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Evaluation of different *Citrus* species for physico-chemical composition of fruits

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Abstract
An experiment was conducted in main experimental station of horticulture farm situated in Acharya Narendra Deva University of Agriculture and Technology (ANDUA&T), Kumarganj, Ayodhya, India to evaluate different *Citrus* spp. for physico-chemical composition of fruits. Seven species of Citrus i.e T_1 (Kinnow), T_2 (Rangpur Lime), T_3 (Pant lemon), T_4 (Kagzi lime), T_5 (Nagpur Mandarin), T_6 (Sweet Orange var. mosambi) and T_7 (Grapefruit var, NRCC Grapefruit-6) were evaluated in completely randomized design with three replications. The results of the experiment revealed that the treatment T_7 (Grapefruit) showed maximum fruit weight (407.52 gm), fruit length (9.12 cm), fruit width (8.90 cm) and Ascorbic acid content (46.04 mg/100 gm). However T_1 (Kinnow) showed maximum TSS (10.61° brix) and Titrable Acidity (9.21%) and T_4 showed maximum specific gravity (1.01 g/cc). Non-reducing (5.76%) and reducing sugars (3.51%) were highest for treatment T_6 (Sweet Orange) and lowest (0.63 % and 0.75%) for treatment T_4 (kagzi lime). Whereas, for Total sugars it was observed that that T_4 was lowest (1.73%) while T_5 (8.44%) showed highest total sugars. So if physico-chemical parameters are to be concerned T_7 (grapefruit) can be regarded as the best species to be used in citrus crop improvement programs while if quality is to be concerned then Kinnow (T_1) and Sweet orange (T_6) would be the best bet for utilization in citrus crop improvement programs.

Keywords: *Citrus* species, physico-chemical, fruits

Introduction
Citrus (*Citrus* spp.) is one of the major fruit crops around the globe. It mostly inhabits the tropical and subtropical regions of the world. It belongs to the family *Rutaceae* and has its origin from the china (Mir *et al*., 2021) [16]. On a global scale, citrus is widely recognized as an important perennial fruit crop. Globally, the production of citrus amounts to 161.8 million tons produced from an acreage of about 10.2 million hectares (FAO, 2023) [7]. India ranks third in citrus production following China and Brazil. In India it is cultivated in an area of about 10.95 million hectares with a production of about 148 million tonnes (Kumar *et al*., 2023) [12]. The major species of *Citrus* include *Citrus aurantifolia* (Lime), *C. limon* (Lemon), *C. maxima* (Pummelo), *C. medica* (Citron), *C. paradise* (Grapefruit), *C. sinensis* (Sweet orange) and *C. reticulata* (Mandarin). Mandarin (*Citrus reticulata*) is the most dominant citrus species in terms of both area and yield because of its exceptional adaptability to various climatic conditions as compared to other *Citrus* species (Koehler *et al*., 2003) [11]. Citrus fruits are crucial in the human diet due to their rich content of vital micronutrients, including vitamins C and E, carotenoids, and flavonoids, which are required for maintaining human health. Citrus species are known for their medicinal and pharmacological properties, such as antibacterial, antioxidant, anticancer, anti-inflammatory, and hypoglycemic effects (Barros *et al*., 2012) [5]. The economic and therapeutic importance of citrus fruits and their by-products is significant due to their numerous applications in the food sector, cosmetics, and traditional medicine (Silalahi, 2002; Saidani *et al*., 2004) [19, 18]. Additionally, it has use in the production of fragrances, cosmetics, bath products, food and drink flavoring, incense, home cleaning goods, and medical purposes (Abdurahman *et al*., 2016) [1]. As a result, upgrading the yield potential of these crops has become important.

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So, changes at genetic level by selection and breeding may help us to develop a relatively enhanced yield performance So for breeding determining the variation among crop species is important. As a result, an understanding of genetic diversity and genetic relationships between different genotypes is crucial for the categorization, usage, and breeding of germplasm resources (Marboh *et al*., 2015) [15]. Currently, qualitative and quantitative characterization of germplasm still remains significant and is used as a first measure for identifying cultivars and assessing variability (Rodriguez-Garay *et al*., 2009) [17]. Hence, this research aims to evaluate the level of diversity and relatedness among citrus genotypes with respect to physico-chemical composition of fruits, which might provide valuable insights for enhancing citrus breeding programs.

Materials and Methods

The experiment was carried out at in main experimental station of horticulture farm situated in Acharya Narendra Deva University of Agriculture and Technology (ANDUA&T), Kumarganj, Ayodhya, India during the year 2023-24. The experiment was laid out in Completely Randomized Design consisting of seven *Citrus* spp. with three replications per treatment. The species of citrus used in the present study has been shown in table 1.

