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Tuber quality assessment of orange-fleshed sweet potato (*Ipomoea batatas*) cultivars and their genetic relatedness as revealed by SDS-PAGE of tuber proteins

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ABSTRACT

Orange-fleshed sweet potato (OFSP) is an important source of β-carotene and can make a major contribution in alleviating vitamin A malnutrition. Replacement of white-fleshed varieties with new β-carotene-rich OFSP cultivars that meet local preference would benefit the children who are at risk for vitamin A deficiency related problems. Twelve OFSP cultivars of diverse origin were analyzed for biochemical and antioxidant composition, viz dry matter, crude protein, crude fat crude fibre, carbohydrate, starch, amylose, reducing sugars, total phenols, ascorbic acid and β-carotene contents of their tubers. Many of the investigated cultivars have high levels of dry matter, crude protein, starch, β-carotene, phenols and ascorbic acid. Cultivars with high dry matter and β-carotene can be utilized to develop cultivars for commercial exploitation. This paper also highlights the genetic relatedness among the OFSP cultivars, assessed by using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of tuber proteins. High genetic similarities were observed among the cultivars originated in India.

Key words: Antioxidants, β-carotene, Orange-fleshed sweet potato, SDS-PAGE, Tuber biochemical composition, Tuber protein diversity

Sweet potato [*Ipomoea batatas* (Lam)] is one of the most important food crops in the world and provides not only staple food but also important as an industrial raw material. Originating in South America, it is now grown all over the world spreading throughout the tropical and subtropical countries. Sweet potato is vital to destitute small-scale farmers with limited land, labour and capital. Besides its importance as food, feed and industrial raw material, it is also recognized as an efficient converter of solar energy (240 × 10^3 Kcal/ha). The area, production and productivity of sweet potato are sharing an increasing trend globally. India is one of the leading producers (1.1 million tonnes) of this crop along with China (81.2 million tonnes), Brazil (0.5 million tonnes) and Peru (0.3 million tonnes) (FAOSTAT 2010). In India it is grown in an area nearing 0.14 million ha concentrated mostly in Odisha, Bihar, West Bengal and Uttar Pradesh, producing approximately 1.17 million. tonnes with a productivity of 8.3 tonnes/ha.

Sweet potato tubers are rich in starch, sugars, minerals and vitamins. Being rich in β-carotene, the OFSPs are gaining importance as a biofortified crop to combat malnutrition in small and marginal farming community (Haskell *et al.* 2004). OFSP has considerable potential to contribute to a food-based approach to prevent the problem of vitamin A deficiency, which is a major public health concern of the poorer sections in developing countries. It is a particularly promising food, because it contains a high level of β-carotene [100-1 600 µg retinol activity equivalent (RAE)/100 g] and is also a good source of energy (293-460 kJ/100 g) (Hagenimana *et al.* 2001). Considerable effort is now being made in different countries to promote the intake of pro-vitamin A through consumption of OFSP along with a variety of suitable plant sources of β-carotene. The bioavailability of β-carotene is affected by several factors (Tanumihardjo 2000). Recent studies showed that minimal fat is necessary for absorption and conversion of β-carotene to retinol (Ribaya-Mercado *et al.* 2007). Although different factors affecting

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bioavailability of carotenoids have been studied, knowledge of their interaction remains limited. Therefore, along with β-carotene content other biochemical constituents of OFSP tubers need to be evaluated for selecting promising cultivars. OFSP is a staple crop targeted for biofortification (Bovell-Benjamin 2007). Efforts to biofortify sweet potato have focused on increasing β-carotene content and improving organoleptic qualities of commonly consumed cultivars. Replacement of white-fleshed sweet potato (WFSP) with orange-fleshed varieties could benefit the children (<6 year of age) at risk of vitamin A deficiency (van Jaarsveld *et al.* 2006). The present study was carried out to evaluate the dry matter, crude protein, crude fat, crude fibre, starch, amylose, carbohydrate, reducing sugars, phenol, ascorbic acid and β-carotene composition of tubers from 12 cultivars. In addition, the genetic relatedness of these cultivars was also assessed on the basis of tuber protein profile.

