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Studies on bio-chemical profiling of Indian gooseberry (*Emblica officinalis*) for genetic diversity

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Abstract

Biochemical profiling of physiologically mature fruits of 51 diverse Indian gooseberry (Emblica officinalis Gaertn) germplasm accessions was collected from Vindhyan hill region of Madhya Pradesh, with a view to select nutraceutically rich genotypes based on important biochemical traits. The mean ascorbic acid and total phenol (tannin) content amongst different accessions was recorded as 496.47mg $100g^{-1}$ and 4.88% with highest value found in CISH A-12 (654.50mg $100g^{-1}$) and CISH A-30 (7.18%), respectively. Apart from the above, wide range of variability in the composition of other important biochemical attributes viz., total soluble solids (8.60-17.70°Brix), acidity (1.61-2.94%), total sugar (4.15-9.17), reducing sugar (2.19-4.45%) and TSS/acid ratio (3.89-8.33) was also recorded. Highest significant and positive correlation was observed between total sugar and TSS (0.895) followed by reducing sugar and TSS (0.882). Significant positive correlation between ascorbic acid and tannins (0.551) was an indication to be associated with binding capacity of ascorbic acid over a longer period of storage.

Key words

Accessions, Aonla, Ascorbic acid, Diversity, Germplasm, Indian gooseberry, Phenol

Introduction

Indian gooseberry (*Emblica officinalis* Gaertn.), locally known as *'Aonla'* or *'Amla'*, is the native to tropical Southeast Asia, particularly central or southern India, Pakistan, Bangladesh, Sri Lanka, Malaya, Southern China and Mascarene Islands and grown all over Asia for its nutritional, pharmacological and commercial significance (Singh *et al*., 2011). It is an important fruit crop of subtropical and tropical regions of India, producing more economical yield (100 to 300kg $plant^{-1}$) than any other fruit tree grown in the region (Goyal *et al*., 2007). Various health rejuvenative products *viz*., Chyavanprash, Trifla, Amrit Kalash, along with other ayurvedic formulations and value added products, are being prepared from its fruits (Sachan *et al*., 2013). Fruit and seed weight varies from 22.29-25.20 g and 1.54-1.82 g respectively amongst the cultivated types (Goyal *et al*., 2007). Total sugar content varies from 7 to 9.6%, reducing sugars from 1.04 to 4.09% and non-reducing sugars from 3.05 to 7.23% among the cultivated varieties. Considerably, higher amount of carbohydrates coupled with many minerals and vitamins like calcium, phosphorus, iron, zinc, anthocynin, vitamin 'B' complex and essential amino acids has been reported. Fresh fruit consumption is not common because of sour, acidic and astringent taste, although it is very popular being richest source of ascorbic acid and polyphenols (tannins) after Barbados cherry (Goyal *et al*., 2008).

Considerable variation in ascorbic acid content of Indian gooseberry fruits (385 to 1800mg $100g^{-1}$) has been reported from various parts of the world (Pareek, 2012). A variety of phytochemicals such as polyphenols (flavonoids, kaempferol, ellagic acid and gallic acid), tannins, terpenoids and alkaloids have reported to indicate various pharmacological properties (Rehman *et al*., 2007; Singh *et al*., 2011). Tannins containing gallic and ellagic acid may help in slow reduction of ascorbic acid and thereby render it the value of fresh and dried pulp as anti-scorbatic (Bhattacharya *et al*., 1999 and Zhang *et al*., 2001). Indian gooseberry germplasm accessions differ among themselves

in the fruit shape, size, weight, pulp: stone ratio and fruit maturity, which offer tremendous scope for improvement of this crop through seedling selection and hybridization, to evolve the new variety rich in antioxidant value, yield and fruit quality. Hence, biochemical profiling of Indian gooseberry germplasm accessions, collected from Vindhyan hill region for potential utilization to nutraceutical based industries or for varietal improvement programme, has been accomplished.

