

Research Article

Studies on the Yield, Quality and Biochemical Characters of Five Less Seeded Strains of Kinnow Mandarin

Imran Muhammad Siddique¹, Naseem Sharif^{1*}, Abdul Ghaffar², Mudassar Naseer¹, Ammara Noreen³, Naseem Akhtar⁴ and Safer ud Din⁵

¹Horticultural Research Station, Sahiwal, Pakistan; ²Mango Research Institute, Multan, Pakistan; ³Horticultural Research Station, Bahawalpur, Pakistan; ⁴Soil and Water Testing Laboratory Sahiwal, Pakistan; ⁵Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan.

Abstract | In the entire scenario of Pakistan citrus sector, kinnow (*Citrus reticulata* Blanco) right from its introduction and successful acclimatization continues to dominate both in area and production besides an exportable commodity. Presence of large seed number in Kinnow is big issue for fresh consumption and as well as for processing industry in the international markets. Visualizing such compulsions of international markets, a systematic study was undertaken by Horticultural Research Station, Sahiwal with identification of five less seeded kinnow strains (1-6 seeds). Results depicted that less seeded kinnow strain, encoded LSKS-2, produced superior external and internal quality attributes. Average fruit weight and size outstripped other contestant strains. It produces average weight per fruit as 170.58gms with length/breadth, as 70.68mm and 68.08 mm respectively. Maximum juice recovery as 46.22% with glucose 48.08gms per liter, fructose 27.72gms per liter was obtained significantly high in less seeded strain LSKS-2. Total soluble solids as 11.70% remained in the same strain (LSKS-2). Vitamin C was 18.43 mg/100gms besides total phenolic compounds 94.78 µgms in LSKS-2. However, somewhat tenacious fruit with more peel thickness was observed in LSKS-4 which was 3.31 mm. Highest firmness remained 2.48kg in LSKS-1 of less seeded kinnow. Conclusively less seeded strain LSKS-2 proved best of all the five strains of less seeded kinnow. The study is worthwhile for exploiting natural mutant kinnow strains bearing less seeded fruit of high quality characters through vegetative method of propagation thereby avoiding inevitable heterozygosity and polyembryonic nature of citrus fruits.

Received | January 13, 2022; Accepted | October 15, 2022; Published | March 27, 2023

*Correspondence | Naseem Sharif, Horticultural Research Station, Sahiwal, Pakistan; Email: seemiaf@gmail.com

Citation | Siddique, I.M., N. Sharif, A. Ghaffar, M. Naseer, A. Noreen, N. Akhtar and S. Din. 2023. Studies on the yield, quality and biochemical characters of five less seeded strains of kinnow mandarin. *Pakistan Journal of Agricultural Research*, 36(1): 9-19.

DOI | <https://dx.doi.org/10.17582/journal.pjar/2023/36.1.9.19>

Keywords | Spontaneous mutation, Parthenocarpy, Biochemical traits, Less seeded, Evaluation, Phenolics, Fructose, Strains



Copyright: 2023 by the authors. Licensee ResearchersLinks Ltd, England, UK.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Introduction

Citrus being a genus of fruit plants belong to family Rutaceae (Eleza *et al.*, 2008) which have attained a great significance for varying attractive

colors, rich and strong fragrance and high nutritional value. Fredrick *et al.* (1990) reportedly traced the history of citrus fruit as early as 2200 BC with the origin in Indo-China. Evidently with the interplay of natural mutations and conventional breeding of

early periods various new citrus cultivars and varieties of commercial importance emerged in citrus sector. Classical and breeding work took strides by the time and pursuant to that an American breeder (Frost, 1935) went successful to develop kinnow employing parentage of King and Willow leaf. Kinnow in the province of Punjab introduced in early fifties transformed scattered groves in regular kinnow crop concentrated areas such as Bhalwal, Sargodha, Faisalabad, Toba Take Singh and many more areas in the province (Khan and Mahmood, 1992). Now in the entire citrus cultivation, 95 percent is contributed by Punjab followed by KPK, Sindh and some parts of Baluchistan (Anonymous, 2019).

China is the largest citrus producer with 42.776 million tons, followed by Brazil with 19.305 million tons and United States of America with 15.212 million tons. Pakistan ranks 12th in terms of citrus production in world, however, in Pakistan citrus is leading fruit crop in term of area and production with annual production of 2.351 million tons over an area of 183.149 thousand hectares. Punjab is leading province with 2.289 million tons production, among which 2113565 metric tonnes is only Kinnow production with 99.79% varietal share (GOP 2019-20).

On account of profuse bearing, blond taste, attractive external color and high juice contents (45-50%), this mandarin variety right from its introduction till now continues to rule the entire domain of Pakistan citrus sector with its substantial share well above 70%. This variety has entered in export from late sixties to seventies stretching to Middle East, Gulf States, Indonesia in south east and central Asian states along with some of the European countries of the world.

However, increasing convenience in direct consumption as a table variety, citrus based byproducts and processing industry, high number of seeds (18-25) have overshadowed its other excellent qualities and the country, for the last many decades is facing problem in export based supply chain with its restrictive impact to keep major volumes of kinnow in conventional markets only. There is thus immense need to develop seedless or less seeded kinnow strains to direct maximum volume of kinnow for its successful export to the high end markets to beef up citrus based foreign exchange earnings. In the process of varietal improvement, thanks to the naturally

occurring spontaneous mutations, detection of superior variations and their exploitation have given high merit citrus cultivars/varieties of commercial acceptability with the desirable unique characters. These mutations in fruit plants particularly in citrus have been identified as chance seedlings, limb sports and chimeras. With reference to citrus seedless fruits various triploids, as a result of natural fusion of haploid gametes and diploids have been reported by Huso *et al.* (2004) ensuring seedless fruits. Phenomenon of natural diversity to develop less seeded fruits (0-10) in kinnow has been discussed by Altaf and Iqbal (2003) in the background of scrupulous testing of kinnow fruit. Such mutations have also been reported by Sherma and Thind (2005) in Ferozpur area of India with the coincidence of good quality attributes.

