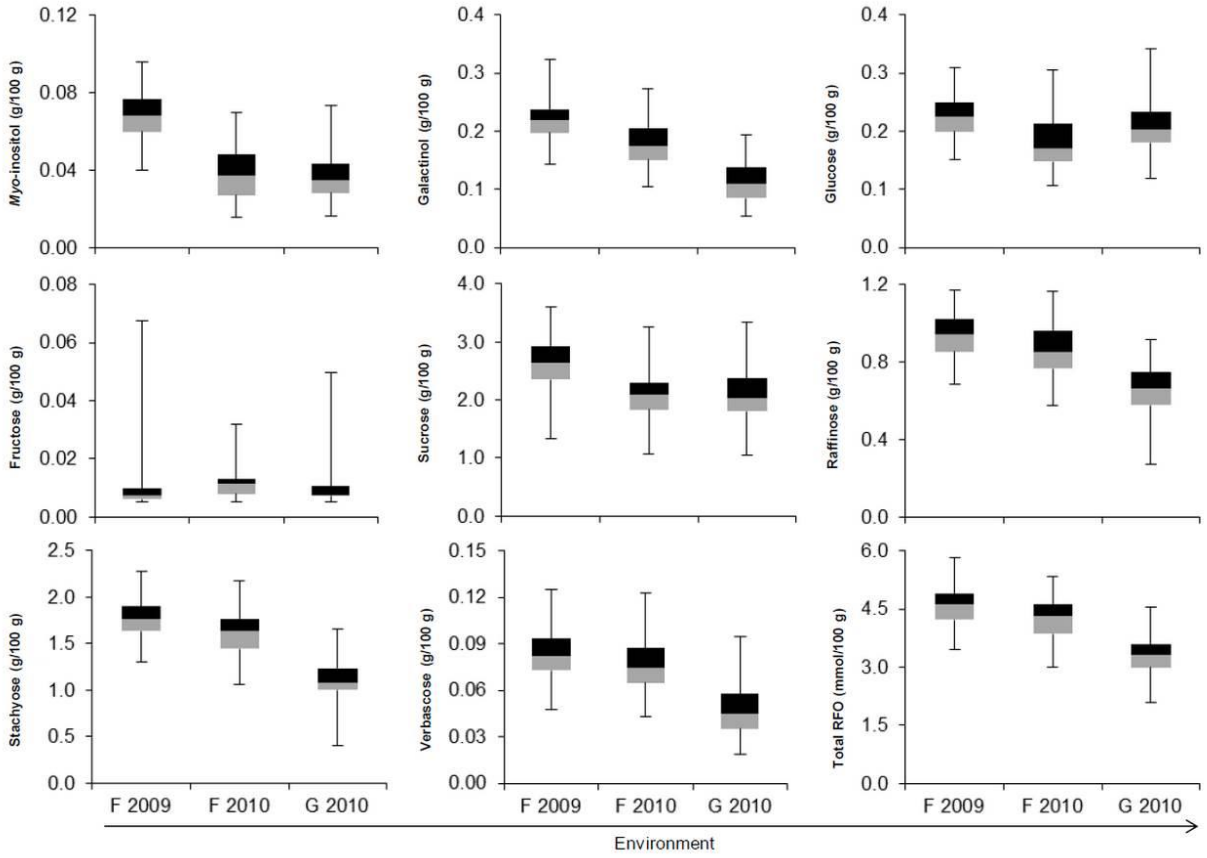

Figure 4. Box plot analysis for selected chickpea seed constituents of kabuli genotypes in different growing environments. Genotypes grown in field during 2008-2009 and 2009-2010 are represented as F 2009 and F2010, respectively whereas G 2010 represents greenhouse genotypes grown in 2010. Upper and lower error bars represent the lowest and highest concentration. Black and grey boxes indicate third and second quartile whereas middle line shows the median of the dataset.

