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Screening of different genotypes of Cape gooseberry (*Physalis peruviana* L.) for their biochemical attributes collected from different parts of India

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Abstract
Cape gooseberry (*Physalis peruviana* L.) is a minor fruit crop belongs to the family Solanaceae. Twelve genotypes of cape gooseberry namely as CITH Sel-I, CITH Sel-III, CITH Sel-V, CITH Sel-VII, CITH Sel-IX, CITH Sel-XI, CITH Sel-XV, CITH Sel-XVI, SS/VK/301, SS/VK/401, SS/VK/501 and SS/VK/601 evaluated for their biochemical attributes such as total soluble sugar (TSS), total acidity, total sugar, reducing sugar, carotenoids, phenolics, flavonoids, ascorbic acid, and antioxidant capacity for harshening maximum yield with high nutritive value. Result revealed that TSS (7.58-16.60°Brix), total acidity (0.40-1.63%), total sugar (2.05-10.86%), reducing sugar (1.04-5.98%) total phenolics (6.33-20.17 mg/100g), flavonoids (2.44-16.60 mg/100g), carotenoids (0.60-5.12 mg/100g), ascorbic acid (18.33-42.90 mg/100g) and antioxidant capacity (21.82-129.3082 µMol trolex eq./100g) varied for different genotypes significantly.

Keywords: Cape gooseberry, genotypes, bioactive, antioxidant

Introduction
Cape gooseberry is botanically known as *Physalis peruviana* L. comes under Solanaceae family native to South America but cultivated in South Africa in the region of Cape of Good Hope during the 19th century. It is commonly called as "Poha" in Hawaii, Golden Berry in South Africa, and Rasbhari, Makoi or Tepari in India (Gupta and Roy, 1980) [7]**.** The first description of *Physalis* genus was made by Linnaeus in 1753. The genus *Physalis* having more than 120 species but only few are of economic value (Licodiedoff *et al*., 2013) [13]. One is the strawberry tomato, husk tomato or ground cherry, *P. pruinosa* L., grown for its small yellow fruits used for sauce, pies and preserves in mild-temperate climates. It is suggested that, Cape gooseberry is tetraploid in nature and having chromosome number 2n = 48 (Menzel 1951) [24]. The fruit type is berry in shape like a small globe with the diameter around 12.5 to 25.0 millimeters and a weight ranges from 4 to 10 g, containing around 150 to 300 seeds (Licodiedoff *et al*., 2013) [13]. During ripening the fruit colour turns from green to orange due to chlorophyll breakdown and carotenoids accumulation and progressive softening occurs (Trinchero *et al.*, 1999; Gutierrez *et al.*, 2008) [36, 8].When the fruit is ripened, calyx shows a brown colour which is for determining the point of harvest (Avila *et al.*, 2006) [2]. Physalis is a climacteric fruit which shows a clear rise in ethylene production during ripening (Trinchero *et al.*, 1999; Majumder and Mazumdar, 2002; Gutierrez *et al*., 2008) [36, 21, 8]. *Physalis* seeds germinate more easily when the temperature lies between 7 to 13°C at night and 22 to 28°C during the day. It can grow around 1.0 to 1.5 meters height. However, with training it can exceed up to 2.0 meters height (Fischer, 2000) [4].

Cape gooseberry is famous for its flavor and having good blend of acid–sugar. The fruits are very attractive in colour at maturity time. *Physalis* fruits show high level of ascorbic acid 36mg $100g^{-1}$ pulp, rich in Vitamin A 1730 IU $100g^{-1}$ of pulp, iron 38mg $100g^{-1}$ of pulp and phosphorus 1.2 mg $100g^{-1}$ of pulp (Fisher, 2000; Ramadan & Morsel, 2007) [4, 26]. The ripe fruits are eaten fresh or can be used for preparation of excellent quality of jam for which it is also called the 'Jam Fruit of India'(Majumdar, 1979) [19]. A number of species in the genus are of horticultural and economic importance due to their high nutritional value in vitamin A, C and B complex (Yen *et al.*, 2010) [41], minerals and phosphorus (Rodrigues *et al.*, 2009; Martinez *et al*., 2008) [30, 22] antioxidants (Wu *et al.*, 2005) [39] as well as potential medicinal properties including anti-bacterial, anti-inflammatory, and anti-cancer properties (Yen *et al.*,

2010; Martinez *et al.*, 2010; Franco *et al.*, 2007) [41, 23, 5].
The analysis of different genotypes for a particular location is one of the most important methods of improvement of any horticultural crops. There are limiting scopes for bringing new area under cultivation and as such as; more emphasis will be given on increasing the yield per unit area. Hence in Indian condition, where population pressure is more and land is inadequate.
As fruits are consumed mostly as fresh and there fruit quality should be more desirable and nutritional quality is essential for *physalis* fruit.

