Biological Forum – An International Journal **5**(2): 114-118(2013)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Diversity and Chemical Composition of some Promising Chestnut *Castanea sativa* Accessions from Kashmir Valley

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(Received 05 February, 2013, Accepted 25 April, 2013)

ABSTRACT: Fifty Chestnut, *Castanea sativa* genotypes comprising of 40 genotypes from Harwan area, 6 from Shalimar (SKAUST-K) and rest from Emporium Garden Lal Chowk were evaluated based on different parameters (bearing habit, number of nuts per bur, number of plump fruits per bur, nut weight, nut size, pellicle adhesion and intrusion, cropping efficiency. The clustering of these accessions grouped them into 12 different clades having less than 25 per cent genetic variability among them. The representative of each clade was evaluated for its chemical composition. On dry matter basis (mg/g) total carbohydrates ranged between 732.4-861.3, total phenols 0.63-4.10, total soluble sugars 190-337, reducing sugars, 28-60, and non reducing sugars 175-281, invert sugar 9.4-12.4, crude cellulose 30.0-48.0, proteins 76.0-120.5, starch 530.57-620.8, sucrose 80.6-210.8, ash 10.0-22.0, total fat 6.7-14.5.

Key words: *Castanea sativa*, Chestnut, chemical composition and diversity analysis.

INTRODUCTION

Chestnut, *Castanea sativa* grows throughout the Himalayas up at altitudes of 2000 to 3000 m asl for its edible nuts. The term 'bread tree' or 'the grain that grows on tree' has been used in some places for chestnuts, which has been one of the fundamental nutrients used in human nutrition. The term "bread tree" has been used in some places for chestnuts, which has been one of the fundamental nutrients used in human nutrition (Bounous *et al*, 2000). The fruit is rich in carbohydrates and low in fat content. This characteristic increases its use in diets. Chestnut is widely used as a food by cooking as well as in cake and candy industry (Anon, 2000). Fresh chestnuts have a high caloric-content (160 K cal per 100 g of edible product), high carbohydrate content (sugar and starch) i.e. 34 g average per 100 g of fresh edible product. It is widely being considered as a valid alternative food for children who are allergic to milk or lactose intolerant and its flour is an ideal carbohydrate alternative for those individuals with cereal intolerance (coelics). Protein content of fresh product is equivalent to that of milk and is of high quality as it contains essential amino acids (tryptophan, lysine and the sulfonated amino acids, methionine and cystenine) and is comparable to the protein content of eggs, considered ideal for amino acid balance. Keeping in view the nutritional potential of these nuts, the first attempt was made to catalogue its available genetic resources from district Srinagar of Kashmir valley and categorize them on the basis of different morphological and chemical parameters. The differences could be detected among the species and the cultivars with respect to their nutritional value. This fact should especially be considered in selection studies. In this way, the genotypes with higher nutritional value as well as high yield and other quality characteristics could be improved. This work was carried out with the aim of determining the chemical composition of some selected important domestic accessions of the chestnut from the valley.

MATERIALS AND METHODS

Fifty genotypes were identified as evaluated comprising of 40 genotypes from Harwan area, 6 from Shalimar (SKAUST-K) and rest from Emporium Garden Lal Chowk based on different parameters (bearing habit, number of nuts per bur, number of plump fruits per bur, nut weight, nut size, pellicle adhesion and intrusion, cropping efficiency). Finally the genetic distance was calculated among the various accessions by construction of dendrogram using SAS. From the 12 different clades each representative accession (HSC026, HSC002, HSC014, HSC040, HSC013, HSC029, HSC044, HSC016, HSC022 and HSC042, SKC044 and HSC036) was evaluated for chemical composition. The fruits were harvested at the end of September through the middle of October. The samples of about 120-150 g fruit that were randomly sampled were squashed with a mortar after their outer shells and seed coat (testa) were removed and analysis were carried out. The dry matter contents of the samples were determined by drying them overnight in the hot-air oven at 105°C. Ash analysis was carried out by burning the sample in muffle furnace at 525°C for 8 h. Total protein quantity was calculated by multiplying the nitrogen content using Kjeldahl method by the coefficient 5.30 (AOAC, 1990).