MATERIALS AND METHODS

Twelve representative OFSP cultivars (Table 1) were grown in the germplasm evaluation plots of All India Coordinated Research Project on Tuber Crops (ICAR), Bidhan Chandra Krishi Viswavidyalaya Centre, West Bengal, India during 2009–10. The field soil texture was silty clay (10.1% sand, 40% silt and 49.9% clay), soil pH and organic matter were 6.8 and 0.88%, respectively. No fertilizers were applied during the whole growth period. The cultivars used in this study were harvested after a growing period of 120 days. Undamaged and uniform shaped tubers were collected immediately after harvest and cleaned with water to remove dirt.

Raw sweet potatoes were peeled and cut into small pieces. The sliced tubers were initially sun-dried, and then dried in a forced-draft oven at 60 ^{O}C until constant weight was reached. The dried chips were powdered and dry weight for each cultivar was determined. The flour samples were stored in airtight containers at room temperature (20–25 °C) until analyzed. The estimation of crude protein, crude fat, crude fibre, starch, amylose, carbohydrate, reducing sugars and phenol content was done on the dry weight basis. The ascorbic acid and β-carotene content were determined on the basis of fresh weight.

Nitrogen, crude fat and crude fiber were determined according to AOAC (1995). The titrimetric Kjeldahl digestion method (method number 955.04D) was used for nitrogen estimation and crude protein contents were calculated as N×6.25. Determinations of crude fat and crude fibre were carried out by using gravimetric method (Method number 920.85 and 920.86, respectively). Starch was determined according to Sadasivam and Manickam (1996) which involve extraction of starch with perchloric acid. The glucose content in the sample was estimated using standard graph and the starch content was obtained by multiplying the value by a factor 0.9. Total carbohydrate was assessed following Sadasivam and Manickam (1996). The amylose content in tuber flour was estimated using a rapid colorimetric method following McGrance *et al.* (1998). The estimation of reducing sugars content was done according to Nelson-Somogyi method (Somogyi 1952).

Total phenolic content was estimated using the Folin-Ciocalteu assay according to the procedure of Makkar *et al.* (1993). Total phenol content was estimated as tannic acid equivalent. The 2,6-Dichloroindophenol titrimetric AOAC method 967.21 was used for ascorbic acid estimation in the samples (AOAC 1995). Sample and acetic acid-metaphosphoric acid were mixed in the ratio of 1 : 1 (v/v). The ascorbic acid content was measured by titrimetry using 2,6-Dichlorophenolindophenol. A blank (distilled water) and ascorbic acid (Sigma-Aldrich Cat A7506) standard were also measured. Determination of β-carotene was done following the AOAC spectrophotometric method (method No. 941.15, AOAC 1995). The retinol activity equivalents (RAE) of the OFSP cultivars were calculated on the basis of conversion rate: 12 μg β-carotene = 1 μg retinol = 1 μg RAE (Institute

Table 1 Varietal characteristics of 12 OFSP cultivars

Cultivar	Origin	Skin colour	Flesh colour	Sweetness
S 61	CTCRI, Trivandrum, Kerala	Pink	Orange	Low
S 594	CTCRI, Trivandrum, Kerala	Pink	Orange	Low
S 1156	CTCRI, Trivandrum, Kerala	Pink	Yellow	Moderate
90/101	BCKV, Kalyani, West Bengal	Pink	Yellow	High
SV 98	CTCRI, Trivandrum, Kerala	Brown	Orange	Moderate
362-7	CTCRI, Trivandrum, Kerala	Brown	Yellow	Low
IGSP 15	IGAU, Raipur, Chhattisgarh	Pink	Yellow	High
CIPSWA-2	CIP, Peru	Pink	Orange	Moderate
187017-1	CIP, Peru	Pink	Orange	High
440127	CIP, Peru	Pink	Yellow	High
ST 14	CTCRI, Bhubaneswar, Odisha	Brown	Orange	Low
Kamala Sundari	BCKV, Kalyani, West Bengal	Brown	Orange	Low

of Medicine, Food and Nutrition Board 2001).