In the natural genesis of mutations for less seeded and seedless citrus Yamanto *et al.* (1997) observed that cytoplasmic male sterility is the potential contributing factor where parthenocarpy accompanied by chromosomal irregularity reflecting the triploid origin give rise to seedless or less seeded fruits. Soost and Camron (1980) supported and exemplified it in Tahiti lime. Jaskani *et al.* (2005) and Usman *et al.* (2008) reported that triploid citrus by changing polidy level ($2n \times 4n$) give rise to triploids ensuring seedless citrus fruits. Navel oranges, Satsuma mandarin and Mukakukishiu grapefruits are seedless on account of triploid character as a result of spontaneous mutation (Fatima *et al.*, 2010). The same was also reported by (Iwamasa, 1966) about Washington Navel, Satsuma mandarin and Tahiti lime being naturally seedless. Salustiana a prominent cultivar of Spanish citrus sector was identified and developed as a limb sport from Communa tree in the orchard of Paul Salustiano. Marrs' Early is also a mutated bud sport of Washington Navel (Cameron and Frost, 1968). It is an established fact that conventional breeding, induced mutation through irradiations, biotechnological techniques and use of phytohormones are the tools being employed and induction of superior and desirable characters are achieved in fruit sector (Khalil *et al.*, 2011; Raza *et al.*, 2003).

However, all these have resulted in a lesser cultivar development as compared to cultivars including seedless types that were introduced through natural mutations. With particular reference to phytohormones such as auxins, cytokinins and giberellins as reported by (George *et al.*, 1984; Talon *et*

al., 1990) parthenocarpy occurred under the influence of phytohormones. Bukvorac and Nakagawa (1967); Fos *et al.* (2000, 2001), Rebers *et al.* (1999), Robinson *et al.* (1971), Rodrigo and Garcia-Maratiness (1998) substantiated the role of cytokinins for the induction of facultative parthenocarpy. However, exogenous application of these phytohormones was very expensive proposition in the face of already ever escalating input cost of citrus orchards. So, natural mutations leading to seedless or less seeded citrus fruits particularly in Kinnow is a viable approach thereby precluding the limitations in conventional breeding and application of other techniques to alter the ploidy level. Pursuant to the objective in view, three less seeded strains encoded as LSKS-1, LSKS-2, LSKS-3 of fully mature bearing trees from Sahiwal and two strains from citrus germplasm unit Arifwala as LSKS-4 and LSKS-5 were evaluated for their seediness and various attributes of quality. Evaluation work on this aspect was carried out during 2019-20 with encouraging results thereby establishing a fair coincidence of less and non-significant number of seeds with high quality of kinnow fruit. The study proved worthwhile to enable the citrus growers and nursery men to produce less seeded plants of kinnow at commercial level. Outcome of this study is considered a breakthrough for further large scale multiplication of less seeded profusely bearing and qualitatively superior strains of Kinnow not only for domestic consumption and also for export purpose ensuring premium prices in the high end markets.

Materials and Methods

Experimental site

An experiment was conducted on 10 year old trees to determine the performance of three less seeded Kinnow strains (i.e. LSKS-1, LSKS-2 and LSKS-3) at Horticultural Research Station Sahiwal and two less seeded Kinnow strains (i.e. LSKS4 and LSKS5) at Citrus Germplasm Unit Muhammad Nagar, Arifwala. Data was collected during the month of January during the year 2019-2020. These research sites were located in Punjab province of Pakistan at geographical coordinates 31°58'28" N 72°19'51" E and 30°17'26.1" N 73°3.944' E. Soil conditions and climatic profiles of experimental sites along with Kinnow strains used in this study are given in Table 1, Figures 1 and 2. Selected strains were chance seedlings or bud sport mutations collected and maintained appropriately after hectic survey programme of citrus groves.

Table 1: Soil profile of citrus orchards at experimental sites in Sahiwal and Arifwala.

Soil characteristics	Horticultural research station, Sahiwal	Citrus germplasm unit Muhammad Nagar, Arifwala
pH	8.2	8.3
Organic Matter %	0.63	0.70
CaCO ₃ %	6.7	8.1
EC (dS/m)	1.9	1.5
Saturation %	36	32
Soil texture	Loamy	Loamy
Available phosphorus (mg/kg)	7.9	9.3
Extractable potassium (mg/kg)	240	200
Zinc (mg/kg)	0.81	0.78
Copper (mg/kg)	3.68	3.71
Iron (mg/kg)	0.15	0.19
Manganese (mg/kg)	0.51	0.46
Boron (mg/kg)	0.40	0.38

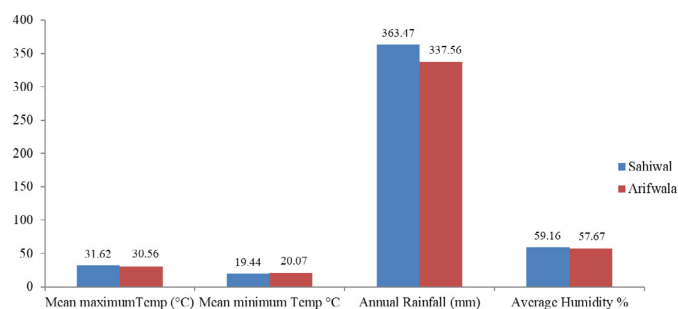


Figure 1: Eco-meteorological distribution data of studied locations (Sahiwal and Arifwala) for the year 2019-2020 (January-December).

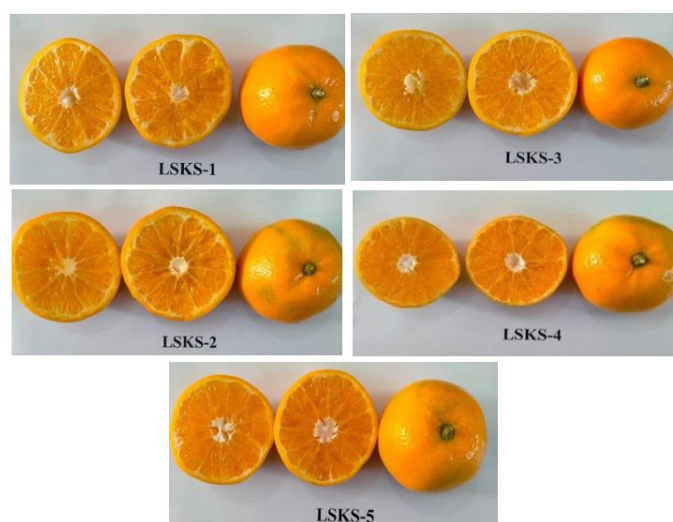


Figure 2: Representation of whole fruit and cross section of five less seeded Kinnow strains used in this study.