Crude cellulose quantity was determined according to the method reported in Association of Official Agricultural Chemists (AOAC) (AOAC, 1990). Total fat quantity was found after extraction with ether for 6 h in soxhelet device (AOAC, 1990). Dinitrophenol method was utilised in the analysis of total carbohydrates, total sugar and invert sugar (Ross, 1959) using the spectrophotometer. Starch quantity was calculated by multiplying the value obtained through subtracting the total sugars from total carbohydrates by the coefficient 0.94. The sugars and sucrose was determined as per method described by Galdon and Rodriguez (Galdon *et al,* 2009). The phenolic content was estimated by colorimetric assay described by Singleton and Rossi (Singleton *et al*, 1965)

RESULTS AND DISCUSSION

Total carbohydrates: Total carbohydrate quantities ranged between 732.4 and 861.3 m g/g depending on cultivars. The maximum carbohydrate content was recorded in HSC013 and this was significantly superior compared to all the accessions followed by HSC029 and HSC042. The latter two were at par with each other and significantly better as compared to all the other accessions. The least carbohydrate content was observed in HSC014 and this was significantly lower than all the other accessions. The chestnut fruits generally contained high rates of carbohydrates; this was 86.26 g/100g in American chestnuts (*C. dentata* Borkh.) (McCarthy *et al* 1988), 87.50 g/100g in the Chinese chestnuts (McCarthy *et al* 1988; Anon, 2003c) and 71.68 - 88.10 g/100g in European chestnuts (McCarthy et al 1988; Künsch *et al*, 1999; Bounous, 1999; Bounous *et al*, 2000). The carbohydrate content in accessions from valley has a wide variation falling in the range of 73.24 – 86.13 g/100g. This value changed nearly 16 per cent in the different materials of *C. sativa* species (Bounous *et al*, 2000; Anon, 2003a.). The accessions in the study also show about 15 per cent variation in carbohydrate content in the highest and lowest one.

Phenols: The total phenolic content between 0.63 and 4.10 mg/g. The maximum phenols were recorded in HSC036 and this was significantly higher than all the other accessions except HSC002 and HSC022. The accession HSC013 recorded the least phenolic content and this was at par with the accession HSC040 and HSC016. The highest o-dihydroxy phenolic content was recorded HSC002 and this was significantly higher than all the other accessions except in HSC029, HSC044 and HSC026. The lowest o-dihydroxy phenolic content was observed in SKC044 and this was further at par with HSC040, HSC013 and HSC042. Our results are in tune with the findings of (Gu *et al*, 2004) reporting that most of the *castanea* tissues are rich in both simple phenolics and complex tannins. Low levels (0.1-0.02 g/100g) have been reported in chestnut fruits (Gu *et al*, 2004). However, other chestnut tissues e.g, leaves, wood and bark, have higher levels of these phenolics (Barreira *et al*, 2008). Epidemiological studies show that many polyphenol compounds present in fruits, vegetables, nuts, wine and tea are partly responsible for their beneficial health effects. Phenol compounds are secondary metabolites with considerable physiological and metabolic importance in plants. These compounds play an important role in growth and reproduction, providing protection against pathogens and predators the higher content of phenols in chestnut might be responsible for its hardy nature and resistance to diseases and insects.