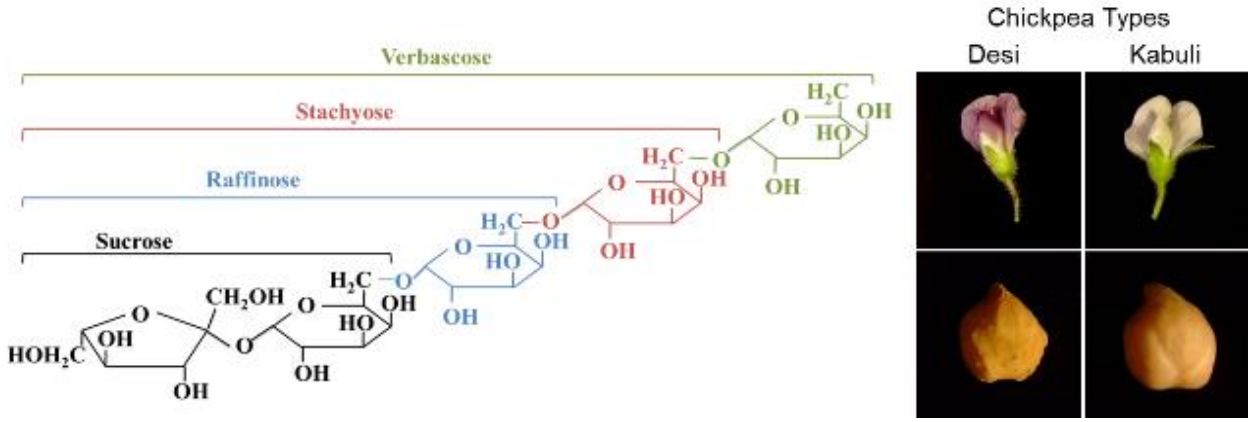

Figure for web

Supporting Table 1. Details of desi genotypes studied

ICC No.	Region	Country	Biological Status	Botanical Types
ICC 6293	Europe	Italy	Traditional cultivar/Landrace	Desi
ICC 7192	Europe	Hungary	Traditional cultivar/Landrace	Desi
ICC 7669	Europe	Greece	Traditional cultivar/Landrace	Desi
ICC 8474	Europe	Spain	Traditional cultivar/Landrace	Desi
ICC 11903	Europe	Germany	Traditional cultivar/Landrace	Desi
ICC 14177	Europe	Germany	Traditional cultivar/Landrace	Desi
ICC 14179	Europe	Germany	Traditional cultivar/Landrace	Desi
ICC 14183	Europe	Germany	Traditional cultivar/Landrace	Desi
ICC 14456	Europe	Yogoslavia (Former)	Traditional cultivar/Landrace	Desi
ICC 16835	Europe	France	Traditional cultivar/Landrace	Desi
ICC 995	MesoAmerica	Mexico	Traditional cultivar/Landrace	Desi
ICC 988	MesoAmerica	Mexico	Traditional cultivar/Landrace	Desi
ICC 5566	MesoAmerica	Mexico	Traditional cultivar/Landrace	Desi
ICC 9557	MesoAmerica	Mexico	Traditional cultivar/Landrace	Desi
ICC 1017	NorthAfrica	Egypt	Traditional cultivar/Landrace	Desi
ICC 1025	NorthAfrica	Algeria	Traditional cultivar/Landrace	Desi
ICC 3335	NorthAfrica	Cyprus	Traditional cultivar/Landrace	Desi
ICC 3336	NorthAfrica	Cyprus	Traditional cultivar/Landrace	Desi
ICC 3429	NorthAfrica	Egypt	Traditional cultivar/Landrace	Desi
ICC 8943	NorthAfrica	Egypt	Traditional cultivar/Landrace	Desi
ICC 9562	NorthAfrica	Algeria	Traditional cultivar/Landrace	Desi
ICC 9567	NorthAfrica	Israel	Traditional cultivar/Landrace	Desi
ICC 15536	NorthAfrica	Morocco	Traditional cultivar/Landrace	Desi
ICC 16343	NorthAmerica	USA	Breeding/Research material	Desi
ICC 4951	SouthAsia	India	Breeding/Research material	Desi
ICC 4918	SouthAsia	India	Advanced/Improved cultivar	Desi
ICC 506-EB	SouthAsia	India	Traditional cultivar/Landrace	Desi
ICC 16382	SouthAsia	India	Advanced/Improved cultivar	Desi
ICC 1431	SouthAsia	India	Traditional cultivar/Landrace	Desi
ICC 4991	SouthAsia	India	Advanced/Improved cultivar	Desi
ICCV 04516	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICC 982	SouthAsia	Sri Lanka	Traditional cultivar/Landrace	Desi
ICC 1471	SouthAsia	India	Traditional cultivar/Landrace	Desi
ICC 2204	SouthAsia	Sri Lanka	Traditional cultivar/Landrace	Desi
ICC 4933	SouthAsia	Sri Lanka	Traditional cultivar/Landrace	Desi
ICC 5186	SouthAsia	India	Traditional cultivar/Landrace	Desi
ICC 5384	SouthAsia	India	Traditional cultivar/Landrace	Desi