Materials and Methods

Collection of fruit samples : Diverse genotypes of Indian gooseberry were collected, based on survey conducted during the year 2006-07 to 2008-09 from Rewa, Panna, Satna, Chitrakoot, Sidhi and Sahdol districts of Madhya Pradesh. Twenty five physiologically mature fruits from each of the 51 accessions were harvested randomly from all the four directions of trees and fruits were stored in 2% perforated LDPE bags. The stored samples were brought to Central Institute of Subtropical Horticulture (ICAR), Lucknow, for analysis of physical and bio-chemical parameters. The stored samples were sorted out and uniform sized fruits free from spots, damages and blemishes were kept for the study. The minimum sample size in each accession was fifteen and five fruits from each accession were randomly selected as replicate.

Biochemical parameters : Juice extracted from fresh fruits by squeezing the homogenized fruit pulp through muslin cloth was used to measure total soluble solids (TSS). It was determined by using ERMA hand refractometer (Japan) for each entry in triplicate. The mean was expressed in ^{0}Brix after temperature correction. Five grams of fresh homogenized pulp was made up to 100 ml and filtered through muslin cloth. Then 10 ml aliquot of filtrate was taken for titration against 0.1N NaOH solution, using phenolphthalein as indicator. Titrable acidity (TA) was expressed as per cent citric acid (Ranganna, 1986).

Powdered sample weighing 100 mg was transferred into test tube and 10 ml alcohol (50%) was added, centrifuged at 5000 rpm for 15-20 min and the supernatant was collected and evaporated on water bath. Finally, 10 ml of CCl_4 was added and kept for 10 min at room temperature. The whole material was transferred into a separating funnel forming two layers. The lower layer was discarded and the upper layer was collected and 10 ml distilled water was added to dissolved sugar. This extract was used for estimation of reducing and total sugar (Miller, 1959). The per cent transmittance of standard (prepared from D-glucose) and samples against reagent blank was read at 575 nm on spectrophotometer (Thermo Spectronic, Helios Epison, USA). Reducing sugar was determined and expressed in percentage (g of reducing sugars $100g^{-1}$ of fresh pulp). Similarly, total sugar content was determined by the method of Dubois *et al.* (1956) using phenol reagent. The intensity of colour was recorded at 480 nm on Spectronic-20 against blank solution. The calculation was done with the help of standard curve prepared from standard glucose solution and the results were expressed as percent (g of total sugar per 100g of edible fresh pulp). TSS/acid ratio was calculated from total acidity (TA) and TSS values. Ascorbic acid content in fresh fruit was determined by the method described by A.O.A.C (1970). Total phenol (Tannins) content was determined using Folin-Ciocalteu's reagent (Singleton and Rossi, 1965). The collected data were statistically analyzed using SAS 9.3.

Results and Discussion

Fifty one Indian gooseberry germplasm accessions were classified into five groups based on fruit weight class intervals (Fig. 1). Wide variation was recorded in fruit weight which, varied from 13.95g to 53.15g. Highest number of Indian gooseberry accessions fall in the class interval grouped II (21.50-29.50g) followed by group I (13.95-21.50g), whereas class interval group III and IV represented 10 genotypes each. Group VI (45.5-53.50g) contained only 03 genotypes and recorded maximum fruit weight. In this group, highest fruit weight was recorded by the selection CISH A-43 (53.15g) followed by CISH A-17 (48.20g) and CISH A-51 (47.10g) were collected from Sahdol, Satna and Panna district of Madhya Pradesh. This variation might be due to high concentration of Indian gooseberry diversity being the hot spots region, as well as, the nutrient status of soil where particular genotypes grown by local tribal communities in the region. Similarly, wide variation in fruit shape, size and surface colour was recorded (Fig. 2). Majority of germplasm accessions were round in shape however, in CISH A-11 one of the accessions collected from Brihaspati kund of Satna district recorded flattened shoulder, which slowly pointed towards distal end giving a view of three distinct segments, although practically six segments are recorded. Similarly, variability in fruit colour was recorded which varied from dull light green to bright light green to golden whitish colour. On few accessions, red blush (CISH-A7 and CISH-A11) on shoulder as well as red tinged (CISH-A24) colour on fruit surface were recorded (Fig. 2).