Materials and Methods
The field experiment was conducted at the experimental field of Horticulture Garden, Bihar Agricultural College, Sabour, Bhagalpur ($87^0$2'42" E, $25^0$15'40" N) at an altitude of 46 m above mean sea level in the heart of vast Indo-Gangatic plains of north India. The climate of this place is sub-tropical of slightly semi-arid in nature and characterized by dry summer, moderate rainfall and cold winter. January and February are usually the coldest months when the mean temperature normally falls as low as 10.4°C whereas April & May are generally the hottest months having the maximum average temperature of 37°C (Supplementary Table1). The data for growth pattern and yields under various treatments are presented in supplementary table 2.

Plant materials
The plant material consists of twelve genotypes of Cape gooseberry **(***Physalis peruviana* L.**)** *viz.*, CITH Sel-1, CITH Sel-3, CITH Sel-5, CITH Sel-7, CITH Sel-9, CITH Sel-11, CITH Sel-15, CITH Sel-16, SS/VK/301, SS/VK/401, SS/VK/501 and SS/VK/601. The genotypes are collected from Srinagar and other local genotypes were collected from the various district of Bihar. Seeds are sown in the protray that consist cocopeat: vermicompost: sand with ratio 2:1:1/2 in the month of August. These seedlings were ready for transplanting after 3-4 weeks and transplanting was done after one month. All the selected plants were almost uniform, healthy and free from pest and diseases. Five plants were selected per treatment in triplicate grown according to Randomized Block Design in twelve treatments.

Reagents
The 2, 6-dichlorophenol indophenols, Folin–Ciocalteu Reagent (FCR), Gallic acid, Meta phosphoric acid, 6-hydroxy-2, 5, 7, 8-tetramethylchromane- 2-carboxylic acid (trolox) were purchased from Hi- media Labs. Pvt. ltd., India. All other chemicals and solvents were of analytical grade.

Total soluble solids (TSS), Titratable acidity and total sugar
The total soluble solids (T.S.S) in ten freshly extracted juice samples of Cape gooseberry was estimated with the help of digital hand refractometer and was expressed as Degree Brix at 20 °C. Titratable acidity was determined by using titration method (Rangana, 2010) [28]. Total sugars were determined by Lane and Eynone (1923) [15].

Total carotenoids content
Total carotenoids content of Cape gooseberry fruit was determined by the method of Roy (1973) [33] with some modifications. In which 5 g of Cape gooseberry pulp was crushed in acetone till the tissue became colourless. Then the extracted solution was poured into a separating funnel. Petroleum ether and small amount of sodium sulphate solution was added and shaken rigorously. Then the separating funnel was kept undisturbed to separate the carotenoids from acetone to petroleum ether layer. After that, coloured solution was separated in a 50 ml volumetric flask and the volume was adjusted with petroleum ether. Finally, the sample absorbance was measured at 452 nm in a (HALO DB- 20S UV-VIS double beam) spectrophotometer, using petroleum ether as blank. The results were expressed as mg 100 g^{-1} FW (Fresh Weight) basis.

Ascorbic acid content
Ascorbic acid was quantitatively determined by Jones and Huges (1983) [10]. For estimation of ascorbic acid fresh harvested fruits was used. Ascorbic acid was quantitatively determined by 2, 6-dichlorophenol indophenols dye method as described by Jones and Hughes (1983) [10] with slight modifications. For each sample, 10 g pulp was homogenized with 10 ml of 3% Meta phosphoric acid. The extract will be made up to a volume of 100 ml and centrifuged at 3000 rpm for 15 min. at room temperature. Ten milliliters of supernatant will be titrated against standard 2, 6-dichlorophenol indophenols dye, which had already been standardized against standard ascorbic acid. Results will be expressed as mg 100 g^{-1} FW basis.