ICC No.	Region	Country	Biological Status	Botanical Types
ICC 5794	SouthAsia	India	Breeding/Research material	Desi
ICC 5912	SouthAsia	India	Traditional cultivar/Landrace	Desi
ICC 8166	SouthAsia	India	Traditional cultivar/Landrace	Desi
ICC 8397	SouthAsia	India	Breeding/Research material	Desi
ICC 10134	SouthAsia	India	Traditional cultivar/Landrace	Desi
ICC 12169	SouthAsia	Nepal	Traditional cultivar/Landrace	Desi
ICC 12184	SouthAsia	Nepal	Traditional cultivar/Landrace	Desi
ICC 12289	SouthAsia	Nepal	Traditional cultivar/Landrace	Desi
ICC 12312	SouthAsia	India	Traditional cultivar/Landrace	Desi
ICC 12511	SouthAsia	India	Breeding/Research material	Desi
ICC 14315	SouthAsia	india	Breeding/Research material	Desi
ICC 14406	SouthAsia	India	Breeding/Research material	Desi
ICC 14497	SouthAsia	Bangladesh	Traditional cultivar/Landrace	Desi
ICC 14575	SouthAsia	Bangladesh	Traditional cultivar/Landrace	Desi
ICC 14592	SouthAsia	Bangladesh	Traditional cultivar/Landrace	Desi
ICC 14674	SouthAsia	India	Traditional cultivar/Landrace	Desi
ICC 16141	SouthAsia	Mynamar	Traditional cultivar/Landrace	Desi
ICC 16173	SouthAsia	Mynamar	Traditional cultivar/Landrace	Desi
ICC 16181	SouthAsia	Myanmar	Traditional cultivar/Landrace	Desi
ICC 16219	SouthAsia	Mynamar	Traditional cultivar/Landrace	Desi
ICCC 37	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCL 81248	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCL 83149	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCL 87207	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 88202	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 89314	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 90201	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 92809	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 92944	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 93952	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 93954	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 94954	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 96836	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCX 820065	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 97105	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 96030	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 07102	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 07104	SouthAsia	India	Breeding line developed at ICRISAT	Desi