Table 1: Different species of citrus used in the study

S. N.	Treatments	Species
1.	T_1	Kinnow
2.	T_2	Rangpur lime
3.	T_3	Pant lemon
4.	T_4	Kagzi lime
5.	T_5	Nagpur Mandarin
6.	T_6	Sweet orange var. mosambi
7.	T_7	Grapefruit var. NRCC Grapefruit-6

Eight quantitative physico-chemical traits were recorded for each replication. Physical Parameters of the fruit such as weight of the fruits (in grams), Length and width of the fruits (in cm), specific gravity of the fruits, number of seeds, juice content (%) and Juice content per fruit (in ml) were recorded by using the standard methods as prescribed for the citrus descriptors by IPGRI (1999) [10]. T.S.S was measured by using a digital refractometer. The titratable acidity (TA) was measured by titrating the juice with normal NaOH until the phenolphthalein endpoint was reached. The TA was quantified as a percentage of citric acid content, using the formula established by the American Organisation of Analytical Chemists (AOAC, 2002) [3].

$$\text{Titrable Acidity } \% = \frac{\text{Titre reading} \times \text{Normality of Alkali} \times \text{Equivalent wt. of Acid}}{\text{volume of sample taken } \times 100} \times 100$$

Ascorbic acid content was measured according to the standard procedure prescribed by AOAC (2002) [3]. Ten grammes of homogenised pulp from the treatments were extracted and put to a 100 ml volumetric flask. The volume was composed of a 3% solution of metaphosphoric acid. After duration of 30 minutes, the solution underwent filtration using Whitman filter paper No.42. The dye (2, 6 dichlorophenol indophenol) was calibrated by performing a titration with standard ascorbic acid, and the dye factor was determined. A 10 ml sample of the supernatant was placed in a 100 ml conical flask and then titrated with a standard dye solution using a burette. The titration was extended until the light pink hue remained for duration of at least 15 seconds.

The ascorbic acid content was determined using the following formula:

$$\text{Ascorbic Acid (mg/100 gpulp)} = \frac{\text{Titrate} \times \text{dye factor} \times \text{volume made up}}{\text{V} \times \text{W}}$$

Where,

V = Aliquot of filtrate taken for estimation

W = Weight or volume of sample taken for estimation.

Reducing sugars: Reducing sugars were measured according to the standard procedure prescribed by AOAC (2002) [3]. The following formula was used to compute the results, which were then expressed in grammes of sugar per 100 grammes.

$$\text{Sugar (gm/100 gm)} = \frac{\text{Dilution factor} \times (\text{T.V.}_{\text{Blank}} - \text{T.V.}_{\text{Sample}}) \times 0.338 \times 100}{\text{Volume of aliquot} \times 1000}$$

Where T.V. = Titre volume

Total Sugars: 4 ml of strong hydrochloric acid was added to 25 ml of sample extract, and the mixture was then heated to 68 °C in a water bath for 15 minutes. A small amount of anhydrous sodium carbonate was added to neutralize the acidity until the effervescence subsided. Following that, the volume was adjusted to 50 ml using distilled water, and the total sugar content was calculated as mentioned in the section on reducing sugars.

Non-reducing sugars: Total sugar –Reducing Sugars

Statistical Analysis

The quantitative data underwent statistical analysis, which involved calculating the mean, critical difference, and coefficient of variation. The outcome of each parameter was determined as either statistically significant or non-significant.

Results and Discussion

1. Weight of the fruit (g)

Significant differences were observed for fruit weight for various species. Highest fruit weight was observed for treatment T_7 containing Grapefruit (407.52 g), while lowest fruit weight was observed for treatment T_4 containing kagzi lime (60.21 g) (Table 2). This shows about the variation that has been observed among the species of Citrus with respect to fruit weight. Similar findings have been observed by Marboh *et al*. (2015) [15] where they experimented on fifty citrus (*Citrus* spp.) genotypes which contained various varieties and hybrids. Significant variation was observed for fruit weight among the species. Similar findings were observed by Usman *et al*. (2020) [20] in germplasms of pigmented grape fruit.