Folin-Ciocalteu reducing capacity and total Phenolics
The total phenolics content of the Cape gooseberry fruit was determined by the Singleton and Rossi method (1965) [35] with some modifications. For this 5 g of Cape gooseberry fruit sample was crushed in 10 ml of 80% ethanol. The homogenate was then centrifuged at 10000 rpm for 20 min at 4°C and supernatant was used for assay of total phenols. Then into 2.89 ml of distilled water, 100 µl sample and 0.5 ml of 2 N Folin- Ciocalteau reagents was added. After 3 min, 2 ml 20% of Na_2CO_3 was added into it. The prepared solution was then kept for some time till it becomes blue-black. Then absorbance was measured at 750 nm using 1 cm cuvette in a Perkin Elmer UV-VIS lambda 25 spectrophotometer. Gallic acid (0-800 mg L^{-1}) was used to produce standard calibration curve. The total phenolics content was expressed in microgram of Gallic acid equivalent per gram of fresh weight (µg Gallic acid equiv. g^{-1}FW).

Total flavonoids content
Total Flavonoids content was determined using aluminum chloride method (Zhishen *et al.*, 1999) [43]. For this an aliquot (0.1 ml) of extract was taken in 10 mL of volumetric flask containing 4 ml of distilled water, 0.3 ml portions of 5% NaNO2, and 0.3 ml portions of 10% AlCl3_6H2O. The mixture was allowed to stand for 6 min at room temperature. Two milliliters of 1 M NaOH was added and the solution was diluted to 10 ml with distilled water. The mixture was mixed well by vortexing. The absorbance was measured immediately at 510 nm using a UV–VIS spectrophotometer. Total flavonoids were expressed as mg of rutin equivalents per 100 g on fresh weight.

Antioxidants capacity (CUPRAC Method)
Antioxidant capacity in the cape gooseberry was determined by following CUPRAC method, which was standardized by (Apak *et al*., 2004) [1]. 1 mL each of copper (II) chloride solution (10-2 M), neocuproine solution of 7.5 × 10-3 M, and

ammonium acetate buffer (pH 7.0) solutions were added in test tube. Antioxidant sample (or standard) solution (x mL) and H_2O (1.1- x mL) was added to the initial mixture so as to make the final volume of 4.1 ml. The tubes were capped and after one hour, the absorbance at 450 nm was recorded against a reagent blank. The standard calibration curve of each antioxidant compound was constructed in this manner as absorbance versus concentration. The molar absorptivity of the CUPRAC method for each antioxidant was recorded from the slope of the calibration line concerned and the antioxidant activity was expressed as µmoltrolox equivalent 100 g-1 fresh weight (µmol TE 100 g-1 FW).

Statistical analysis and interpretation of data

The experimental data recorded from different plots and were subjected to statistical analysis using DSAASTAT software and significant effects ($p< 0.05$) were noted. Further, Duncan's Multiple Range Test (DMRT) was done for pair-wise comparison of genotypes and the effects which are significantly different were represented by different alphabets. The genotypes which get same letter grouping are at par and the genotypes pairs getting different letter grouping are significantly different

Result and Discussion

Total soluble solids (TSS), Titratable acidity and Total sugar

It was revealed from the data pertaining to quality of fruit like TSS was maximum with SS/VK/401, 16.60 °Brix and minimum with CITH Sel-IX, 7.58 °Brix. With regard to chemical composition of fruits, TSS, acidity and total sugar, TSS was not affected either by room temperature or low temperature storage (Javanmardi & Kubota, 2006) [9]. Several scientists suggested that TSS varied from 13 to 15°Brix respectively (Kour & Bakshi 2006; Resterpo, 2008; Labarca *et al*., 2013; Lopez *et al*., 2013) [12, 29, 13]. However, with regard to acidity, the acidity factor has been identified as an important variable in the process of ripening and flavor of the fruit (Rodrigues *et al*., 2006) [31]. The highest percentage of total titratable acidity was found in the cultivated genotypes 1.63% in SS/VK/301 and minimum acidity was found in CITH Sel-V, 0.40%. Several Scientists reported that the acidity varies from 0.90 to 2.10 ± 0.26% (Mazumdar & Bose, 1979; Ramadan & Moersel, 2007; Botero, 2008; Resterpo *et al*., 2008) [20, 26, 29] the total sugar was found to be maximum with SS/VK/301, 10.86% and minimum with CITH Sel-XI, 2.05% which are presented in Table 1 and Fig.1.The total sugar in Cape gooseberry varies from 9 to 10% (Panayotov & Popova, 2014) [25].