Proteins were extracted from freeze dried tubers using 1 mL Tris-HCl buffer (*p*H 6.8), and the decanted supernatants after centrifugation (14 000 rpm at 4 °C for 20 min.) were taken for SDS-PAGE analysis. Prior to loading on gel, the extracted proteins were mixed well with sample buffer (2X stock: 2.5 mL of 4X Tris-HCl buffer, *p*H 6.8 + 0.4 g of SDS + 2 mL of 100% glycerol + 400 µL of 5% bromophenol blue + 0.2 M DTT + water to 10 ml) and heated in boiling water for 5 min. The electrophoresis was performed according to Laemmli (1970). The electrophoresed gel was stained overnight with coomassie brilliant blue, then destained and photographed.

The data on different biochemical parameters were submitted to one-way analysis of variance (ANOVA) and expressed as mean of duplicate measurements. The means were compared using Duncan's multiple range test (DMRT). Differences were considered statistically significant at P=0.05. Principal component analysis (PCA) of the mean data was also performed. Analyses were carried out using statistical software, SPSS version 16.0 for windows.

Protein bands were scored for their absence as 0 and presence as 1. The similarity matrix was estimated using the similarity coefficient (Sokal and Michener 1958) and NTSYSpc (version 2.1, Rohlf 2000). The similarity matrix = m/n, where m = shared present fragments + shared absent fragments and n = total of obtained fragments. The dendogram based on similarity matrix was constructed using the unweighed pair-group method with arithmetic averages (UPGMA) by using NTSYSpc software package.

RESULTS AND DISCUSSION

The observations on skin and flesh colour of 12 OFSP cultivars are given in Table 1. The spectrum of skin colour of the tubers was observed among the cultivars. The tuber flesh colour was mostly orange with deviation in contrast, though, some of the cultivars possessed yellow coloured flesh. Variation was also observed for the sweetness of tuber flesh (Table 1).

Descriptive statistics

Significant (P<0.05) differences were observed among OFSP cultivars with regard to all the parameters studied. The mean, range, standard deviations and coefficients of variation of 11 biochemical parameters of 12 OFSP cultivars are presented in Table 2. The dry matter content of tubers ranged from 21.5-29.4%. Unlike traditional orange-fleshed cultivars with lower dry matter content than white-fleshed cultivars, some of the orange-fleshed cultivars (187017-1, CIPSWA 2 and SV 98) had higher dry matter content (27–29%). Orange-fleshed cultivars with higher dry matter content had also been reported earlier (TheVITAAProject 2001). These OFSP cultivars with higher dry matter can be used to develop cultivars with higher dry matter content because there has been a strong consumer preference for types with higher dry matter. The crude protein content of the cultivars varied between 4.4 and 7.8 g/100 g dry weight with a average value of 6.3 g/100 g dry weight. Highest crude protein content was recorded for cultivar S 1156 which was closely followed by cultivars like S 594, CIPSWA 2, 440127, 362-7, 90-101, IGSP 15 and SV 98. The crude fat and fiber content of tubers ranged between 1.1 and 2.1 g/100 g and 1.9 and 3.3 g/100 g on dry weight basis respectively (Table 3).

Table 2 Biochemical characteristics of 12 OFSP cultivars

Trait	Mean	SD	Max	Min	CV (%)
Dry matter (% w/w)	25.4	2.17	29.4	21.5	4.4
Carbohydrate (g/100 g dw)	6.3	0.84	7.8	4.4	6.1
Starch (g/100 g dw)	1.6	0.28	2.1	1.1	8.8
Reducing sugar (g/100 g dw)	2.6	0.41	3.3	1.9	8.1
Amylose (g/100 g dw)	65.4	8.47	80.1	54.4	9.6
Crude protein (g/100 g dw)	19.4	1.93	23.5	16.4	5.4
Crude fat (g/100 g dw)	29.5	4.23	37.1	21.2	8.1
Crude fibre (g/100 g dw)	3.7	0.45	4.7	2.8	5.9
Total phenol (g/100 g dw)	1.0	0.22	1.6	0.5	11.0
Ascorbic acid (mg/100 g fw)	19.0	4.20	29.4	14.8	11.5
β-carotene (mg/100 g fw)	7.2	2.13	12.2	4.1	14.4