ICC No.	Region	Country	Biological Status	Botanical Types
ICCV 07105	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 07108	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 07109	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 07110	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 07113	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 07115	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 07116	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 07117	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICC 283	SouthAsia	India	Traditional cultivar/Landrace	Desi
ICC 1882	SouthAsia	India	Traditional cultivar/Landrace	Desi
ICC 4958	SouthAsia	India	Advanced/Improved cultivar	Desi
ICCV 94916-4	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 94916-8	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 98901	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 98902	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 98903	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICCV 98904	SouthAsia	India	Breeding line developed at ICRISAT	Desi
ICC 1026	SouthwestAsia	Iraq	Traditional cultivar/Landrace	Desi
ICC 2234	SouthwestAsia	Iraq	Traditional cultivar/Landrace	Desi
ICC 2935	SouthwestAsia	Iran	Traditional cultivar/Landrace	Desi
ICC 3485	SouthwestAsia	Jordan	Traditional cultivar/Landrace	Desi
ICC 3867	SouthwestAsia	Iran	Traditional cultivar/Landrace	Desi
ICC 3935	SouthwestAsia	Iran	Traditional cultivar/Landrace	Desi
ICC 4482	SouthwestAsia	Turkey	Traditional cultivar/Landrace	Desi
ICC 4902	SouthwestAsia	Turkey	Traditional cultivar/Landrace	Desi
ICC 6152	SouthwestAsia	Jordan	Traditional cultivar/Landrace	Desi
ICC 9125	SouthwestAsia	Iran	Traditional cultivar/Landrace	Desi
ICC 10090	SouthwestAsia	Iran	Traditional cultivar/Landrace	Desi
ICC 10600	SouthwestAsia	Pakistan	Traditional cultivar/Landrace	Desi
ICC 16436	SouthwestAsia	Pakistan	Traditional cultivar/Landrace	Desi
ICC 1163	SubSaharanAfrica	Nigeria	Traditional cultivar/Landrace	Desi
ICC 11886	SubSaharanAfrica	Sudan	Traditional cultivar/Landrace	Desi
ICC 12123	SubSaharanAfrica	Malawi	Traditional cultivar/Landrace	Desi
ICC 12554	SubSaharanAfrica	Ethiopia	Traditional cultivar/Landrace	Desi
ICC 12620	SubSaharanAfrica	Ethiopia	Traditional cultivar/Landrace	Desi
ICC 12787	SubSaharanAfrica	Ethiopia	Traditional cultivar/Landrace	Desi
ICC 13941	SubSaharanAfrica	Ethiopia	Traditional cultivar/Landrace	Desi
ICC 14176	SubSaharanAfrica	Ethiopia	Traditional cultivar/Landrace	Desi

ICC No.	Region	Country	Biological Status	Botanical Types
ICC 16298	SubSaharanAfrica	Malawi	Traditional cultivar/Landrace	Desi
ICC 16833	SubSaharanAfrica	Uganda	Traditional cultivar/Landrace	Desi
ICC 17083	SubSaharanAfrica	Tanzania	Traditional cultivar/Landrace	Desi