A wide variation in TSS content of fruit was observed in Indian gooseberry accessions (Table 1). Highest TSS was found in Indian gooseberry accessions CISH–A-51, CISH A-8, CISH A-25 (17.70^{o}brix) followed by CISH A-50 and CISH A-4. Among the accessions, lowest TSS was observed in CISH A-9 (8.60^o Brix). Variation in TSS content of Indian gooseberry is in agreement with the previously published reports (Aulakh *et al*., 2013). Although, maximum TSS, reported in the established Indian gooseberry cultivars, was

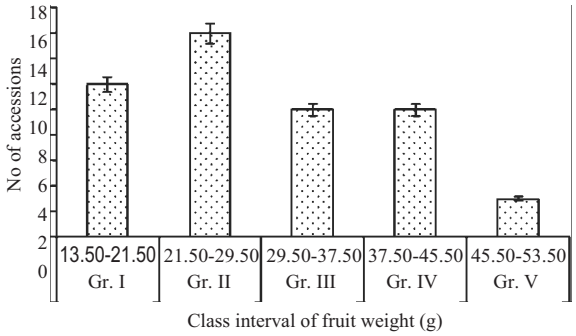

Fig. 1 : Classification of Indian gooseberry germplasm based on fruit weight (g) class interval

Fig. 2 : Variability pattern in fruit shape, size and colour of Indian gooseberry germplasm accessions amongst 51 accessions collected from Vindhyan hill region of Madhya Pradesh, India

higher than that in the present study and further substantiate the vast diversity existing in the Vindhyan hill region of India.

Acidity content of Indian gooseberry accessions showed wide variation, ranging from 1.61% to 2.94%. Almost similar work was reported earlier by Goyal *et al.* (2008). Maximum titrable acidity found in CISH A-8 was significantly higher over rest of the accessions, while minimum was recorded in CISH A-20. Many accessions were found to have lower acidity levels and statistically at par with CISH A-8. Generally, total acidity in Indian gooseberry was low at immature stage and slowly increased with advancement of maturity (Pathak, 2003). However, in the present study, all precautions were taken to harvest physiologically mature fruits and this wide variation in acidity might be due to greater diversity exist in the Vindhyan hill region.

TSS/acid ratio is an economically important biochemical parameter, as it determines taste, sugar acid blend and acceptability of product by consumers in the market. Although, commercial acceptability of Indian gooseberry as fresh fruit is very meager since it possess sour astringent taste. In the present investigation, maximum TSS/acid ratio was associated with accessions CISH A-51 (8.33) and least was noticed in CISH A-21 (3.89). TSS content of CISH A-35, CISH A-31 and CISH - 41 was more than 12.98°Brix, their TSS/acid ratio was lower on account of their higher acidity content (Table 1). Similar findings were reported by Aulakh *et al*. (2013) in the performance evaluation of Indian goose beery cultivars under agro climatic conditions of Punjab. On the other hand, CISH A-51 with highest TSS above 17.70°Brix, showed highest TSS/acid ratio which could be attributed to genetic makeup of the accessions. Generally, TSS is indicative of sugar level in fruit and low acidity, and high sugar are responsible for sweet taste of fruits.

Total sugar and reducing sugar content in Indian gooseberry accessions ranged from 4.15% (CISH A-9) to 9.17% (CISH A-1) and 2.19% (CISH A-10) to 4.45% (CISH A-51), respectively (Table 1). Similar findings were reported in commercial Indian gooseberry cultivars NA-6, NA-7, Krishna and Kanchan by Goyal *et al*. (2008) and Sahu (2013). No significant difference was observed among CISH A-13, CISH A-15, CISH A-32, CISH A-42 and CISH A-50 with respect to total sugars.