Experimental design and cultural practices

The experiment was laid out in RCBD with five treatments and 10 replications making total number

of 50 plants involving in the trial. The treatments were as follows; [Less seeded Kinnow strain1 (LSKS-1), Less seeded Kinnow strain 2 (LSKS-2), Less seeded Kinnow strain3 (LSKS-3), Less seeded Kinnow strain4 (LSKS-4) and Less seeded Kinnow strain 5 (LSKS-5)]. All selected plants were of the same age (ten years), grafted on rough lemon rootstock and planted in square system at 20 x 20 feet. Data was collected for two consecutive years i.e. 2019 and 2020 and fruit evaluation was performed after its attainment of physiological and commercial maturity. The trees were subjected to standard agricultural practices. Fifty fruits (five fruits from each plant) were harvested for each strain to collect and characterize data.

Soil analysis

Soil samples were taken up to 30 cm depth randomly with the help of augur from field, mixed properly by following the guidelines of soil sampling and sample preparation (Andreas and Micheal, 2005). After collecting, samples were dried in shade, pulverized softly and passed through 2.0 mm sieve to get invariable sample. Later on standard soil analysis methods were used to analyze physico-chemical characteristics of soil. Saturated paste was used to determine saturation percentage and then it was dried at 105°C for 24 hours and then it was computed with the help of following formula.

$$\text{Saturation Percentage} = \frac{\text{Loss in Weight of Soil}}{\text{Weight of Wet Soil}} * 100$$

The pH was measured in soil: water (1:1) solution by pH meter (HANNA-HI 2210) and electrical conductivity (EC) was measured in soil: water (1:10) by using EC meter (HANNA-edge^{ec}) according to criteria described by Malik *et al.* (1984). Soil organic matter was measured by titration method (Nelson and Sommers, 1982), available phosphorus was extracted by using 0.5N Na₂CO₃ solution (Olsen and Sommers, 1982) and then Spectrophotometer (PD-303S) was used to measure it. Potassium was measured on Flame photometer (JENWAY PFP-7) using 1M NH₄-acetate at normal pH i.e. 7 (Helmke and Sparks, 1996). Analysis of micronutrients was carried out from soil and water testing laboratory for research Multan.

Physical traits analysis

Physical traits including fruit weight (g), fruit length

(mm), fruit breadth (mm), peel thickness (mm), juice contents (%), viable seed number, aborted seed number, fruit firmness (kg), total number of segments per fruit and yield (number of fruits/tree) were measured. The traits were measured with recommended indicators i.e. Digital weighing balance (Model UniB1C., SHIMADZU, U x 320g, Min.0.02g, e=0.01 g and d=0.001 g), Vernier caliper (Model: KBD-MT 0014, Cingda Industry China with an accuracy of 0.01cm) and Penetrometer (Model B059M-01).

Biochemical traits analysis

Juice pH, specific gravity, total soluble solids (°Brix), titrate able acidity (%), vitamin C contents (mg/100g) and phenolic compounds (mg GAE/100 mL) were measured to evaluate the fruit quality of selected less seeded Kinnow strains. The manual extractor was used to squeeze juice and Whatman® filter paper No.1 was used to filter the juice. The juice pH was measured with bench-type digital calibrated pH meter at 20 °C as described by the Anwar *et al.* (2007). Digital refractometer (ATAGO, RS- 5000) was used for measuring the total soluble solids (°Brix) from the juice. To determine titratable acidity (%), 10 mL juice was taken and titrated against 0.1 N NaOH and phenolphthalein was used as indicator to attain the end-point of pink colour (Hortwitz, 1960). Vitamin C contents (mg/100ml) were determined according to the method described by Ruck (1969). The total phenolic compounds (mg GAE/100 mL) were calculated by using a modified method that involved Folin Ciocalteure agent (FCR) (Ghafoor *et al.*, 2012).

Carbohydrate analysis

High performance liquid chromatography (HPLC) technique was used to measure glucose, fructose and sucrose. Kinnow juice from selected strains was extracted and filtered to remove pulp and seeds. Juice was centrifuged (at 300rpm) for 15 minutes to get supernatant. Then supernatant was diluted with HPLC grade water. It was again filtered (with 0.45 µm membrane filter) prior to inject in HPLC (Qureshi *et al.*, 2021).

Statistical analysis

Collected data for less seeded Kinnow strains was analyzed by constructing analysis of variance (ANOVA) employing statistical software Statistix 8.1. Means were compared by using least significant

difference (LSD) test at significance level of 0.05 (Steel *et al.*, 1997).

Results and Discussion

Physical traits

Individual fruit weight (g) significantly varied ($p \leq 0.05$) among the selected less seeded Kinnow strains. It appeared that maximum fruit weight (170.58g) remained in LSKS-2 followed by LSKS-3 (165.53g) and LSKS-5 (158.64g) whereas minimum fruit weight (150.73g) was recorded in LSKS-1 followed by LSKS5 (158.64g) (Table 2). These results are in conformity with Hussain *et al.* (2017) who evaluated the different sweet orange cultivars under varying climatic conditions and found significant variation in fruit weight from 129.33g (Faisalabad conditions) to 169.22g (Sahiwal conditions). Similar results were reported by Altaf *et al.* (2008) showing variability in fruit weight (157.40g-244.20g per fruit) of different Kinnow strains. These results were further verified by the findings of Altaf and Khan (2009), who reported a variation of 151.45g-191.70g in average fruit weight of Kinnow fruit. Maximum fruit length (70.68mm) was shown by LSKS-2 followed by LSKS-3 (66.48mm) whereas minimum fruit length was counted for LSKS-1 (56.57mm) (Table 2). These results are in accordance with the findings of Altaf *et al.* (2008) who investigated that different strains of Kinnow had higher level of variation with respect to their fruit length differed from 7.20 cm to 8.55 cm. Maximum fruit breadth (68.08 mm) was depicted by LSKS-2 and LSKS-3 (65.61mm) followed by LSKS-5 (59.66mm), while minimum fruit breadth was observed in LSKS-1 (55.07mm) and LSKS-4 (51.83mm) (Table 2). As different fruits had different fruit length so their breadth also varied greatly. These results were supported by the findings of Altaf *et al.* (2008) who reported a variation of 5.18cm to 6.63 cm in fruit breadth of studied Kinnow strains. Maximum peel thickness was computed in LSKS-4 (3.31mm) followed by LSKS-1 (3.13mm) and LSKS-5 (3.11mm), while minimum peel thickness was recorded in LSKS-3 (2.91mm) (Table 2). These results were in accordance with the findings of Hussain *et al.* (2017) who investigated that peel thickness varied among different sweet orange cultivars from 4.16mm to 5.50mm. These results were also supported by the findings of Altaf *et al.* (2008) who reported a variation of 2.10 mm to 3.50 mm in peel thickness of different seeded Kinnow strains. Similar results were