Supporting Table 2. Details of kabuli genotypes studied

ICC No.	Region	Country	Biological Status	Botanical Types
ICC 6263	Europe	Russian Federation	Traditional cultivar/Landrace	Kabuli
ICC 4861	Europe	Yogoslavia (Former)	Traditional cultivar/Landrace	Kabuli
ICC 6231	Europe	Spain	Traditional cultivar/Landrace	Kabuli
ICC 7263	Europe	France	Traditional cultivar/Landrace	Kabuli
ICC 7570	Europe	Bulgaria	Traditional cultivar/Landrace	Kabuli
ICC 16774	Europe	Portugal	Traditional cultivar/Landrace	Kabuli
ICC 16820	Europe	Portugal	Traditional cultivar/Landrace	Kabuli
ICC 8261	Europe	Turkey	Traditional cultivar/Landrace	Kabuli
ICC 17109	MesoAmerica	Mexico	Breeding line developed at ICRISAT	Kabuli
ICC 5116	NorthAfrica	Israel	Traditional cultivar/Landrace	Kabuli
ICC 6334	NorthAfrica	Egypt	Traditional cultivar/Landrace	Kabuli
ICC 7292	NorthAfrica	Tunisia	Traditional cultivar/Landrace	Kabuli
ICC 7294	NorthAfrica	Tunisia	Traditional cultivar/Landrace	Kabuli
ICC 7298	NorthAfrica	Tunisia	Traditional cultivar/Landrace	Kabuli
ICC 8527	NorthAfrica	Algeria	Traditional cultivar/Landrace	Kabuli
ICC 11553	NorthAfrica	Egypt	Traditional cultivar/Landrace	Kabuli
ICC 15367	NorthAfrica	Morocco	Traditional cultivar/Landrace	Kabuli
ICC 15380	NorthAfrica	Morocco	Traditional cultivar/Landrace	Kabuli
ICC 15388	NorthAfrica	Morocco	Traditional cultivar/Landrace	Kabuli
ICC 6283	SouthAmerica	Peru	Traditional cultivar/Landrace	Kabuli
ICC 11795	SouthAmerica	Chile	Traditional cultivar/Landrace	Kabuli
ICCV 2	SouthAsia	India	Advanced/Improved cultivar	Kabuli
ICCV 05530	SouthAsia	India	Breeding line developed at ICRISAT	Kabuli
ICC 4969	SouthAsia	India	Advanced/Improved cultivar	Kabuli
ICC 5270	SouthAsia	India	Breeding/Research material	Kabuli
ICC 14533	SouthAsia	Bangladesh	Traditional cultivar/Landrace	Kabuli
ICC 16216	SouthAsia	Mynamar	Traditional cultivar/Landrace	Kabuli
ICCV 3	SouthAsia	India	Breeding line developed at ICRISAT	Kabuli
ICCV 91302	SouthAsia	India	Breeding line developed at ICRISAT	Kabuli
ICCV 93512	SouthAsia	India	Breeding line developed at ICRISAT	Kabuli
ICCV 95311	SouthAsia	India	Breeding line developed at ICRISAT	Kabuli
ICCV 95332	SouthAsia	India	Breeding line developed at ICRISAT	Kabuli
ICCV 06301	SouthAsia	India	Breeding line developed at ICRISAT	Kabuli
ICCV 06302	SouthAsia	India	Breeding line developed at ICRISAT	Kabuli
ICCV 06306	SouthAsia	India	Breeding line developed at ICRISAT	Kabuli
ICCV 07304	SouthAsia	India	Breeding line developed at ICRISAT	Kabuli
ICCV 07311	SouthAsia	India	Breeding line developed at ICRISAT	Kabuli

ICC No.	Region	Country	Biological Status	Botanical Types
ICCV 07312	SouthAsia	India	Breeding line developed at ICRISAT	Kabuli
ICCV 07313	SouthAsia	India	Breeding line developed at ICRISAT	Kabuli
ICCV 07118	Southwest Asia	Iran	Traditional cultivar/Landrace	Kabuli
ICC 6169	SouthwestAsia	Iraq	Traditional cultivar/Landrace	Kabuli
ICC 6969	SouthwestAsia	Iran	Traditional cultivar/Landrace	Kabuli
ICC 7241	SouthwestAsia	Lebanon	Traditional cultivar/Landrace	Kabuli
ICC 8273	SouthwestAsia	Lebanon	Traditional cultivar/Landrace	Kabuli
ICC 10674	SouthwestAsia	Turkey	Traditional cultivar/Landrace	Kabuli
ICC 15779	SouthwestAsia	Syria	Traditional cultivar/Landrace	Kabuli
ICC 15807	SouthwestAsia	Syria	Traditional cultivar/Landrace	Kabuli
ICC 16453	SouthwestAsia	Pakistan	Traditional cultivar/Landrace	Kabuli
ICC 16528	SouthwestAsia	Pakistan	Traditional cultivar/Landrace	Kabuli
ICC 16626	SouthwestAsia	Pakistan	Traditional cultivar/Landrace	Kabuli
ICC 1164	SubSaharanAfrica	Nigeria	Traditional cultivar/Landrace	Kabuli
ICC 11901	SubSaharanAfrica	Sudan	Traditional cultivar/Landrace	Kabuli
ICC 12121	SubSaharanAfrica	Malawi	Traditional cultivar/Landrace	Kabuli
ICC 14913	SubSaharanAfrica	Sudan	Traditional cultivar/Landrace	Kabuli
ICCV 89509	SubSaharanAfrica	Sudan	Breeding line	Kabuli