Indian gooseberry is medicinally more important because of high antioxidant content, especially ascorbic acid and total phenols (tannins). Ascorbic acid content in Indian gooseberry accessions exhibited a wide range of variation from 349mg $100g^{-1}$ (CISH A-27) to 654.50mg $100g^{-1}$ (CISH A-12) being average value 496.97mg/100g edible portion. The values are in conformity with the earlier published works (Goyal *et al*., 2008; Pareek, 2012). No statistical difference in ascorbic acid content was noticed among CISH A-12, CISH A-3, CISH A-14, CISH A-15, CISH A-26, CISH A-30, CISH A-37 and CISH A-51. The average value of ascorbic acid was found to be significantly higher than the established cultivars (Table 1). Further, majority of the accessions contain higher amount of tannins from average value and natural tannins present in these accessions further prevent the oxidation of ascorbic acid as suggested by Bhattacharya *et al*. (1999). Wide variation in ascorbic acid content in the present study further substantiates wide variability in Indian gooseberry germplasm accessions collected from diverse agro-ecological environments of Vindhyan hill regions of India (Pathak, 2003). Since Indian gooseberry is a commercial crop for nutraceutical industry, the present diversity can be gainfully utilized for future breeding programme.

Total phenol content was measured as tannic acid and presented as tannins. Wide variation was recorded in total

Table 1 : Mean values of total soluble solids (TSS), acidity, TSS: acid ratio, sugars, tannins and ascorbic acid in 51 accessions of Indian gooseberry collected from Vindhyan region of Madhya Pradesh