dw, Dry weight; fw, fresh weight

Starch, the storage form of carbohydrate was the predominant fraction of the dry matter. Cultivars like S-594, CIPSWA 2, 362-7, SV 98, 187017-1 of higher dry matter content had recorded higher level of starch (Table 3). The average starch content of the tubers was recorded to be 65.4 g/100 g dry weight. Amylose content affects the textural properties of starch and influences the pasting quality. Pasting is an important property in determining starch behaviour in food products, affecting product quality such as texture, stability and digestibility. In this study, the average amylose content of OFSP tubers (19.4 g/100g dry weight) was lower than that of previously reported OFSP clones from CIP, Peru (Brabet *et al.* 1999). The amylose content of the OFSP cultivars included here ranged from 16.4 to 23.5 g/100 g dry weight. The cultivars like SV 98, 362-7, ST 14 and 187017-1 had higher amylose content (Table 3). Carbohydrates which exist as free sugars and polysaccharides are the important

Table 3 Proximate composition of biochemical characters in OFSP tubers[a]

Cultivar	Dry matter (% w/w)	Crude protein (g/100 g dw)	Crude fat (g/100 g dw)	Crude fibre (g/100 g dw)	Starch (g/100 g dw)	Amylose (g/100 g dw)	Carbohydrate (g/100 g dw)	Reducing sugars (g/100 g dw)	Total phenol (g/100 g dw)	Ascorbic acid (mg/100 g fw)	β-carotene (mg/100 g fw)	RAE µg/ 100 g
S 61	22.1^{g}	5.6^{c}	1.3^{fg}	1.9^{g}	58.0^{de}	19.3^{cd}	28.9^{c}	3.5^{ef}	1.2^{a}	15.8^{bc}	11.0^{a}	941.3
S 594	26.1^{bcde}	6.7^{b}	1.4^{ef}	2.5^{de}	71.6^{b}	19.7^{c}	30.7^{bc}	3.3^{fg}	1.1^{ab}	14.8^{a}	5.8^{cde}	917.9
S 1156	25.2^{de}	7.7^{a}	1.7^{bc}	2.5^{ef}	64.5^{c}	16.6^{f}	32.5^{b}	3.8^{bcde}	1.1^{ab}	15.2^{ab}	6.3^{cd}	522.3
90/101	22.6^{fg}	6.6^{b}	1.5^{de}	2.4^{f}	55.4^{e}	17.4^{e}	29.9^{bc}	4.5^{a}	1.3^{a}	19.3^{e}	4.6^{e}	577.1
SV 98	27.7^{abc}	6.4^{b}	1.2^{g}	2.5^{de}	79.1^{a}	23.4^{a}	30.9^{bc}	3.6^{ef}	0.9^{abc}	28.9^{h}	6.8^{cd}	570.6
362-7	25.0^{cde}	6.7^{b}	2.0^{a}	2.6^{d}	77.7^{a}	21.2^{b}	31.9^{b}	3.4^{efg}	0.5^{c}	17.6^{d}	9.0^{b}	463.8
IGSP 15	24.2^{ef}	6.7^{b}	1.6^{cd}	2.8^{c}	58.8^{d}	18.8^{d}	22.8^{d}	4.1^{abcd}	1.2^{a}	16.2^{c}	5.6^{d}	530.0
CIPSWA 2	28.0^{ab}	6.4^{b}	1.6^{cd}	3.1^{b}	71.6^{b}	18.8^{d}	31.3^{bc}	3.8^{bcde}	0.9^{abc}	17.4^{d}	6.9^{c}	482.5
187017-1	29.0^{a}	6.5^{b}	1.8^{b}	3.0^{b}	70.0^{b}	20.9^{b}	24.4^{d}	4.3^{ab}	1.1^{ab}	18.7^{e}	6.4^{cd}	477.5
440127	24.6^{def}	6.8^{b}	1.4^{ef}	1.9^{g}	56.4^{de}	18.8^{d}	22.3^{d}	4.1^{abc}	1.1^{ab}	17.6^{d}	5.7^{cde}	384.6
ST 14	24.0^{efg}	4.6^{d}	2.0^{a}	2.6^{de}	57.0^{de}	20.9^{d}	36.4^{a}	3.0^{g}	1.0^{ab}	22.4^{f}	11.3^{a}	568.3
Kamala Sundari	26.5^{bcd}	5.0^{cd}	1.3^{fg}	3.2^{a}	64.0^{c}	17.0^{ef}	31.8^{b}	3.6^{ef}	0.7^{bc}	24.3^{g}	6.9^{cd}	751.9