2. Length of fruits (cm)

Significant variations were also observed for length of fruits of Citrus species. Highest length of fruits were observed for treatment T_7 containing Grapefruit (9.12 cm), while lowest fruit length was observed for treatment T_4 containing kagzi lime (4.53 cm) (Table 2). The treatments T_2 (Rangpur lime) and T_3 (Pant Lemon) were statistically at par with each other. Similar results were reported by Kumar *et al*. (2010) [13] where they studied different citrus species under hilly conditions of Arunachal Pradesh and found that fruits with maximum length were recorded for Washington Malta Sweet orange. Similar results were reported by Marboh *et al*. (2015) [15] and Mir *et al*. (2021) [16].

3. Width of the fruits (cm)

Significant variations were also observed for width of fruits of Citrus species. Highest fruit width was observed for treatment T_7 containing Grapefruit (8.90 cm) (Table 2), while lowest fruit width was observed for treatment T_4 containing kagzi lime (3.98 cm). The treatments T_1 (Kinnow) and T_3 (Pant Lemon) were statistically at par with each other. Similar results were reported by Kumar *et al.* (2010) [13] where maximum width was reported for Washington Malta Sweet orange. Similar results were reported by Marboh *et al*. (2015) [15] and Mir *et al*. (2021) [16].

4. Specific Gravity (g/cc)

From Table 2, it can be observed that highest specific gravity was observed for treatment T_4 containing kagzi lime (1.01 g/cc), while lowest specific gravity was observed for treatment T_7 containing Grapefruit (0.87 g/cc). Treatment T_2 was statistically at par with treatment T_1 (Kinnow) and T_3 (Pant Lemon) and Treatment T_5 (Nagpur mandarin), T_6 (Sweet Orange) and T_7 (Grapefruit) were statistically at par with each other. Hazarika *et al*. (2013) [9] also reported similar findings where they reported that Maximum specific gravity was recorded in MZU-H-6 (1.27 g/cc) and the minimum was recorded in MZU-H-15 (1.04 g/cc). Similar results were reported by Dorji and Yapwattanaphun (2011) [6].

5. Number of seeds

From Table 2, it can be observed that highest number of seeds (21.54) was observed for treatment T_1 containing Kinnow, while lowest was observed for treatment T_2 (Rangpur lime). Significant variation for number of seeds was reported among the genotypes. Hazarika *et al*. (2013) [9] also reported similar findings where they reported that Maximum number of seeds was observed for MZU-H-3 (23.67) while lowest was reported for MZU-H-1. Similar results have also been reported by Angami *et al*. (2022) [2].

6. T.S.S (Total Soluble Solids)

From Table 2, it was observed that TSS was highest (10.61 °brix) in treatment T_1 (Kinnow) while lowest TSS (7.15 °brix) was observed for treatment T_3 (Pant lemon). The treatment T_4 containing kagzi lime was statistically at par with treatment T_6 (Sweet Orange). Similarly, Akhter *et al.* (2019) [22] assessed the physico-chemical properties of 20 specific citrus fruit germplasm. Germplasm No. 20 had a greater concentration of total soluble solids at 12.23 °brix. Similar findings were also reported by Marboh *et al*. (2015) [15] and Bankar *et al*. (2021) [4].

7. Titrable Acidity (%)

From Table 3, it was observed that Titrable Acidity was highest (9.21%) for treatment T_1 (Kinnow), while it was lowest (0.62%) for treatment T_6 (Sweet Orange). The treatment T_2 (Rangpur lime), was statistically at par with treatment T_3 (Pant lemon) and T_4 (kagzi lime). The mandarins exhibit significant geographical variation due to their high levels of heterozygosity and cross fertility. In a study conducted by Kundan *et al*. (2010) [14], the researchers assessed the acidity levels of Sikkim mandarin fruits. They found that mandarins from the western regions of Sikkim had the lowest acidity. Gaikwad *et al*. (2015) [8] also reported maximum titrable acidity in pummelo cultivar CG-29. Similar findings were also reported Angami *et al*. (2022) [2].

8. Ascorbic Acid Content (mg/100 g)

From Table 3, it was observed that Ascorbic acid content was highest (46.04) in treatment T_7 containing Grapefruit and lowest (28.81) in treatment T_1 containing Kinnow. The treatments T_2 (Rangpur lime) and T_3 (Pant lemon) were statistically at par with each other. However, stark variations were observed in ascorbic acid content among the species. Marboh *et al*. (2015) [15] experimented on fifty citrus (*Citrus spp*) genotypes which contained various varieties and hybrids. Significant variations were observed for Ascorbic acid content. The species karna khata and sweet orange showed maximum ascorbic acid content. Similar results were reported by Kundan *et al*. (2010) [14] and Gaikwad *et al*. (2015) [8] in citrus.