Accessions code	TSS (°Brix)	Acidity (%)	TSS/ acid ratio	Total sugar (%)	Reducing sugar (%)	Tannins (%)	Ascorbic acid (mg $100g^{-1}$)	Accession code	TSS (°Brix)	Acidity (%)	TSS/ acid ratio	Total sugar (%)	Reducing sugar (%)	Tannins (%)	Ascorbic acid (mg $100g^{-1}$)
CISH-A 1	15.10	2.42	6.24	9.17	4.25	6.10	471.83	CISH-A 27	13.80	2.70	5.11	6.46	3.47	2.87	349.00
CISH-A 2	9.85	2.34	4.20	5.58	2.39	5.38	455.00	CISH-A 28	12.80	2.75	4.65	6.95	2.94	6.09	582.50
CISH-A 3	12.00	2.41	4.98	6.56	2.80	5.00	643.67	CISH-A 29	10.80	2.70	4.00	5.08	2.51	4.71	474.83
CISH-A 4	16.35	2.87	5.69	7.75	4.25	4.53	425.34	CISH-A 30	10.80	2.03	5.31	6.09	2.90	7.18	637.50
CISH-A 5	12.55	2.60	4.81	6.90	3.25	5.29	518.67	CISH-A 31	12.90	2.62	4.92	6.97	3.45	5.46	513.50
CISH-A 6	11.65	2.49	4.68	5.30	3.10	3.39	448.50	CISH-A 32	12.90	2.34	5.53	6.43	3.08	4.70	394.00
CISH-A 7	14.25	2.53	5.63	7.87	3.54	4.15	563.50	CISH-A 33	12.30	2.50	4.90	5.28	3.05	3.34	451.65
CISH-A 8	17.70	2.94	6.03	8.37	4.40	4.35	585.00	CISH-A 34	11.40	2.29	4.97	6.31	2.48	7.16	405.00
CISH-A 9	8.60	1.83	4.71	4.15	2.58	6.02	592.50	CISH-A 35	13.05	2.52	5.16	6.40	3.11	4.69	412.00
CISH-A 10	8.70	2.08	4.18	4.16	2.19	5.12	454.50	CISH-A 36	15.25	2.54	6.12	7.31	3.52	4.34	409.50
CISH-A 11	14.05	2.76	5.09	6.56	3.52	2.95	349.66	CISH-A 37	12.00	2.87	4.18	5.74	3.45	4.38	628.00
CISH-A 12	14.80	2.78	5.32	7.92	4.35	5.05	654.50	CISH-A 38	15.70	2.60	6.03	7.49	3.78	4.74	361.00
CISH-A 13	12.70	2.68	4.73	6.79	2.94	6.13	584.00	CISH-A 39	12.80	2.68	4.76	7.13	3.52	5.59	538.65
CISH-A 14	11.25	2.69	4.19	6.10	2.80	5.90	634.67	CISH-A 40	10.60	2.50	4.23	5.20	2.72	4.56	482.00
CISH-A 15	10.70	2.70	5.17	6.06	2.95	7.11	635.33	CISH-A 41	13.10	2.40	5.43	6.78	3.46	4.05	356.00
CISH-A 16	12.25	2.41	5.09	6.98	2.80	5.76	595.50	CISH-A 42	12.40	2.73	4.55	7.04	2.90	6.37	593.84
CISH-A 17	14.50	2.21	6.55	6.75	3.23	4.32	479.50	CISH-A 43	13.75	2.56	5.37	6.59	3.45	3.48	413.33
CISH-A 18	13.00	2.39	5.44	6.40	3.72	4.63	392.44	CISH-A 44	12.30	2.62	4.68	6.89	3.37	6.33	592.17
CISH-A 19	10.55	2.61	4.03	5.09	2.39	4.02	404.00	CISH-A 45	14.90	2.55	5.83	7.28	3.50	4.40	459.50
CISH-A 20	9.60	1.61	5.94	5.49	2.78	3.89	424.00	CISH-A 46	12.90	2.31	5.58	6.51	3.14	4.69	405.50
CISH-A 21	10.30	2.64	3.89	4.99	2.44	4.60	472.67	CISH-A 47	11.80	2.81	4.20	6.20	3.89	5.04	581.50
CISH-A 22	12.50	2.88	4.33	6.12	2.85	4.48	421.17	CISH-A 48	12.70	2.33	5.45	6.57	3.12	4.62	407.00
CISH-A 23	15.00	2.49	6.02	7.19	3.66	4.27	409.50	CISH-A 49	15.00	2.46	6.10	7.30	3.49	4.33	416.00
CISH-A 24	15.10	2.50	6.04	7.35	3.58	4.30	410.50	CISH-A 50	16.45	2.79	5.90	8.63	4.36	4.33	583.67
CISH-A 25	17.70	2.90	6.09	8.40	4.40	4.45	586.50	CISH-A 51	17.70	2.12	8.33	8.90	4.45	4.37	623.83
CISH-A 26	11.60	2.83	4.10	6.01	2.85	5.90	636.15	LSD (p=0.05)	4.29	0.54	1.64	2.15	1.15	1.97	186.91

Table 2 : Variability pattern of Indian gooseberry accessions collected from Vindhyan hill region of Madhya Pradesh, India

Variables	Mean	Range		Standard Error of Mean	Variance	Skewness	Kurtosis	LSD (p=0.05)
		Min.	Max.					
Total soluble solids (°Brix)	12.98	8.60	17.70	0.307	4.807	0.289	-0.169	4.29
Acidity (%)	2.51	1.61	2.94	0.38	0.073	-1.138	1.934	0.54
TSS/ acid ratio	5.18	3.89	8.33	0.117	0.702	0.947	2.394	1.64
Total sugars (%)	6.62	4.15	9.17	0.154	1.213	0041	0.181	2.15
Reducing sugars (%)	3.27	2.19	4.45	0.082	0.345	0.394	-0.459	1.15
Tannins (%)	4.88	2.87	7.18	0.141	1.019	0.454	0.036	1.97
Ascorbic acid (mg $100g^{-1}$)	496.47	349.00	654.50	13.35	9094.61	0.198	-1.422	186.91

