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Assessment of the variations in selected industrially desirable morphological and biochemical traits of eleven *Citrus* species in Sri Lanka

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Abstract

The fruits with nutraceutical and medicinal values are having a greater significance in modern food industry. Out of many fruit tree bearing genera, the genus *Citrus* is extremely important in this regard. It contains well known species such as orange, lime, mandarin, lemon and many other underutilized species. The nutritive value of *Citrus* is given by many bioactive compounds present in the fruits such as alkaloids, tannins, phenols and saponins. In Sri Lanka, the available *Citrus* germplasm is not characterized in detail. Especially underutilized *Citrus* species are yet to receive a proper taxonomic identity. Therefore in the present study we report some of the important morphological and biochemical traits of eleven *Citrus* species. The fruit size traits; weight, height and width were very highly variable among the studied genotypes and positively correlated to each other (P<0.05). However, juice volume was not highly correlated with the other fruit size traits. The juice volume per unit fruit weight can be given as a better indicator for the selection of these species for industrial applications. Pummelo, a popular edible *Citrus* fruit, showed the lowest volume to weight ratio of 0.06. The size of the endocarp cells (i.e. juice sacks) was also very highly variable among genotypes / species studied. The brix value, a parameter of total soluble solids, was highest in Sidaran fruits (8.07%) and lowest in cooking types such as lemon and lime (3.27% and 3.76% respectively). Similarly the ascorbic acid concentration was also lowest in lime and lemon (1.9 and 1.5 mM respectively). The sweet orange cultivar Sisila (5.9 mM) and Pummelo (6.9 mM) had the highest ascorbic acid concentrations. The results reported here are very useful to select these genotypes / species for potential industrial applications, future genetic and breeding studies and to further characterize at molecular level to establish an accurate taxonomic scheme for Sri Lankan *Citrus* germplasm which will be the next focus of our group.

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Keywords:Citrus spp. ; Brix percentage; Ascorbic acid concentration; *Citrus sinensis*; *Citrus auriantium*, Underutilized fruit species

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1. Introduction

The genus *Citrus* comprises a wide range of species with significant economic and medicinal values. The taxonomy of *Citrus* species is not fully resolved yet especially when the underutilized species within the family are considered. The well-known examples of the *Citrus* species are orange, lime, mandarin, lemon and grapefruit. The fruit is the most important part of the plant but the other parts such as leaves and stems also contain significant amounts of bioactive compounds. Most of the *Citrus* fruits are highly preferred as a fresh juice[1] and as a flavour enhancer in food indicating the very high economic value of the plant. The nutritive value of *Citrus* is given by a multitude of compounds such as alkaloids, tannins, phenols and saponins[2]. Moreover, *Citrus* species contain antimicrobial compounds which make them frequently being used in medicinal preparations. Exploitation of the bioactive compounds in various parts of the *Citrus* plants for the treatment of disorders in respiratory and digestive tracts has been documented. In addition, the phytonutrients in *Citrus* plants account for the anti-cancer[3], antidepressant and anti-inflammatory activities[4].

Citrus plants have been widely cultivated in many parts of the world in large scale, exceeding eight billion hectares in 2012. However, in Sri Lanka, despite the higher abundance of *Citrus* germplasm, the industry-based cultivation of *Citrus* species is underdeveloped. Furthermore, utilization of *Citrus* plants for the large scale extraction of bioactive compounds with medicinal values is also not well developed. This may be partly due to the limited studies conducted to properly classify the local *Citrus* germplasm and to explore the potential benefits of this important genus.

According to our knowledge, detailed studies have not been carried out to assess the morphological parameters and nutritive values of the diverse *Citrus* spp. available in Sri Lanka although such a within species analysis was reported for sweet orange[5]. Therefore, the present study was conducted as a part of an on-going project to assess eleven *Citrus* species for fruit morphological and important biochemical parameters to be used in potential industrial applications.

2. Materials and Methods

2.1. Plant material

Two sweet orange cultivars and ten other *Citrus* species (total of 11 species) (Table 1) were collected from the three Regional Agricultural Research and Development Centers at Bandarawela, Monaragala, Bibila, Sri Lanka and from home-gardens in Kandy and Polonnaruwa in May, 2015. Three to five randomly selected matured fruits (unripe) were harvested and taken from each tree. Fruits were collected from three to five randomly selected trees from each cultivar/ species, so that, at least a total of nine fruits taken from each cultivar/species were subjected to the morphological and biochemical analyses.

2.2. Fruit parameters

The diameter (both width and height axis) and weight were recorded for each fruit collected. The number of cells was counted along a radius of the cross section and cell size was determined by using the microscopic images of the fruit cross sections. The juice volume of each fruit was measured using a measuring cylinder after squeezing the each fruit by applying a uniform force. The pH was measured using a pH meter and color of the juice was determined using a standard color chart and normalized using Adobe Photoshop CS6 to obtain the universal # color codes. The soluble sugar content was measured in terms of brix percentage obtained using a refractometer at 24 ^{0}C. The ascorbic acid concentration was determined using a titrimetric method where a known volume of juice from each fruit was titrated against a standard KI/I_2 solution in the presence of starch as the indicator. All the fruit parameters were analyzed using the ANOVA and CORR Procedures in the Statistical Package SAS 9.1.3 (SAS Institute, NC, Cary, USA).