reported by Altaf and Khan (2009) who reported 4.10 mm peel thickness in Kinnow fruits. Highest juice percentage was shown by LSKS-2 (46.32%) and LSKS-5 (43.54%) followed by LSKS-3 (40.37%), LSKS-1 (40.32%) and LSKS-4 (38.44%) (Table 2). These results were supported by the findings of Khalid *et al.* (2018), who conducted an experiment on quality estimation of Kinnow mandarin by growing it under different geographical conditions. They reported that juice percentage varied from 37.7% to 40.9% in studied Kinnow strains. Number of viable seeds was found non-significant among selected less seeded kinnow strains (Table 2). Data collected for aborted seeds was found significant. Maximum number of aborted seeds was shown by LSKS-3 and LSKS-5 i.e. 5.0 followed by LSKS-1 and LSKS-2 i.e. 4.0 (Table 2). These results are verified by the findings of Altaf *et al.* (2008) who concluded that variations exist for aborted seed number in Kinnow mandarin. It was reported that number of aborted seeds per fruit varied from 1.1 to 5.1 in seeded Kinnow strains. Highest fruit firmness was shown by LSKS-1 (2.48 kg) followed by LSKS-3 (1.88 kg), LSKS-2 (1.66kg) and LSKS-4 (1.27 kg) whereas minimum fruit firmness was observed by LSKS-5 i.e. 1.33 kg (Table 2). These results are verified by the findings of Khalid *et al.* (2018), who concluded that a greater level of variation exists in Kinnow mandarin. They reported that fruit firmness in Kinnow varied from 2.11kg to 2.30kg.

Number of segments (also known as locules) was also found to be significant which most important portion of kinnow is as they hold pulp i.e. the edible portion of fruit. Maximum number of segments (13) was counted in LSKS-3 followed by LSKS-1 (12), LSKS-4 and LSKS-5 (11.0) whereas minimum number of segments was counted in LSKS-2 i.e. 10.0 (Table 2). These results are in accordance with the findings of Altaf *et al.* (2008) who reported about 8.0 to 12.0 number of segments per Kinnow fruit among studied strains. These results were further verified by the findings of Altaf and Khan (2009) who reported 9-14 number of segments per fruit in Kinnow mandarin. Highest yield (974.00 number of fruits/tree) was counted in LSKS-2 followed by LSKS-3 (880.00) whereas lowest yield was calculated for LSKS-1 (765.00 number of fruits/tree) and LSKS-4 (766.00 number of fruits/tree) (Table 2). These results are verified by the findings of Altaf and Khan (2009) who reported a yield variation in seeded kinnow strains.

Table 2: Physical characteristics of five less seeded Kinnow strains under Sahiwal and Arifwala conditions.

Less Seeded Kinnow Strains	Viable seed number	Aborted seed number	Fruit firmness (Kg)	Total number of segments/fruit	Yield (Number of fruits/tree)
Less Seeded Kinnow Strain 1 (LSKS-1)	3.20±1.23a	4.00±0.39ab	2.48±0.31a	12.0±0.23ab	765.00c
Less Seeded Kinnow Strain 2 (LSKS-2)	3.60±0.87a	4.00±0.45ab	1.66±0.21ab	10.0±0.11c	974.00a
Less Seeded Kinnow Strain 3 (LSKS-3)	4.00±0.54a	5.00±0.56a	1.88±0.43ab	13.0±0.17a	880.33b
Less Seeded Kinnow Strain 4 (LSKS-4)	3.60±0.61a	3.00±0.19b	1.278±0.16ab	11.0±0.19b	766.00c
Less Seeded Kinnow Strain 5 (LSKS-5)	4.60±0.34a	5.00±0.64a	1.334±0.42b	11.0±0.32b	765.333bc
Less Seeded Kinnow Strains	Fruit weight (g)	Fruit length (mm)	Fruit breadth (mm)	Peel thickness (mm)	Juice contents (%)
Less Seeded Kinnow Strain 1 (LSKS-1)	150.73±2.67d	56.57 ±3.56d	55.07±3.05c	3.13±0.42b	40.32±3.61b
Less Seeded Kinnow Strain 2 (LSKS-2)	170.58±3.78a	70.68±2.71a	68.08±3.78a	3.01±0.39c	46.22±3.98a
Less Seeded Kinnow Strain 3 (LSKS-3)	165.53±2.87b	66.48±3.45b	65.61±2.45a	2.91±0.64d	40.37±2.67b
Less Seeded Kinnow Strain 4 (LSKS-4)	146.61±3.12e	54.51±4.13d	51.83±2.98c	3.31±0.33a	38.44±3.24b
Less Seeded Kinnow Strain 5 (LSKS-5)	158.64±3.99c	61.13±3.29c	59.66±4.34b	3.11±0.54b	43.54±1.65a

Means sharing same letters in a group show non-significant differences ($p \leq 0.05$).

Similar variations in Kinnow yield were reported by Modise *et al.* (2009) and Ashraf *et al.* (2012). Castle (1995) and Treeby *et al.* (2007) found variation in number of fruits among different sweet orange varieties and stated that this variation is due to environmental and genetic constitution of each variety.

Biochemical traits

As a result of various biochemical attributes, maximum pH in juice was recorded in LSKS-4 (4.76%) and LSKS-2 (4.19%) followed by LSKS-5 (3.98%), whereas minimum was counted in case of LSKS-1 (3.28) (Figure 3). These results were supported by the findings of Altaf *et al.* (2008) who concluded that a greater level of pH variation existed in various Kinnow mandarin strains. It was reported that pH of juice varied from 3.69 to 4.17 in Kinnow strains. Our results are further verified by Altaf *et al.* (2014) who concluded that biochemical parameters of seeded and seedless Kinnow differ at non-significant level.