[a]Values are expressed mean of duplicate measurements. Mean values of each column followed by different superscript letter significantly differ when subjected to DMRT (P=0.05)

components of storage and structural materials in plants. Among the cultivars included in this study, ST 14 registered highest level of carbohydrate content (36.4 g/100 g dry weight), while, the lowest value was recorded for 440127, and the total carbohydrate content varied between 21.2 and 37.1 g/100 g dry weight (Table 3). The reducing sugar (glucose, galactose, lactose and maltose) content ranged from 2.8 to 4.7 g/100 g dry weight (Table 3).

The content and variation in antioxidant substances are reported in Table 3. The level of total phenols, a class of molecules having antioxidant properties varied between 0.5 and 1.6 g/100 g dry weight. The highest phenol content was recorded for cultivar 90/101, closely followed by S 61, IGSP 15, S 594, CIPSWA 2, S 1156, ST 14 and 440127. Considerable variation in ascorbic acid content (CV = 11.48%) of the tubers, ranging from 14.8 to 29.4 mg/100 g fresh weight, was recorded among the cultivars. A few genotypes like SV 98, Kamala Sundari, ST 14 and 90/101 had higher level of ascorbic acid content. Rautenbach *et al.* (2010) also reported similar ranges of ascorbic acid in OFSP cultivars.

The OFSP cultivars showed substantial variation in β-carotene content (CV = 14.43%) with a range of 4.1 to 12.2 mg/100 g fresh weight. Vitamin A value in retinol activity equivalents (RAE) of these OFSP cultivars varied between 384.6 and 941.3 µg RAE/100 g (Table 3). The values for vitamin A of these OFSP cultivars were lower than the OFSP cultivars reported by van Jaarsveld *et al.* (2006), though, the level of vitamin A in present OFSP cultivars is quite higher than the level recommended by the Food and Agriculture Organization (FAO)/ World Health Organization (WHO) (FAO/WHO 2001). The mean β-carotene content of the studied cultivars showed ST 14 = S 61 > 362-7 > CIPSWA 2 = Kamala Sundari = SV 98 = 187017-1 = S 1156 = S 594 = 440127 > IGSP 15 > 90/101.

The bioavailability of β-carotene that depends upon the presence of inhibitors and enhancers (like fibre and fat, respectively) in the tubers will be higher in the cultivars having lower fibre and higher fat contents. Thus the β-carotene in the tubers of S 1156, ST 14, S 61, SV 98 and 362-7 will be more bio-available than other OFSP cultivars. In addition, high dry matter content in tubers enhances conversion of β-carotene to vitamin A.

PCA on biochemical traits

The PCA variable loadings, percentage of variance explained and cumulative variance for the first four principal components are given in Table 4. The first four principal components accounted for 81% of the total variance. The first principal component (PC1) accounted for 33% of the total variance and had factors with high contribution as dry matter, carbohydrate, starch, crude fibre, ascorbic acid and β-carotene content. Most of the traits except, reducing sugar, crude protein and total phenol contributed positively towards PC1. The second principal component (PC2) accounting for

Table 4 Factor loadings of the 11 biochemical characters for the first four principal components and the percentage variance accounted for each component