9. Non-reducing, reducing and Total sugars

From table 3, it was observed that non-reducing (5.76%) and reducing sugars (3.51%) were highest for treatment T_6 **(**Sweet Orange) and lowest (0.63 %and 0.75%) for treatment T_4 (kagzi lime). Whereas, for Total sugars it was observed that that T_4 was lowest (1.73%) while T_5 (8.44%) showed highest total sugars. In a study conducted by Kundan *et al.* (2010) [14], the researchers assessed the reducing sugars, non-reducing sugars and total sugars of the fruits. They reported that mandarin fruits of the northern district of Sikkim showed maximum reducing sugars (6.20%), non-reducing sugars (3.29%) and Total sugars (9.49%). Similar findings were also reported by Gaikwad *et al*. (2015) [8] and Angami *et al*. (2022) [2] in citrus.

10. Total juice per fruit and Juice content

From table 3, it was observed that juice content % was highest (48.88%) in T_1 (Kinnow) and lowest (37.91%) in treatment T_3 (Pant lemon). Total juice per fruit was found to be lowest (28.17 ml) in treatment T_4 (kagzi lime) and highest (185.85ml) in T_7 (Grapefruit). Zandkarimi *et al.* (2011) [21] also studied on 19 genotypes of lemon and limes and reported that juice content was significantly affected by the genotypes. Marboh *et al.* (2015) [15] reported that juice content was higher in sweet oranges Valencia and Mosambi and grapefruits. Similar results have also been reported by Kundan *et al*. (2010) [14] and Gaikwad *et al*. (2015) [8] in citrus.

Table 2: Physico-chemical characteristics of different *Citrus* spp.

Sl. No.	Treatments	Weight (g)	Length	Width	Specific Gravity	Number of seed	T.S.S.
1	T_1	141.00	5.04	5.31	0.95	21.54	10.61
2	T_2	158.00	5.63	5.58	0.98	3.95	7.61
3	T_3	129.10	5.48	5.23	0.92	15.08	7.15
4	T_4	60.21	4.53	3.98	1.01	8.57	8.82
5	T_5	127.33	5.95	6.63	0.95	5.67	10.02
6	T_6	174.89	7.02	7.24	0.91	16.00	8.77
7	T_7	407.52	9.12	8.90	0.87	5.33	8.04
Overall Mean		171.15	6.11	6.12	0.94	10.88	8.72
C.V. %		3.14	2.12	3.11	2.81	3.52	2.75
C.D. (5%) Ai-Aj		9.41	0.23	0.33	0.05	0.67	0.42
F (Prob)		0.00	0.00	0.00	0.00	0.00	0.00

Table 3: Quality attributes of different *Citrus* spp.

Sl. No.	Treatments	Titratable Acidity	Ascorbic Acid	Non-reducing sugar	Reducing Sugar	Total Sugar	Juice content	Total juice/fruit
1	T_1	9.21	28.81	5.63	3.18	7.33	48.88	85.50
2	T_2	1.19	37.83	2.04	3.19	3.74	40.54	108.14
3	T_3	1.11	38.50	0.68	1.42	2.12	37.91	44.16
4	T_4	1.29	42.45	0.63	0.75	1.73	46.95	28.17
5	T_5	0.99	34.05	4.52	3.46	8.44	40.79	77.02
6	T_6	0.62	42.05	5.76	3.51	6.84	44.30	87.60
7	T_7	4.04	4604	2.26	2.42	5.07	46.40	185.85
Overall Mean		2.63	38.53	3.07	2.56	5.04	43.68	88.06
C.V. %		3.31	1.66	3.23	2.66	3.33	3.19	8.47
C.D. (5%) Ai-Aj		0.15	1.12	0.17	0.12	0.29	2.44	13.07
F (Prob)		0.00	0.00	0.00	0.00	0.00	0.00	0.00

Conclusion

From the experiment it can be concluded that there existed a significant amount of variability among the *Citrus* spp. studied. Investigations concerning crop improvement of citrus are becoming more frequent as global consumption and utilisation of citrus, particularly oranges, continues to increase. Key characteristics for the development of new kinds are superior fruit quality in terms of flavour, size, and texture. So improving these traits through proper breeding programs is necessary. Characterizing germplasm is crucial for obtaining information on the attributes of accessions, ensuring optimal utilisation of the germplasm collection by end-users. It facilitates the easy organization of accessions, creation of essential collections, identification of missing items, and retrieval of significant germplasm for breeding programmes. This leads to a more comprehensive understanding of the collection's composition and genetic diversity of *Citrus* Spp. So focusing on the characterization of these germplasms of citrus can help in improving and planning the citrus breeding programs.

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