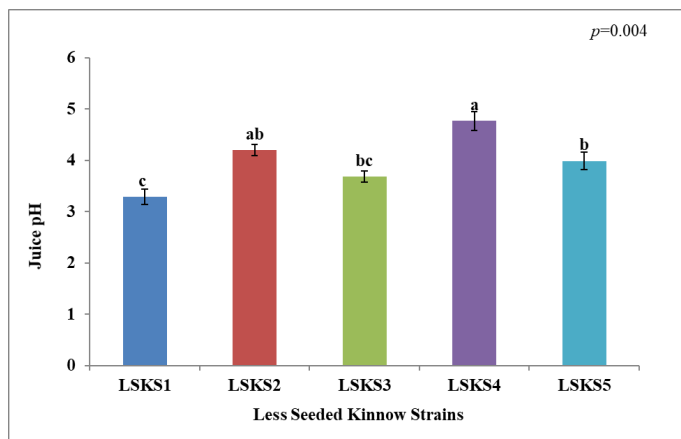


Figure 3: Juice pH of five less seeded Kinnow strains under Sahiwal and Arifwala conditions.

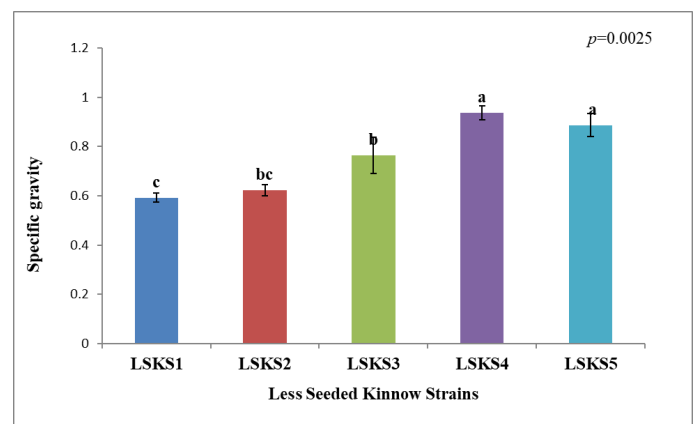


Figure 4: Specific gravity of five less seeded Kinnow strains under Sahiwal and Arifwala conditions.

Maximum specific gravity value was shown by LSKS-4 (0.93) and LSKS-5 (0.88) followed by LSKS-3 (0.76), LSKS-2(0.62) and LSKS-1(0.59) (Figure 4). These results are verified by the findings of Kumar *et al.* (2017), as reported that specific gravity of Kinnow deviate from 0.93 to 1.02.

Highest total soluble solid (TSS) contents were shown by LSKS-2 (11.70°Brix) followed by LSKS-3 (9.62°Brix), whereas minimum TSS value was observed in LSKS-4 (7.84°Brix) (Figure 5). These results are in accordance with the findings of Altaf *et al.* (2008) who also reported that a greater level of variation exists in TSS of seeded Kinnow mandarin. They reported a variation of 9.7°Brix to 11.00°Brix in studied Kinnow strains. These results are further confirmed by the findings of Altaf and Khan (2009) who reported total soluble solids in Kinnow mandarin with a range of 8.5°Brix to 14.5°Brix. Treeby *et al.* (2007) worked on different citrus varieties and

concluded such changes might have occurred due to variation in photosynthates transportation and diverse nutrients accumulation. Riaz *et al.* (2015) evaluated citrus germplasm and concluded that deviation in biochemical traits is linked to inherent features of scion.

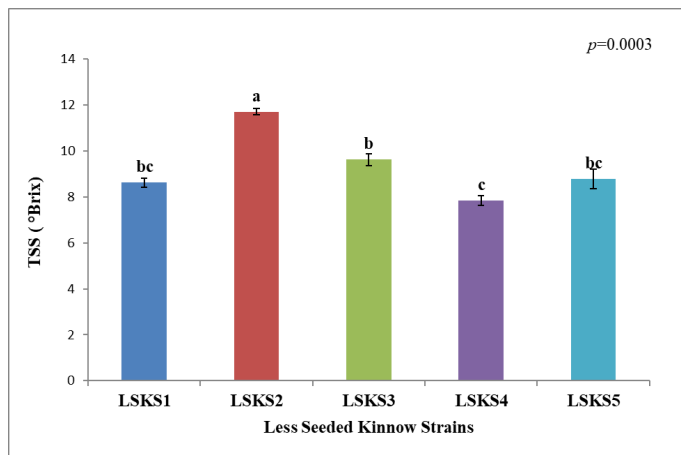


Figure 5: Total soluble solids (°Brix) of five less seeded Kinnow strains under Sahiwal and Arifwala conditions.

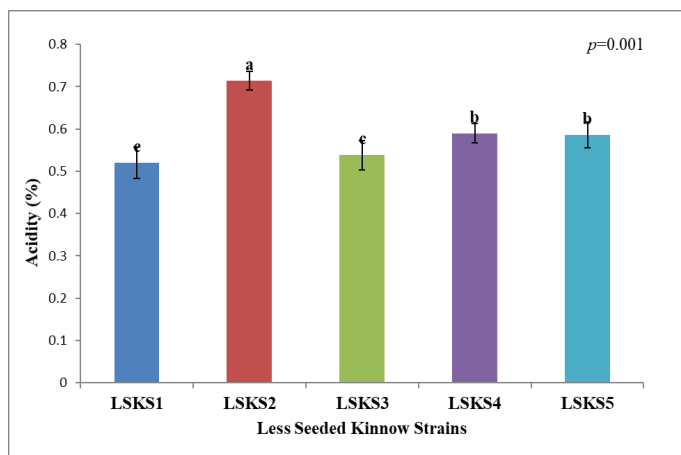


Figure 6: Acidity (%) of five less seeded Kinnow strains under Sahiwal and Arifwala conditions.

Maximum results for acidity were shown by LSKS-2 (0.714 %) followed by LSKS-4(0.59 %) and LSKS-5 (0.58%) however, minimum result was depicted by LSKS-1 (0.52%) (Figure 6). These findings are supported by the findings of Khalid *et al.* (2018) who concluded that a greater level of variation existed in acidic contents of Kinnow mandarin strains. It was reported that acidity of juice varied from 0.78% to 0.81% in Kinnow strains. Similar results were also reported by Altaf and Khan (2009) with titratable acidity ranging from 0.7% to 1.0% in Kinnow mandarin strains.

Regarding vitamin C, highest value was shown by LSKS-2 (18.43mg/100g) and LSKS-3 (17.36

mg/100g) followed by LSKS-1 (15.65 mg/100g), LSKS-4 (14.79 mg/100g) and LSKS-5 (13.75 mg/100g) (Figure 7). These results are in conformity with the findings of Khalid *et al.* (2018) who reported that a greater level of variation exists in vitamin C of Kinnow mandarin. They reported a variation of 56.8mg/100g to 67 mg/100g in studied Kinnow strains. In case of phenolic compounds, maximum value remained in LSKS-2 (94.69µg GE/mL) followed by LSKS-3 (93.02 µg GE/mL) and LSKS-1 (90.74 µg GE/mL) while minimum values were recorded in LSKS-5(82.65 µg GE/mL)) (Figure 8). These results stand in corroboration of Hussain *et al.* (2017) as they reported total phenolic compounds with a range of 43.6 µg GE/mL to 96.3µg GE/mL.