Sugars: Total sugar soluble contents changed between 190 and 337 mg/g. The maximum sucrose content was observed in HSC044 and this was significantly higher than that observed in all the other accessions followed by HSC002 and HSC022, the latter two were significantly different from each other and superior as compared to all the other accessions. The lowest total sugar content was observed in the SKC044 and it was significantly lower as compared to all the other accessions. This range of sucrose content in the studied accessions was similar to those obtained by Bounous *et al.,* (2000) and Bounous *et al*., (2000) which were 14.01-20.60 and 20.38 g/100 g, respectively. The invert sugar content of the cultivars ranged between 9.4 and 13.3 mg/g. The invert sugar content among the various accessions was at par with each other. Pavaia *et al,* (1993) found the invert sugar quantity of the cultivars between 0.82 and 3.56 g/100g. The invert sugar contents of the cultivars examined were somewhat lower than these values as the share of invert sugar in total sugars was quite low, ranging between 3.67 and 5.68 for the accession with highest (HSC044) an lowest (SKC044) lowest total soluble sugars. The maximum amount of reducing sugar was observed in HSC029 and this was significantly higher as compared to that observed in all the other accessions followed by HSC026, the later was at par with SKC044. The lowest reducing sugar content was observed in HSC014 and this was significantly lower as compared to all the other accessions except HSC016. The maximum non-reducing sugar content was observed in the HSC002 and this was significantly higher as compared to all the accessions except HSC042. The reducing sugar content in the chestnut comprised of 51.9 to 88.94 percent of the total sugar accession with highest (HSC044) and lowest (SKC044) lowest total soluble sugars. The lowest non-reducing sugar content was observed in SKC044 and this was further at par with that observed in HSC014 and HSC044.

Starch: Starch quantities ranged from 530.57 to 620.8 mg/g with among the different accessions. The maximum starch content was observed in HSC042 and this was significantly higher as compared to all the other accessions followed by HSC013 and HSC029, the latter two were further at par with SKC044 and HSC036. The lowest starch content was recorded in HSC002 and it was significantly lower compared to all the accessions except HSC044. The starch content reported in chestnut was generally ranged between 49.60 and 65.40g /100g in different species (Bounous *et al*, 2000; Liu, 1993; Bounous *et al*, 2000) and this was in tune with our findings.

However, lower (29.80 g/100g) or higher (80 g/100g) starch content than observed in the present studies has been reported by Üstün *et al* (1999) and Demiate *et al* (2001), respectively. A part of starch changes into sugars during storage, thus the ratio of sugars increases and that of starch decreases (Soylu *et al*, 1987). The correlation between the starch and sugar content reflects a strong negative correlation of -0.78, which may be indicative of the variable level of conversion of starch into sugars in these accessions.

Sucrose: The sucrose quantities of the cultivars changed between 80.6 and 210.8 mg/g. The maximum sucrose content was observed in HSC022 and this was significantly higher compared to all the accessions except HSC013. The lowest sucrose content was observed in HSC016 and this was significantly lower as compared to that observed in all the other accessions. The sucrose content have a positive correlation with the total sugars and it was observed to be 0.54 for these accessions. Pavaia *et al* (1993) reported the sucrose content between 10.45 and 19.74 g/100g while Künsch *et al* (1999) observed 12.40 g/ 100g; the accessions from Kashmir valley were slightly in a broader range of 20.08 to 8.06 g/100g.

Ash quantity: The ash content ranged between 10.0 and 22.0 mg/g among accessions. The maximum was observed in HSC040 and this was at par with that observed in HSC013, HSC016, HSC026 and HSC036. The minimum quantity of ash was reported in HSC022 and this was significantly lower compared to all the other accessions except HSC044. The quantity of ash reported in chestnut ranged between 0.83 and 4.92 g/100g in various species and genotypes (Brighenti *et al*, 1998; Üstün *et al*, 1999; Demiate *et al*, 2001; Anon, 2003b, Sundriyal & Sundriyal, 2001; Anon, 2003c.), however the ash quantity in accessions from Kashmir valley had a very narrow range of 1.0 to 2.2 g/100 g.

Table 1: Biochemical profile (mg g^{-1} Dry Weight) of representative chestnut accessions.