phenol content, being maximum in CISH A-30 (7.18%) followed by CISH A-34 (7.16%) where as minimum was recorded in CISH A-27 (2.87%). Total phenol content in CISH A-30, CISH A-34, CISH A-42, CISH A-44, CISH A-28, CISH A-15 and CISH A-1 did not differ significantly from each other and the amount of phenol (tannins) present in these accessions was more than the average value of Indian gooseberry germplasm accessions studied (Table 1). Moreover, these accessions were also coupled with higher amount of ascorbic acid from the average value, which further substantiated that tannin contained gallic, ellagic acid and glucose which might retard oxidation of ascorbic acid and render its value as anti ascorbutic in fresh and dried conditions as suggested by Bhattacharya *et al.* (1999), Rehman *et al*. (2007) and Zhang *et al*. (2001).

Distribution of skewness values of different traits *viz*, TSS (0.289), total acidity (-1.13), TSS/acid ratio (0.97), total sugars (0.041), reducing sugars (0.394), tannins (0.454) and ascorbic acid (0.198) showed that TSS, TSS/acid ratio, total sugar, reducing sugar, tannin and ascorbic acid were positive and skewness showed mass concentration values on left side

Table 3 : Correlation coefficient of quality attributes among the eight variables of fruit weight of 51 accessions of Indian gooseberry accessions collected from Vindhyan hill region of Madhya Pradesh, India

Variables	Fruit weight (g)	TSS (^{0}Brix)	Acidity (%)	Tannins (%)	Ascorbic acid (mg 100g^{-1})	Reducing sugars (%)	Total Sugars (%)	TSS: Acid ratio
Fruit weight (g)	1.000							
Total Soluble Solids (^{0}Brix)	-0.131	1.000						
Acidity (%)	-0.429**	0.423**	1.000					
Tannins (%)	-0.162	-0.334**	-0.180	1.000				
Ascorbic acid (mg/100g)	-0.043	-0.056	0.109	0.551**	1.000			
Reducing sugars (%)	-0.149	0.882**	0.369**	-0.276**	0.085	1.000		
Total Sugars (%)	-0.165	0.895**	0.331**	-0.015	0.146	0.840**	1.000	
TSS/acid ratio	0.195	0.757**	-0.263**	-0.234**	-0.119	0.670**	0.715**	1.000

** Significant at p (0.05)

of the curve, whereas acidity skewness negative value (-1.13) was indicated on the right side of graph. The traits with kurtosis values *viz*, TSS (0.0), acidity (1.93), TSS/acid ratio (2.39), total sugar (0.181), reducing sugar (0.0), tannin (0.036) and ascorbic acid (0.0) indicate a peak shaped curve would be more flatter than normal curve since all the values for all traits were less than three (Table 2). The estimates of genotypic correlation coefficient between fruit weight and biochemical attributes were worked out (Table 3). The correlation coefficient revealed that acidity, total soluble solids (TSS), tannins, TSS/acid ratio and sugar: acid ratio was significantly and positively associated with each other. Highest significant and positive correlation was observed between total soluble solids (TSS) and total sugars (0.895) followed by reducing sugar (0.882), as well as, between total sugar and reducing sugars (0.840) followed by total soluble solids and TSS/acid ratio (0.757). Similar observation with TSS/acid ratio was recorded in mango (Wongkhot *et al.*, 2012), and in different fruit juices (Eissa *et al*., 2014), where as negative correlation with fruit weight and TSS/acid was also recorded in jackfruit (Maiti, 2012). Significant positive correlation between ascorbic acid and tannins (0.551) was also observed which indicated that tannins might contributed towards ascorbic acid, sugars and total soluble solids. A positive correlation between biochemical attributes could be useful measure of fruit maturity for future selection of genotypes.

The present investigation deciphers information on genetic diversity pattern of biochemical traits and some of the accessions such as CISH A-12, CISH A-17, CISH A-30, CISH A-43 and CISH A-51 can be commercially exploited directly as they contain high antioxidant value.

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