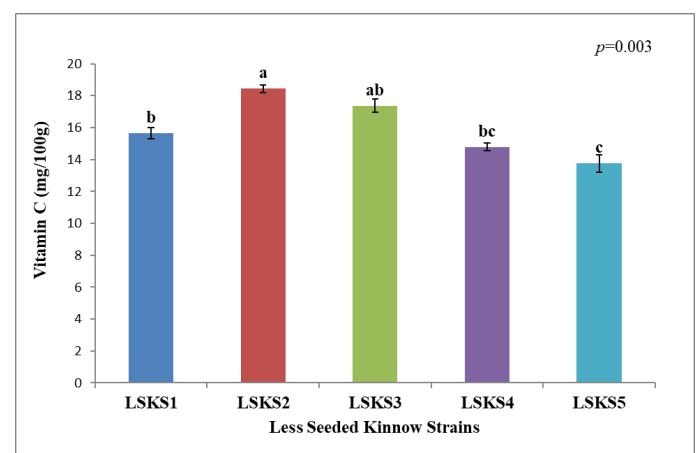


Figure 7: Vitamin C contents (mg/100g) of five less seeded Kinnow strains under Sahiwal and Arifwala conditions.

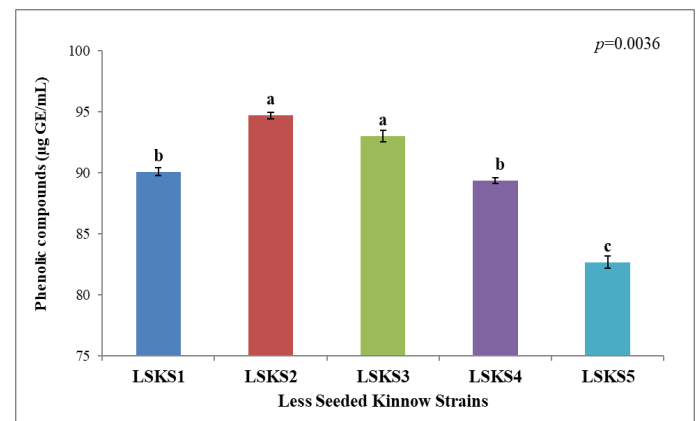


Figure 8: Phenolics compounds (µg GE/mL) of five less seeded Kinnow strains under Sahiwal and Arifwala conditions.

Sugar contents

Individual fruit sugar contents significantly varied ($p \leq 0.05$) among the selected less seeded Kinnow strains. In case of sucrose contents, results revealed that maximum fruit sucrose concentration (48.08g/L) was shown by LSKS-2 followed by LSKS-3 (43.14g/L) and LSKS-1 (41.52g/L) whereas minimum sucrose

Table 3: Sugar contents (g/L) in juice of five less seeded Kinnow strains under Sabiwal and Arifwala conditions.

Less Seeded Kinnow Strains	Sucrose (g/L)	Fructose (g/L)	Glucose (g/L)
Less Seeded Kinnow Strain 1 (LSKS-1)	41.52±1.53b	19.61±1.95bc	5.44±1.94B
Less Seeded Kinnow Strain 2 (LSKS-2)	48.08±2.57a	27.72±2.88a	4.41±3.61B
Less Seeded Kinnow Strain 3 (LSKS-3)	43.14±3.45b	21.82±3.94b	6.42±2.89Ab
Less Seeded Kinnow Strain 4 (LSKS-4)	38.50±4.56c	17.09±1.67c	8.94±3.34A
Less Seeded Kinnow Strain 5 (LSKS-5)	41.12±1.54bc	19.42±3.37bc	4.44±4.12B

Means sharing same letters in a group show non-significant differences ($p \leq 0.05$).

concentration was observed in LSKS-4 (38.50g/L) followed by LSKS-5 with value of (41.12g/L) (Table 3). Our results are supported by the findings of Qureshi *et al.* (2021), who evaluated sugar contents of Kinnow mandarin on different rootstocks. They reported that glucose concentration varied from 24.4 (g/L) to 48.3 (g/L) with an average of 37.9 (g/L). Regarding fructose contents, maximum results were shown by LSKS-2 (27.07g/L) followed by LSKS-3 (21.82g/L) and LSKS-1 (19.61g/L) whereas, minimum value was recorded in LSKS-4 (17.09g/L)(Table 3). Our findings are in accordance with that of the Qureshi *et al.* (2021), who evaluated sugar contents of Kinnow mandarin on different rootstocks. They reported fructose concentration varied from 14.3 (g/L) to 26.3(g/L) with an average of 20.2 (g/L). In case of glucose contents, maximum results were illustrated by LSKS-4 with (8.94g/L) followed by LSKS-1 (5.44g/L) and LSKS-3 (6.42g/L) and minimum value for glucose was shown in LSKS-2 (4.41g/L) (Table 3). Our results are at par with the findings of Qureshi *et al.* (2021), who evaluated sugar contents of Kinnow mandarin on different rootstocks. They reported that glucose concentration varied from 4.4 (g/L) to 9.9 (g/L) with an average of 6.5 (g/L).

Conclusions and Recommendations

Diversification of Pakistan citrus industry with less seeded Kinnow strains is highly valuable to boost the trade. Investigated strains showed good physiochemical attributes. Less seeded Kinnow strain-2 showed highest values for most of the evaluated traits like fruit weight, fruit length, fruit breadth, highest juice contents, highest fruit yield, total soluble solids and vitamin-C. Results revealed that juice obtained from selected strains is of highly good quality being nutritionally rich with the scope for processing industry. These strains of less seeded Kinnow may be suitable for its commercial cultivation in local environment of country. Findings of this research endeavor will go a

long way to expand the less seeded strains of kinnow mandarin through vegetative propagation supplying the bud wood to registered nurseries of the Punjab province. Moreover, identification, confirmation and observations to retain less seeded trait in a consistent manner precluding the application of mutagens to alter the genetic makeup have opened bright prospects to improve the inferior citrus cultivars to superior ones with the intended and desired characters prominently the seedless or less seeded ones to earn a place in the competitive trade of citrus fruits.