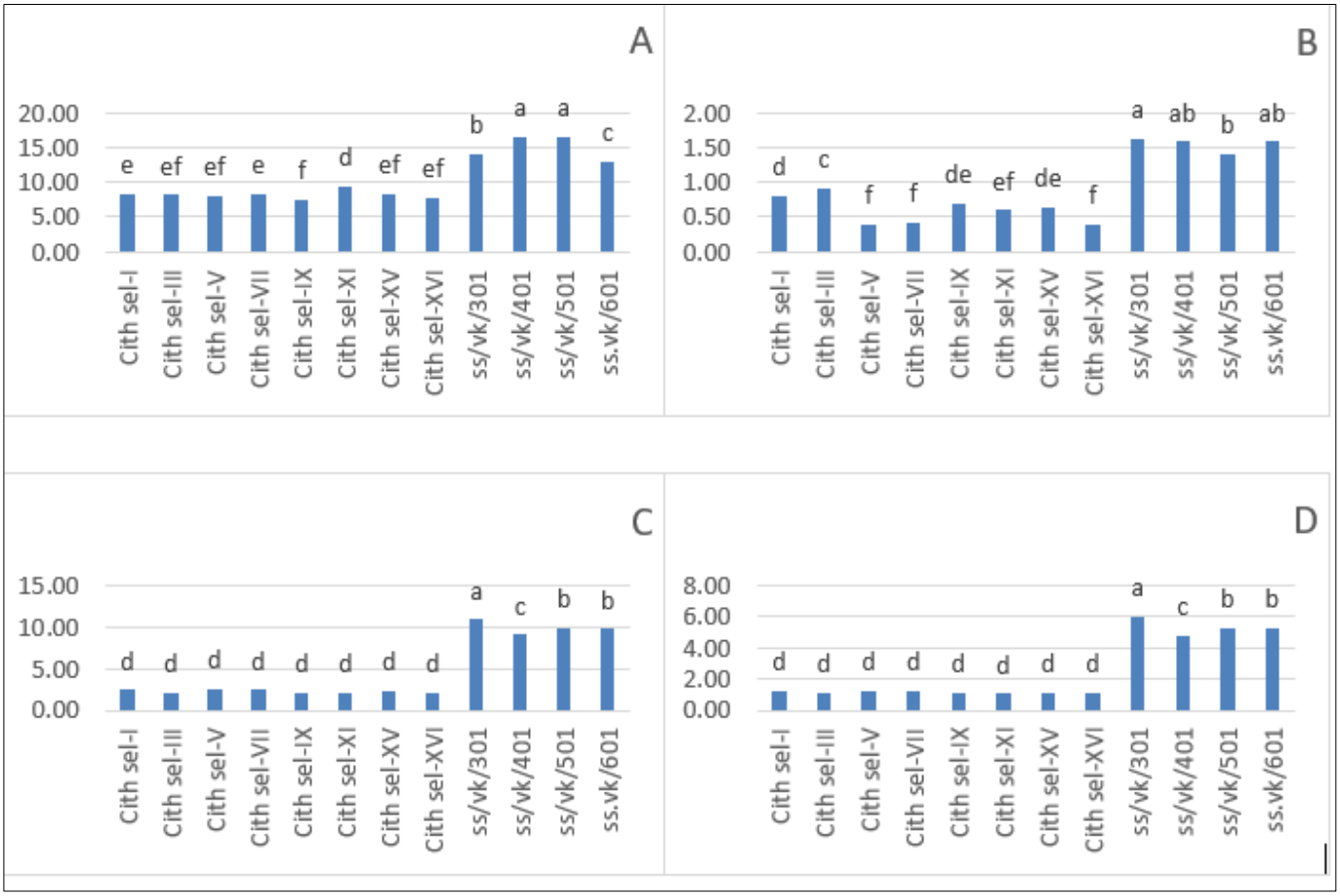

Fig A, B, C, D 1: In which A graph shows the TSS, B graph shows the Acidity, C graph shows the total sugar and D graph shows the reducing sugar of Cape gooseberry

Ascorbic acid

The ascorbic acid content was recorded highest in SS/VK/501, 42.90 mg/100g followed by SS/VK/601, 42.69 mg/100g and minimum in CITH Sel-XVI, 18.33 mg/100g. Literature revealed that 24.31 mg/100g ascorbic acid found in CITH Sel-VI (Singh *et al*., 2011) [34] and 43-54.58 mg/100g ascorbic acid in fruit of Cape gooseberry (Khan and Gowder, 1955; Ramadan *et al*., 2011; Ramadan & Moersel 2007; Zhang *et al*., 2013; Botero *et al*., 2008) [11, 27, 26, 42, 3]. Vitamin C is the most abundant antioxidant which reduces the aging problem in human.