Trait	PC1	PC2	PC3	PC4
Dry matter (% w/w)	0.516	0.779	0.002	–0.063
Carbohydrate (g/100 g dw)	0.621	–0.393	0.142	–0.271
Starch (g/100 g dw)	0.663	0.601	0.112	0.314
Reducing sugar (g/100 g dw)	–0.694	0.530	–0.124	–0.130
Amylose (g/100 g dw)	0.621	0.093	–0.175	0.633
Crude protein (g/100 g dw)	–0.477	0.596	0.388	0.369
Crude fat (g/100 g dw)	0.274	–0.107	0.790	–0.112
Crude fibre (g/100 g dw)	0.461	0.522	0.092	–0.652
Total phenol (g/100 g dw)	–0.779	–0.225	–0.146	0.004
Ascorbic acid (mg/100 g fw)	0.564	0.081	–0.710	–0.117
β-carotene (mg/100 g fw)	0.534	–0.739	0.136	0.160
Variance (%)				
Proportion	33.501	24.134	12.774	10.973
Cumulative	33.501	57.634	70.408	81.381

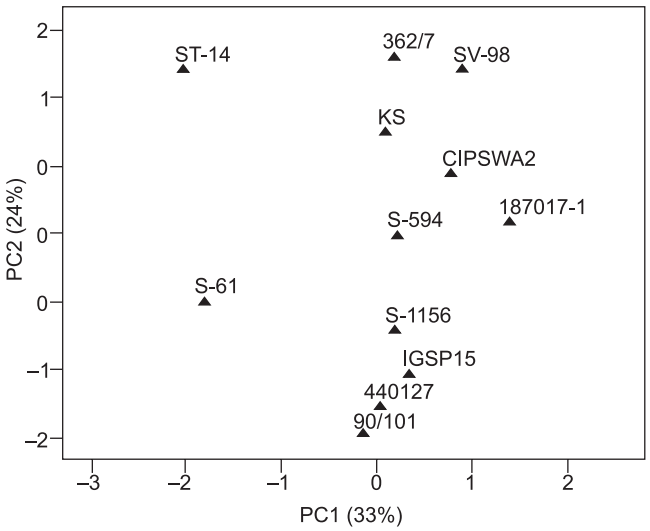

Fig 1 Configuration of 12 OFSP cultivars under principal axes 1 and 2

an additional 24% of the total variation, depicted primarily the pattern of variation in dry matter, starch, reducing sugar, crude protein and crude fibre content. Finally, third and fourth principal components (PC3 and PC4) contributed around 12% and 11%, respectively of the variation among the cultivars for the traits studied. The PC1 distinguished those cultivars that have higher contents of dry matter, starch, amylase, ascorbic acid and β-carotene. In contrast, PC2 distinguished cultivars having high dry matter, starch, crude protein and reducing sugar content (Fig 1). PCA is an effective method in grouping genotypes according to their phenotypes and better understanding of existing variability that facilitate genotypic selection for crop improvement (Roy *et al*. 2011).

Genetic diversity on the basis of electrophoresis pattern of tuber protein

The banding pattern of tuber proteins from 12 OFSP cultivars are shown in Fig 2a. A total of 29 polypeptide bands were detected with molecular masses ranging from 11.2 to 111 kDa. The genetic similarities based on the SDS-PAGE bands are shown in Table 5. The dendogram revealed (Fig 2b) that cultivars from CIP (CIPSWA 2 and 440127) along with IGSP 15 from India were distantly related with the other OFSP cultivars. High similarity was observed between pink-