3. Results and Discussion

The fruit cross sections of the each studied *Citrus* genotype / species are shown in Figure 1. Fruit weight, height and width of the 12 studied *Citrus* genotypes were very highly variable among the species. Fruit weight and height were positively and significantly correlated with the Pearson Correlation Coefficient of 94.51 %. Whereas, the traits, weight vs. width (90.24 %) and height vs. width (98.79 %) were also positively and significantly correlated to each other. However, mean fruit weight and respective juice volumes were not significantly correlated implying that higher fruit weight is not always attributed to the amount of juice present inside the fruit. Juice volume per unit fruit weight can be considered as a better parameter to explain the industrial value of the fruit. Juice volume to fruit weight ratio was highest in lime (0.60) and lowest in Pummelo (0.06) ($P<0.05$). The least juice volume in Pummelo is not a big problem as it is being sold and consumed as a fresh fruit. The pH is significantly lowest in lime and lemon (2.37 and 2.54 of pH) and the highest pH was observed in Pummelo attributing its popularity as a fresh fruit. The endocarp cell size was also very highly variable among the tested *Citrus* genotypes but the correlation between juice volume and the cell size was 12.06 % implying the independence of the available juice from the number of juice sacks available within the fruits. The juice color, which is an industrially important parameter, of each genotype / species are indicated as # color codes given by Adobe Photoshop CS6. These color codes can be used by the juice or jam manufacturers to decide whether artificial color enhancement is required in industrial processing.

The brix percentage, amount of total soluble solids, was highest in Sidaran and lowest in lime (3.67 %), lemon (3.27 %) and Citramelo (3.87 %). In sweet orange cultivars and in all the other *Citrus* species studied, the brix percentage was higher and in the range between 4.80 % to 8.07 %. The brix percentage is extremely important for industrial processing of these *Citrus* fruits in to commercial beverages and jams. A higher brix value of 7.55 % in sweet orange cultivar Sisila is an encouraging sign for it to use as an industrial sweet orange cultivar. The ascorbic acid content is extremely important as it marks the amount of Vitamin C present in *Citrus* fruits. Pummelo had the highest ascorbic acid concentration (6.9 mM) indicating it's very high significance as a fresh fruit. The species used in cooking such as lime and lemon exhibited low ascorbic acid concentration of 1.9 mM and 1.5 mM respectively. The sweet orange cultivar Sisila exhibited 5.9 mM of ascorbic acid concentration further highlighting its significance as an industrially useful sweet orange cultivar (Table 1). The Correlation between brix percentage vs. pH was 5 %, pH vs. ascorbic acid concentration was 44.68 %, brix percentage vs. ascorbic acid concentration was 6 %. These lower correlation values imply the possible independent genetic mechanisms controlling the biochemical properties of fruit juice in *Citrus.*

In conclusion, the recorded data set for the selected species of Sri Lankan *Citrus* germplasm reflects a variation in measured morphological and biochemical parameters indicating their suitability for different industrial applications. However, the general industrial applicability should be determined by considering all these measured parameters rather than relying on one or few parameters. Furthermore, it may have a great significance for future bio-prospecting programs leading to potential industrial biotech applications. This will undoubtedly lift the wild *Citrus* spp. from their underutilized status while significantly contributing to the development of rural economies. Also collected germplasm in this study will be immensely useful to further characterize at molecular level to establish an accurate taxonomic scheme for Sri Lankan *Citrus* germplasm which will be the next focus of our group.