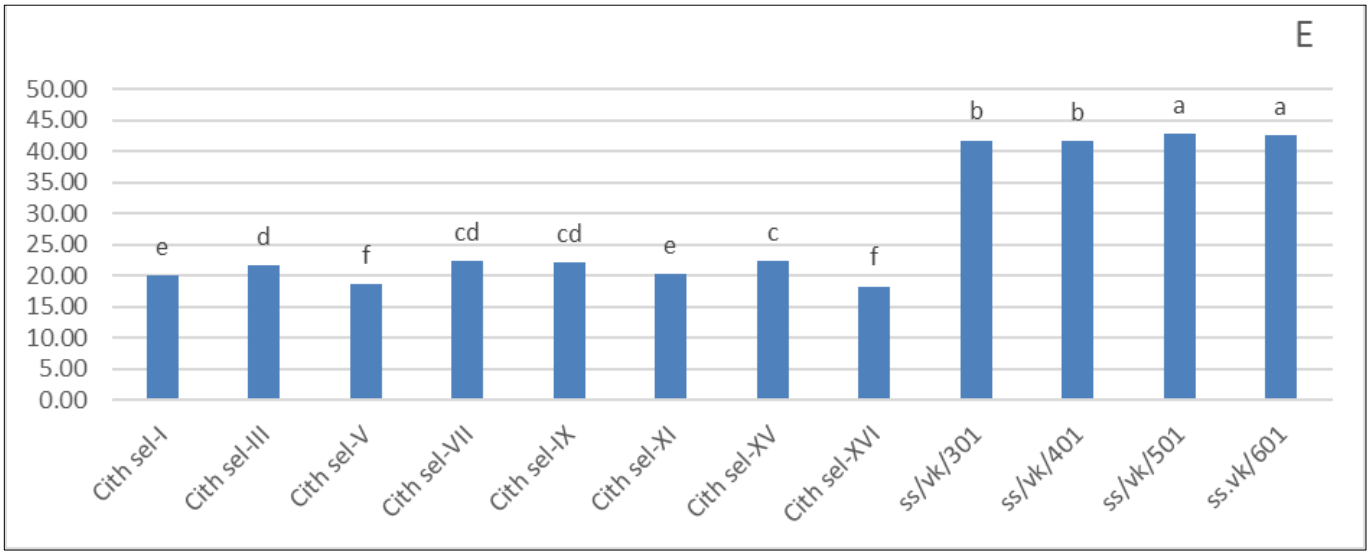

Fig 2: Shows the graph Ascorbic acid percentage in Cape gooseberry

Total carotenoids content

The colour of juices is the first quality factor appreciated by consumers and has a remarkable influence on its acceptance.Carotenoids content revealed that maximum carotenoids found in SS/VK/501, 5.12 mg/100g followed by SS/VK/601, 4.98 mg/100g.While Lopez *et al.* (2013) [17] identified β-carotene 722.30-783.16 mg/ 100 g sample. CITH Sel-XI, 0.60 mg/100g shows that lower amount of carotenoids content in the fruits. Further, higher amount of carotenoids in fruit enhance the fruit quality statuswhich are presented in Table 1 and Fig.2.