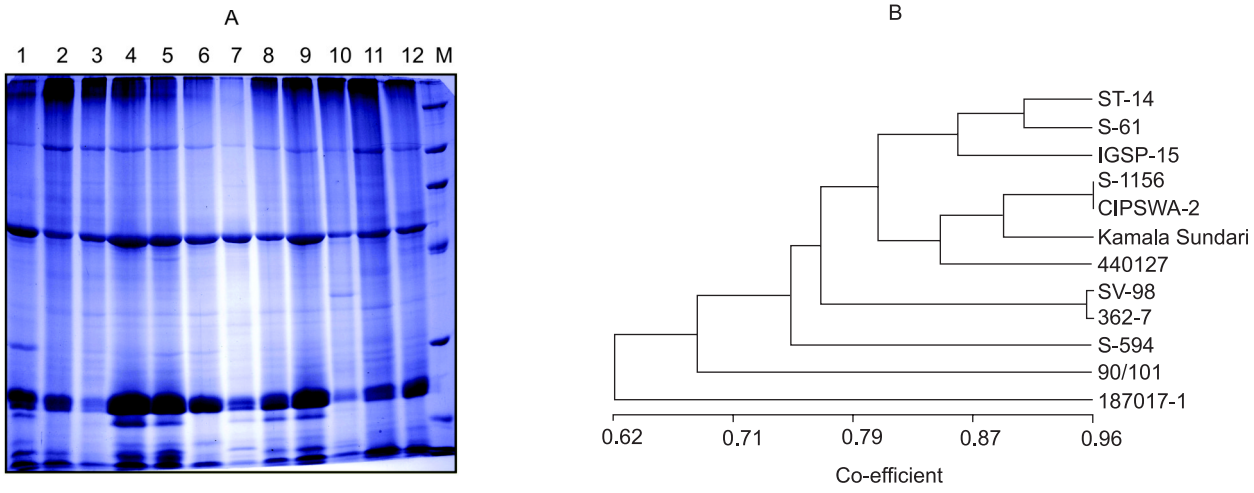

Fig 2 SDS-PAGE analysis of tuber proteins of 12 OFSP cultivars. (*A*) Electrophoregram showing protein banding pattern in 12 OFSP cultivars S 61, S 594, S 1156, 90/101, SV 98, 362-7, IGSP 15, CIPSWA 2, 187017-1, 440127, ST 14 and Kamala Sundari respectively. Lanes 1–12 refer to the cultivars mentioned in Fig 1. M-molecular weight marker, (*B*) dendogram of 12 OFSP cultivars based on similarity matrix of tuber proteins

Table 5 Similarity matrix based on tuber protein banding pattern of 12 OFSP cultivars. Number 1–12 represent the cultivars denoted in Table 1

	1	2	3	4	5	6	7	8	9	10	11	12
1												
2	0.91											
3	0.83	0.91										
4	0.79	0.87	0.96									
5	0.79	0.87	0.88	0.91								
6	0.83	0.86	0.79	0.75	0.75							
7	0.64	0.64	0.65	0.62	0.62	0.74						
8	0.69	0.77	0.78	0.74	0.74	0.73	0.73					
9	0.74	0.82	0.82	0.86	0.86	0.77	0.63	0.85				
10	0.79	0.72	0.67	0.63	0.69	0.68	0.56	0.60	0.64			
11	0.78	0.86	0.79	0.75	0.75	0.74	0.54	0.73	0.77	0.68		
12	0.75	0.83	0.76	0.72	0.79	0.71	0.52	0.70	0.74	0.72	0.95	

skinned cultivars S 1156 and 90/101 and brown-skinned cultivars ST 14 and Kamala Sundari. A cluster of seven cultivars was divided into two sub-clusters: S 61, S 594, and 362-7, and S 1156, 90/101, S 98 and 187017-1. The tuber protein similarities among the cultivars ranged from 0.52 to 0.96 (Table 5). From the similarity coefficient table it is clear that the cultivars from India had higher similarities among themselves. Unlike the cultivars from CIP which appeared to be somewhat distant from rest of the cultivars. The cultivars under study, collected from the field gene bank of AICRP on tuber crops, BCKV Centre, ICAR, were representing the cultivars developed or selected in different research centers. It might be the fact that the narrow genetic base of OFSP cultivars from India resulted due to lack of genetic diversity as cultivars were developed by intensive selection and subsequent exclusion of much genetic variation from germplasm stocks.

In view of the results, it is suggested that the orange-fleshed sweet potatoes can serve as good source of protein, starch and antioxidants and it had the potentiality to nurture the poorer and hunger part of human societies. Biofortification efforts can focus on increase in dry matter, β-carotene and dietary antioxidants in OFSP cultivars. Regular intake of OFSP will have a positive impact on nutrition status and will check the childhood blindness among the children in developing countries. The SDS-PAGE of tuber protein suggested a narrow genetic base of the cultivars, which was also reflected from the low variability for most of the parameters studied. The parameters such as β-carotene content, total phenol content and ascorbic acid content with considerable amount of variation can be improved through selective breeding methods.

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