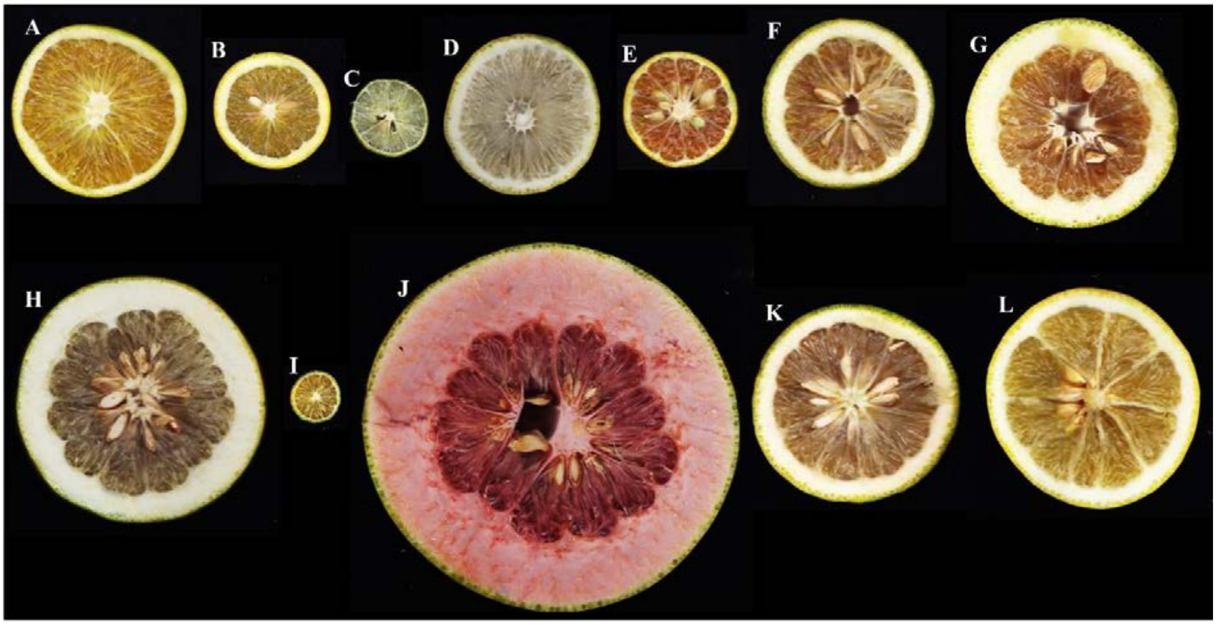

Fig. 1. Cross sections of the Citrus fruits studied A:Arogya, B: Sisila, C: Lime, D: Lemon, E: Mandarin, F: Sour orange, G: Sidaran, H: Grapefruit, I: Heen-naran, J: Pummelo, K: Marmalade, L: Citramelo

Table 1. Morphological and biochemical parameters of the *Citrus* fruits.

Vernacular Name	Species name	Weight (g)	Height (cm)	Width (cm)	pH	Volume (ml)	Volume per unit Weight	Juice color$^{\$}$	Cell size (cm)	Brix %	Ascorbic acid conc. (mM)
Arogya*	*C.sinensis*	183.17^{c}	7.24^{c}	7.24^{d}	3.20^{b}	56.86^{b}	0.31^{b}	c3972c	0.68^{c}	5.78^{d}	3.30^{c}
Sisila*	*C.sinensis*	94.05^{c}	5.84^{d}	5.91^{e}	2.98^{b}	27.07^{c}	0.29^{b}	cea962	0.80^{c}	7.55^{b}	5.90^{b}
Lime	C. *aurantifolia*	32.61^{d}	4.22^{e}	4.05^{f}	2.37^{c}	19.5^{c}	0.60^{a}	a8ac8b	1.28^{b}	3.76^{f}	1.90^{c}
Lemon	*C.limon*	125.84^{c}	5.89^{d}	5.54^{e}	2.54^{c}	28.2^{c}	0.22^{b}	ccc5a9	1.77^{b}	3.27^{f}	1.50^{c}
Mandarin	*C.reticulate*	39.50^{d}	4.07^{e}	4.36^{f}	2.89^{b}	14.33^{c}	0.36^{b}	c27e59	0.55^{c}	6.14^{c}	2.80^{c}
Sourorange	*C.auriantium*	173.47^{c}	7.23^{c}	7.23^{d}	2.88^{b}	39.33^{c}	0.23^{b}	8d5b28	1.07^{b}	5.05^{e}	3.10^{c}
Sidaran	*C.medica*	292.80^{c}	8.57^{c}	8.9^{c}	3.03^{b}	76.44^{b}	0.26^{b}	a47847	1.28^{b}	8.07^{a}	2.80^{c}
Grapefruit	*C.paradisi*	449.18^{b}	10.03^{b}	9.9^{b}	3.42^{b}	127.22^{a}	0.28^{b}	a9977f	1.21^{b}	4.25^{f}	3.10^{c}
Heen-naran	*C.crenatifolia*	31.86^{d}	3.91^{e}	4.17^{f}	3.23^{b}	5.45^{d}	0.17^{c}	a69054	0.59^{c}	6.36^{c}	2.10^{c}
Pummelo	*C.maxima*	1163.33^{a}	15.88^{a}	14.24^{a}	3.98^{a}	74.25^{b}	0.06^{d}	d29197	2.65^{a}	4.80^{e}	6.90^{a}
Marmalade	*C.* sp	242.72^{c}	7.71^{c}	7.69^{d}	2.88^{b}	39.5^{c}	0.16^{c}	ad8a6d	1.69^{b}	5.72^{d}	2.70^{c}
Citramelo	*C.* sp	135.80^{c}	6.73^{c}	6.49^{e}	2.77^{b}	48.86^{b}	0. 36^{b}	aa8b4a	1.39^{b}	3.87^{f}	3.70^{c}

*Sweet orange cultivars commonly grown in Sri Lanka

$^{\$}$The juice color is indicated as # color code given by Adobe Photoshop CS6

Means denoted by same letters within each column are significantly different at $P < 0.05$

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