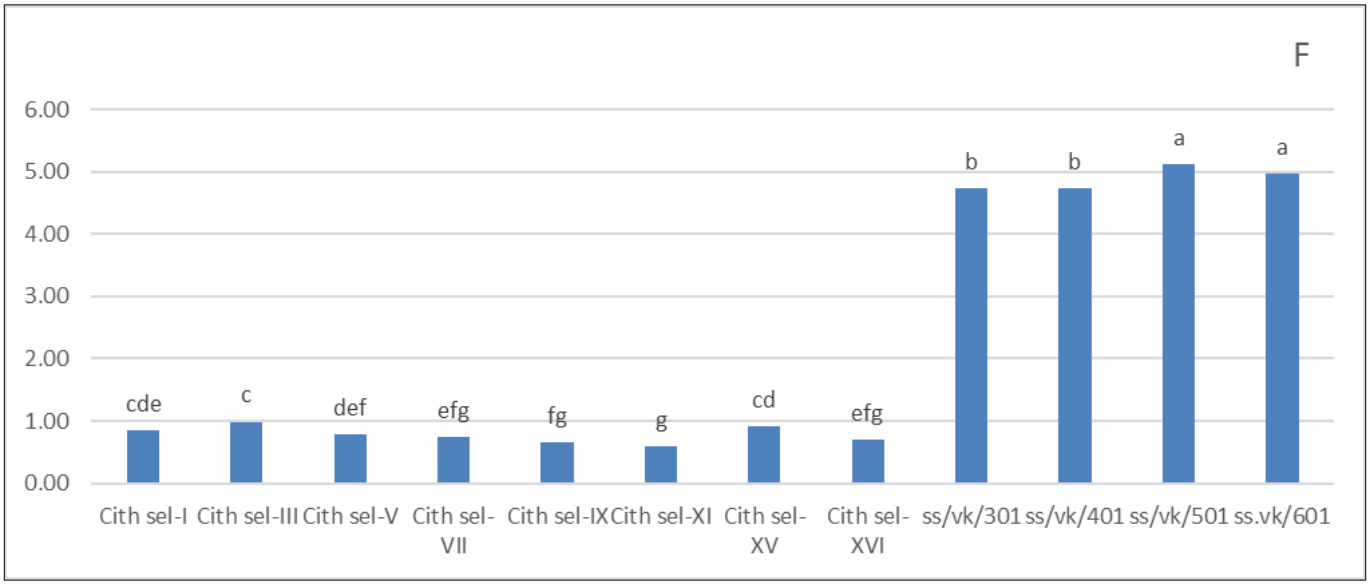

Fig 3: Shows the graph Total carotenoids content in Cape gooseberry

Total phenolics

The highest phenolic content 20.17 mg/100g was in CITH Sel-I& CITH Sel-XVand lowest phenolic content 6.33 mg/100g was observed in SS/VK/501. Phenolics content of local genotypes varies from 6 to 8 mg/100g but other genotypes which are collected from CITH, Srinagar varies from 16 to 20 mg/100g (Table 1 and Fig. 2). Phenolics compound found in plants play important role in human due to their antioxidant properties. Moreover, the loss of total phenols could be due to the oxidation, degradation of phenolic compounds and the polymerization with proteins (Wang *et al*., 2012) [37]. Phenolic compounds are secondary metabolites and these antioxidant compounds donate an electron to the free radical and convert it into aninnocuous molecule. The antioxidants present in fruits, such as phenolic acids are frequently associated with health benefits (Fu *et al*., 2011) [6]. From studies, it has been found out that fruits are not only the source of micro-nutrient sand fibers but it also contain several photo chemicals that alone or in combination provide health benefits. (Yahia, 2009) [40].