Accessions	Total phenols	o-dihydroxy phenol	Total soluble sugars	Reducing sugar	Non reducing sugar	Invert sugar
HSC026	2.44	1.97	251	52	199	9.8
HSC002	3.80	2.24	322	41	281	10.0
HSC014	2.10	1.62	202	28	175	11.0
HSC040	0.83	0.78	273	37	236	11.5
HSC013	0.63	0.33	259	37	222	10.0
HSC029	1.63	2.01	243	60	203	12.2
HSC044	2.05	2.00	337	35	175	12.4
HSC016	0.67	1.11	261	29	190	10.0
HSC022	3.76	1.31	305	43	256	13.3
HSC042	2.20	0.54	210	38	267	12.1
SKC044	1.87	0.43	190	47	169	10.8
HSC036	4.10	1.18	245	31	243	9.4
CV	**16.83**	**21.99**	**3.21**	**10.72**	**4.18**	**14.78**
LSD (0.05)	**0.56**	**0.47**	**13.91**	**7.29**	**15.06**	**NS**

Table 2: Biochemical profile (mg g^{-1} Dry Weight) of representative chestnut accessions.

Accessions	Total carbohydrate	Crude cellulose	Proteins	Starch	Sucrose	Ash	Total fat
HSC026	805.4	48.0	97.8	521.1	140.5	20.0	8.5
HSC002	763.6	44.0	84.4	415.1	180.1	16.8	11.6
HSC014	732.4	56.0	115.5	498.6	100.4	19.0	10.5
HSC040	802.5	40.0	88.9	497.7	163.3	22.0	5.6
HSC013	861.3	30.0	93.3	566.2	194.7	19.9	7.5
HSC029	845.3	47.2	110.6	566.2	155.4	18.0	14.5
HSC044	790.2	44.7	76.0	426.0	132.2	11.0	12.9
HSC016	754.5	35.8	99.5	463.9	80.6	20.0	10.7
HSC022	803.9	41.5	109	469.0	210.8	10.0	6.7
HSC042	834.6	33.8	87.0	587.1	120.5	17.3	9.5
SKC044	783.6	44.3	120.5	558.0	105.5	16.7	12.7
HSC036	824.7	37.1	101.6	544.9	99.06	21.1	14.0
CV	**0.95**	**7.61**	**2.51**	**1.37**	**3.66**	**11.74**	**15.43**
LSD (0.05)	**12.73**	**5.33**	**4.15**	**11.64**	**8.58**	**3.47**	**2.67**

Crude cellulose: The crude cellulose quantities of the cultivars ranged from 33.8 to 56.0 mg/g. The maximum crude cellulose content was reported in HSC014 and this was significantly higher compared to all the accessions. However in most of the accessions such as HSC026, HSC002, HSC029, HSC044 and SKC044 the crude cellulose content was in a closer range of 44.0 to 48.0 mg/ g and all these were at par with each other. The least cellulose content was observed in HSC013 and it was significantly lower as compared to all the other accessions except HSC042. Demiate *et al* (2001) found the crude cellulose quantity in Brazilian cultivars (*C. sativa)* as 2.34 g/100g however, Sundriyal and Sundriyal (2001) reported the crude cellulose content in American, European and Chinese chestnuts between 1.00 and 2.00 g/100g. The accessions from valley showed the notable differences in crude cellulose quantity between 3.0 and 5.6 g/100g.

Total fat: The total fat content of the samples ranged from 5.6 to 14.0 mg/g. The maximum fat content was observed in HSC029 and it was significantly higher compared to all other accessions except HSC044, SKC044 and HSC036. Minimum fat content was observed in HSC022 and this was significantly lower as compared to all the other accessions except HSC040, HSC013 and HSCO26. This fat content ranging between 0.66 and 5.59 g/100g has been reported in the cultivars belonging to the species *C. sativa* Mill. (Ferreria-Cardoso *et al*, 1993; Demiate *et al*, 2001; Soylu *et al*, 1987; Anon, 2003b). Fat content was determined as 1.98 mg/g in the Chinese chestnuts (Anon, 2003c) and as 0.38 g/100g in some Australian cultivars (Sundriyal & Sundriyal, 2001). Only few of the Indian chestnuts showed the fat content as reported in Australian cultivars. However most of them showed higher fat content but this was less as reported in Chinese cultivars.