Acknowledgements

Author is highly grateful and acknowledge the contributions of whole team of researchers for support and kind guidance.

Novelty Statement

This study will facilitate to develop the less seeded Kinnow orchards in country as kinnow bud wood will be available for asexual propagation. Study manifested superior kinnow strains with high quality attributes to replace the inferior varieties.

Author's Contribution

IMS: Conceived the research idea

NS: Conducted research, performed statistical analysis and wrote manuscript.

MN: Helped in data collection.

AG and SD: Critically reviewed

AN and MN: Analyzed data and helped in writing manuscript.

All authors discussed the results and contributed to final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

References

- Altaf, N. and A.R. Khan. 2009. Growth and development of low seeded Kinnow mandarin fruit in dense plantation. *J. Agric. Sci. Technol.*, 11: 191–198.
- Altaf, N. and M.M. Iqbal. 2003. Towards a seedless cultivar of Kinnow mandarin III. Variability of developed and undeveloped seed number in seedless/low seeded fruits. *Pak. J. Bot.*, 35: 339–342.
- Altaf, N., A.R. Khan and J. Hussain. 2008. Fruit variability in Kinnow mandarin. *Pak. J. Bot.*, 40: 599–604.
- Altaf, S., M.M. Khan, M.J. Jaskani, I.A. Khan, M. Usman, B. Sadia, F.S. Awan, A. Ali and A.I. Khan. 2014. Morphogenetic characterization of seeded and seedless varieties of Kinnow Mandarin (*Citrus reticulata* Blanco). *Aust. J. Crop Sci.*, 8: 1542–1549.
- Andreas, P. and W.B. Micheal. 2005. Soil sampling and storage. In: (eds. R. Margesin and F. Schinner). *Manual for soil analysis monitoring and assessing soil bioremediation*. Chapter. 1. p. 1–45.
- Anonymous, 2019. Fruit, vegetable and condiments statistics of Pakistan. Ministry of national food security and research Islamabad.
- Anonymous, 2020. Fruit, vegetable and condiments statistics of Pakistan. Ministry of national food security and research Islamabad.
- Anwar, F., S. Latif, R. Przybylski, B. Sultana and M. Ashraf. 2007. Chemical composition and antioxidant activity of seeds of different cultivars of Mungbean. *J. Food Sci.*, 72: 503–510. <https://doi.org/10.1111/j.1750-3841.2007.00462.x>
- Ashraf, M.Y., A. Gul, M. Ashraf, F. Hussain and G. Ebert. 2012. Improvement in yield and quality of Kinnow (*Citrus deliciosa* × *Citrus nobilis*) by potassium fertilization. *J. Plant Nutr.*, 33: 1625–1637. <https://doi.org/10.1080/01904167.2010.496887>
- Bukvorac, M.J. and S. Nakagawa. 1967. Comparative potency of gibberelins in inducing parthenocarpic fruit growth in *Malus sylvestris* Mill. *Experientia*, 23: 865–871. <https://doi.org/10.1007/BF02146896>
- Cameron, J.W and H.B. Frost. 1968. Genetics, breeding and nucellar embryony. In: (W. Reuther, L.D. Batchelor and H.J. Webber). *The citrus industry*. Vol. II. University of California Press, Berkeley. pp. 325–370.
- Castle, W.S., 1995. Rootstock as a fruit quality factor in citrus and deciduous tree crops. *N. Z. J. Crop Hortic. Sci.*, 23: 383–394. <https://doi.org/10.1080/01140671.1995.9513914>
- Eliza, R., H. Heven and N. Carmi. 2008. Introduction of seedlessness in citrus from classical techniques and emerging biotechnological approaches. *J. Am. Soc. Hortic. Sci.*, 133: 117–128. <https://doi.org/10.21273/JASHS.133.1.117>
- Fatima, B., M. Usman, I.A. Khan, M.S. Khan and M.M. Khan. 2010. Exploring citrus cultivars for underdeveloped and shriveled seeds: A valuable resource for spontaneous polyploidy. *Pak. J. Bot.*, 42: 189–200.
- Fos, M., F. Nuez and J.H. Martinez. 2000. The gene pat-2 which induces natural parthenocarpy alters the gibberellins contents in un-pollinated tomato ovaries. *Plant Physiol.*, 122: 471–480. <https://doi.org/10.1104/pp.122.2.471>
- Fos, M., K. Proano, F. Nuez and G. Martinez. 2001. Role of Gibberelins in parthenocarpic fruit development induced by the genetic system pat-3/ pat-4 in tomato. *Plant Physiol.*, 111: 545–550. <https://doi.org/10.1034/j.1399-3054.2001.1110416.x>
- Frederick, G.G., J. Jaon and X. Hu. 1990. The possible role of Yunnan, China, in the origin of contemporary citrus species (Rutaceae). *Econ. Bot.*, 44: 267–277. <https://doi.org/10.1007/BF02860491>
- Frost, H.B., 1935. Four new citrus varieties the Kara, Kinnow and Wilking mandarins and the Trovita orange. *Calif. Agric. Exp. Stat. Bull.*, 597: 14.
- George, W.L., J.W. Scott and W.E. Splittstoesser. 1984. Parthenocarpy in tomato. *Hortic. Res. Am. Soc. Hortic. Sci.*, 6: 65–84. <https://doi.org/10.1002/9781118060797.ch2>
- Ghafoor, K., F. Al-Juhaimi and Y.H. Choi. 2012. Supercritical fluid extraction of phenolic compounds and antioxidants from grape (*Vitis labrusca* B.) seeds. *Plant Food Hum. Nutr.*, 67: 407–414. <https://doi.org/10.1007/s11130-012-0313-1>
- Government of Pakitan. 2020. Finance division. Economic survey of Pakistan.
- Helmke, P.A., and D.L. Sparks. 1996. Lithium, sodium and potassium In: *Methods of soil analysis*. part 2. chemical and microbial