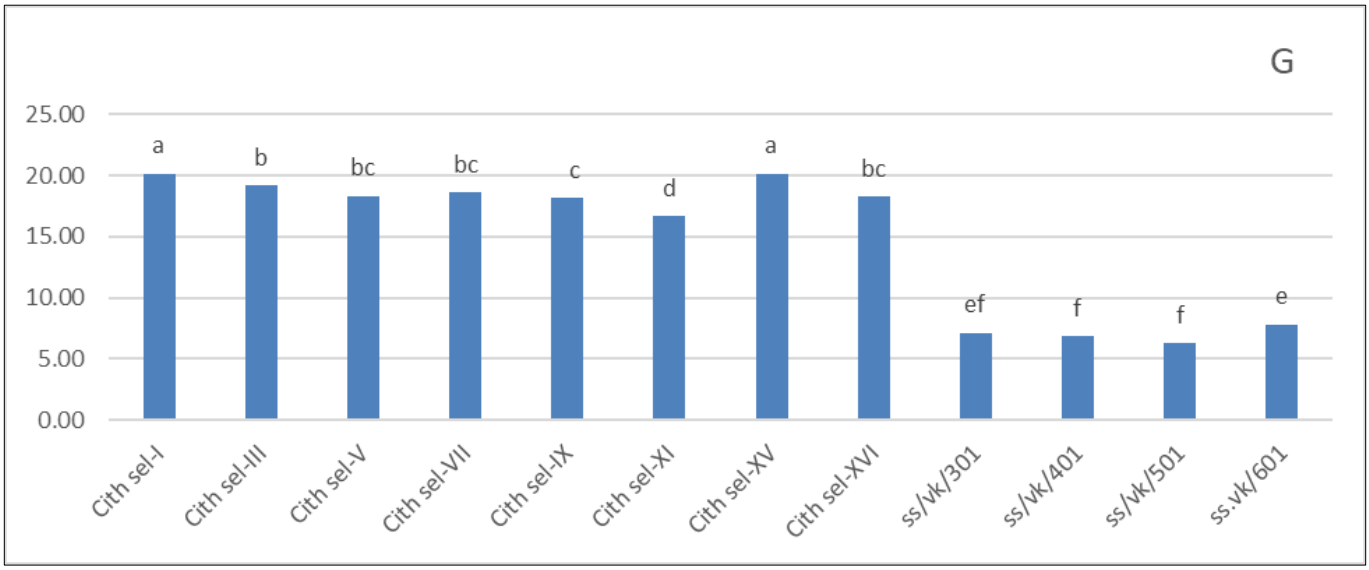

Fig 4: Shows the graph Total phenolic content in Cape gooseberry

Total flavonoids content

Flavonoids are another important polyphenols that naturally occurs in fruits. A total flavonoids range in fruits of subtropical to temperate region was varied from 2 to 17 mg/100g. The highest flavonoid was found in CITH Sel-XV, 16.60 mg/100g followed by CITH Sel-XVI, 16.02 mg/100g (Table 1). Flavonoids or bioflavonoids are natural antioxidants and these are widely distributed in fruits (Lampila, Lieshout, Gremmen & Lahteenmaki, 2009) [14]. These substances have apparent roles in plant stress defence, such as in the protection against damage caused by pathogens, wounding or excess UV light (Winkel-Shirley, 2002) [38]. The range of flavonoids varies from 100 to 145 mg quercitin equivalents 100g DW (Lopez *et al.*, 2013) [18].