Total protein: Total protein quantity ranged between 76.0 and 120.5 mg/g among the various accessions. The maximum protein content was reported in SKC044 and this was significantly higher than all other accessions followed by HSC014 and HSC029, the latter two were significantly different from each other. The lowest protein content was reported in HSC044 and this was significantly lower as compared to all the other accessions. The protein content was reported between 3.43 and 13.28 g/100g in *C. sativa* Mill. Cultivars (Pavaia *et al*, 1993; Ferreria-Cardoso *et al*, 1993; Brighenti *et al*, 1998; Bounous, 1999; Üstün *et al*, 1999; Anon, 2003a). This range was narrower in the Chinese chestnuts being between 2.12 and 7.49 g/100g (McCarthy *et al*, 1988; Anon, 2003c). However higher range was observed in Indian-Kashmir chestnuts i.e between 7.6 to 12.05 g/100g.

The accessions identified offer a wide variety of chestnut diversity and very promising sources based on chemical profile. The study offers a first and basic platform to identify the promising accessions which can be utilized for breeding programme keeping in view the nutritive potential of this fruit. From a general point of view, the chemical composition of chestnut may vary depending on the source from which the fruits were taken. However it can be stated that fruit of chestnut contained mainly carbohydrates, mostly in starch and sucrose form. It is also good source of antioxidants indicated by the phenolic content as well as rich source of proteins. From the various composition and health studies it is clear that chestnut fruits, and potentially other extracts from chestnut trees, have considerable potential as functional foods or as food ingredients, *e.g.* chestnut polyphenolic extracts as a natural source of antioxidants and other beneficial compounds such as gallic, ellagic acids and ellagitannins. The benefits that this fruit can provide for human and animal health are numerous, but it is clear that improvements can be made for both production and quality of chestnut products, e.g. genetic selection and optimizing industrial processing.

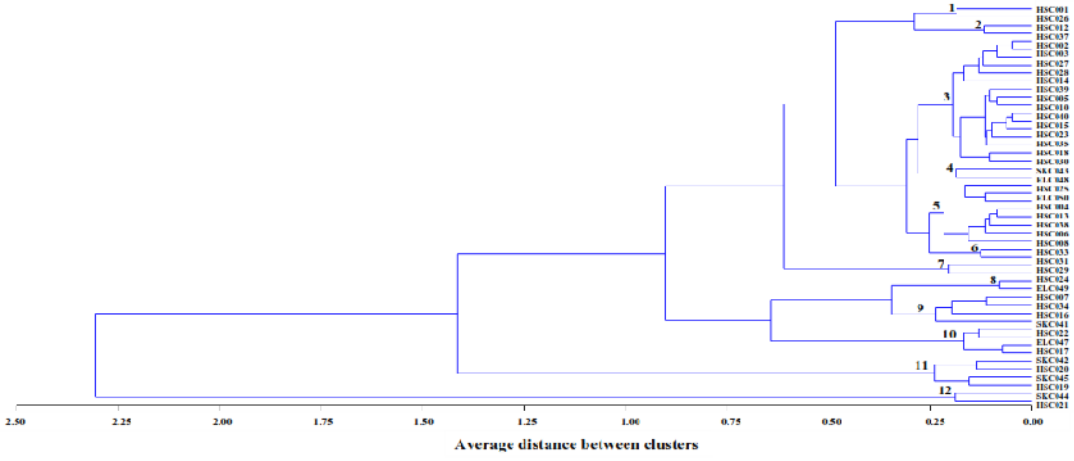

Fig 1. Cluster analysis of chestnut accessions based on different morphological parameters clubs them into twelve different clades.

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