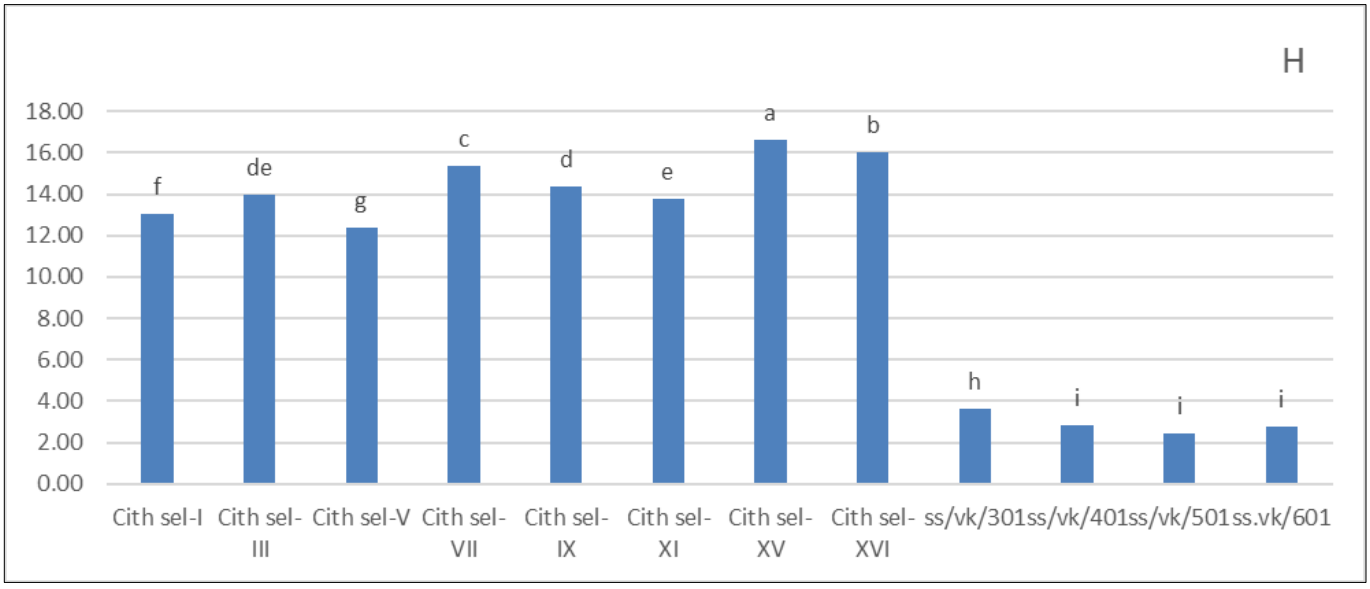

Fig 5: Shows the graph Total flavonoids content in Cape gooseberry

Total antioxidant activity

Antioxidant capacity was maximize found in CITH Sel-I, 129.30 µMol trolex eq./100g and the minimum was found in SS/VK/601, 21.82 µMol trolex eq./100g(Table 1). A high antioxidant capacity has been demonstrated for goldenberry juice and synergistic effect of different antioxidants has been suggested (Ramadan and Morsel, 2007) [26]. Rop *et al.* (2012) [32] recommended antioxidant that varied from 7 to 9 (grams of AAE kg^{-1} FM) by DPPH test.

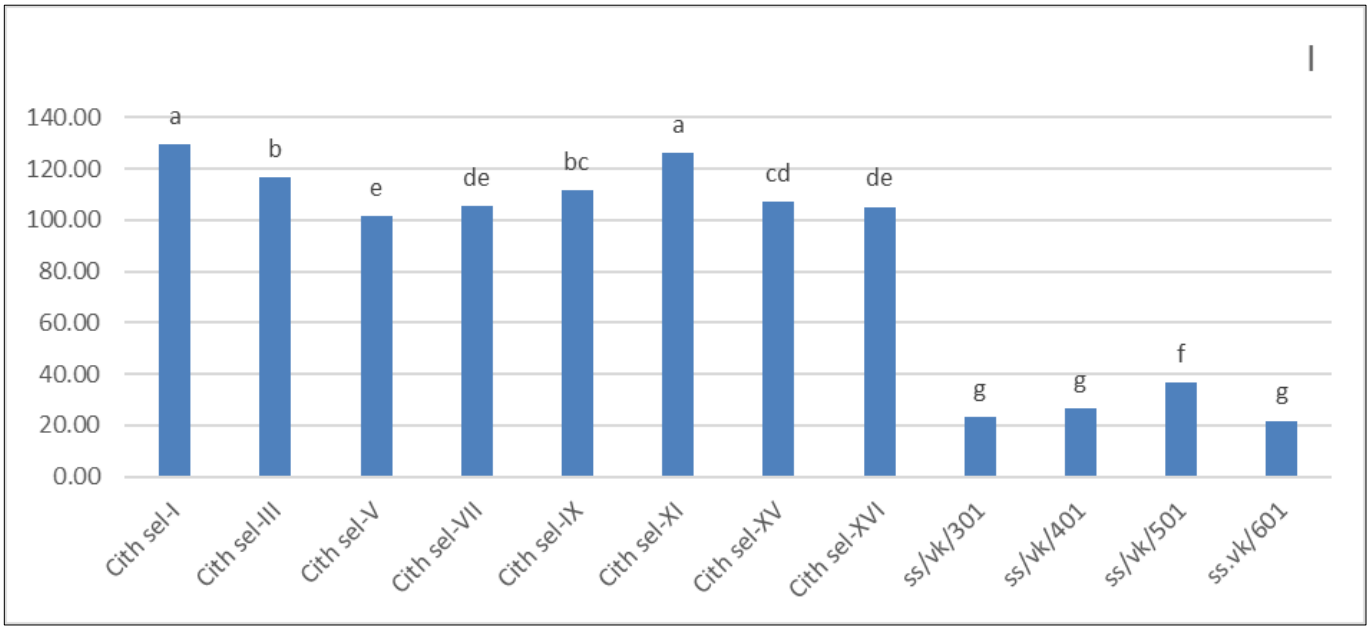

Fig 6: Shows the graph Total antioxidant activity in Cape gooseberry

Conclusion

In conclusion, all the parameter regarding biochemical attributes such as: TSS, total acidity, total sugar, reducing sugar, total phenolics, flavonoids, carotenoids, ascorbic acid and antioxidant capacity varied for different genotypes significantly. *physalis*have a bright future as a fresh fruit as well as functional food due to its high quality and quantity of its bioactivities compound. The phenolic content, ascorbic acid content and antioxidant properties of Cape gooseberry of different genotypes which were affected due to climatic condition. Consequently, The information about cape gooseberry which can be used in food industry as quality-freshness markers and developing new products from this fruit.

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