- properties (eds. A.L. Page, O.L. Helmke, P.N. Sultnanpur, M.A. Tabatbhai and M.E. Summer). SSSA. Wisconsin, USA. p. 551-575.
- Hortwitz, W., 1960. Official and tentative methods of analysis, 9th Ed. Association of Official Agric. Chemists, Washington, USA.
- Huso, F., F. Maddy, C. Jacquemond, Y. Froelichel, R. Morithion, D. Rist and P. Ouollitraul. 2004. Identification and evaluation of diploidy in clamantine for use on breeding. *Acta Hort.*, 663: 844-848.
- Hussain, S.B., M.A. Anjum, S. Hussain, S. Ejaz and M. Ahmed. 2017. Physico-chemical profiling of promising sweet orange cultivars grown under different agro-climatic conditions of Pakistan. *Erwerbs-Obstbau*. 59: 315-324. <https://doi.org/10.1007/s10341-017-0327-9>
- Iwamasa, M., 1966. Studies on the sterility in genus *Citrus* with special reference to seedlessness. *Bul. Hortic. Res. Stat. Japan Min. Agric. For. Ser.*, B6 1-77.
- Jaskani, M.J., I.A. Khan and M.M. Khan. 2005. Fruit set, seed development and embryo germination in interploid crosses of citrus. *Sci. Hortic.*, 107: 51-57. <https://doi.org/10.1016/j.scienta.2005.06.011>
- Khalid, S., A.U. Malik, Z. Singh, S. Ullah, B.A. Saleem and O.H. Malik. 2018. Tree age influences nutritional, pectin, and anatomical changes in developing 'Kinnow' mandarin (*Citrus nobilis* Lour × *Citrus deliciosa* Tenora) fruit. *J. Plant Nutr.*, 41: 1786-1797. <https://doi.org/10.1080/01904167.2018.1462378>
- Khalil, S.A., A. Sattar and R. Zamir. 2011. Development of sparse-seeded mutant Kinnow (*Citrus reticulata* Blanco) through budwood irradiation. *Afr. J. Biotechnol.*, 10: 123-134. <https://doi.org/10.5897/AJB10.1810>
- Khan, A.R., 2007. Varietal spectrum of *Citrus* nurseries published proceedings of workshop on the production of disease free citrus nursery plants. pp. 12-13.
- Kumar, A., A. Mirza, A. Singh, Vikas, J. Rafie, P. Kumar, B. Kumar and P. Verma. 2017. Improvement in physico-chemical properties of kinnow (*Citrus reticulata* Blanco) after the foliar application with micronutrients and growth regulators. *Int. J. Curr. Microbiol. App. Sci.*, 6: 389-394. <https://doi.org/10.20546/ijcmas.2017.611.044>
- Mahmood, N.M., and I.A. Khan. 1992. Citriculture in Pakistan. Proceedings of 1st international seminar on citriculture in Pakistan. In: I.A. Khan (Ed). Department of Horticulture University of Agriculture, FSD, pp.17. ISBN96982 37-007.
- Malik, D.M., M.A. Khan and D.A. Choudhary. 1984. Analysis manual for soil, water and plants. Director Rapid Soil Fertility Lahore.
- Modise, D.M., A.S. Likuku, M. Thuma and R. Phuti. 2009. The influence of exogenously applied 2, 4-dichlorophenoxyacetic acid on fruit drop and quality of navel oranges (*Citrus sinensis* L.). *Afr. J. Biotech.*, 8: 2131-2137.
- Nelson, S.W. and I.E. Sommers. 1982. The total carbon, organic carbon and organic matter. In: Methods of soil analysis. Chemical and microbial properties. *Agron.* 9 Part 2, 2nd Ed. A.L. Page (Ed.). USA. pp. 539-580.
- Olsen, S.O. and I.E. Sommers. 1982. Phosphorus. In: methods of soil analysis. A.L. Page, (Eds.). chemical and microbial properties. Part2, 2nd Edition, American Society of Agronomy. Medison, Wisconsin, United States of America. pp.403-430. <https://doi.org/10.2134/agronmonogr9.2.2ed.c24>
- Qureshi, M.A., M.J. Jaskani, A.S. Khan, M.S. Haider, W. Shafqat, M. Asif and A. Mehmood. 2021. Influence of different rootstocks on physico-chemical quality attributes of Kinnow Mandarin. *Pak. J. Agric. Res.*, 7: 346-365.
- Raza, H., M.M. Khan and A.A. Khan. 2003. Seed lessness in citrus (review). *Int. J. Agric. Biol.*, 7: 1560-8530.
- Rebers, M., T. Kaneta, H. Kawaide, S. Yamaguchi, Y.Y. Yang, R. Imai, H. Sekimao and Y. Kamiya. 1999. Regulation of biosynthesis genes during flower and early fruit development of tomato. *J. Plant*, 17: 241-250. <https://doi.org/10.1046/j.1365-313X.1999.00366.x>
- Riaz, M., T. Zamir, N. Rashid, N. Jamil, Z. Masood, U. Jabeen, F. Mandokhel, F. Behlil, F. Mengal, and M. Khan. 2015. Quality assessment in different stages of maturity of fruits, mandarins kinnow and feutrell's early collected from the fruit market of Quetta city at in relation to their benefits for human health. *Am.-Eurasia. J. Toxicol. Sci.*, 7: 203-208.
- Robinson, R.W., D.J. Cantliffe and S. Shannon. 1971. Morphactin-induced parthenocarpy in the cucumber. *Science*, 171: 1251-1252. <https://doi.org/10.1126/science.171.3977.1251>
- Rodrigo, M.J. and J.L. García-Martínez. 1998.

- Hormonal control of parthenocarpic ovary growth by the apical shoot in pea. *Plant Physiol.*, 116: 511–518. <https://doi.org/10.1104/pp.116.2.511>
- Ruck, J.A., 1969. Chemical methods for analysis of fruits and vegetables products. Department of Agriculture. Canada SP 50 Summerland Research Station, Ontario.
- Sharma, J.N. and S.K. Thind. 2005. An approach for the establishment of Seed lessness in Kinnow Mandarin. *Indian J. Hortic.*, 62: 8–11.
- Soost, R.K. and J.W. Camosan. 1985. Mologold a triploid pummelo grapefruit Hybrid. *Hortic. Sci.*, 20: 1134–1135. <https://doi.org/10.21273/HORTSCI.20.6.1134>
- Steel, R.G.D., J.H. Torrie and D.A. Dicky. 1997. Principles and procedures of statistics, a biometrical approach. 3rd Edition, McGraw Hill, Inc. Book Co., New York, pp. 352–358.
- Talon, M., L. Zacarias and E. Primo-Millo. 1990. Hormonal changes associated with full set and development in mandarins differing in parthenocarpic ability. *Plant Physiol.*, 79: 400–406. <https://doi.org/10.1034/j.1399-3054.1990.790227.x>
- Treeby, M.T., R.E. Henriod, K.B. Bevington, D.J. Milne and R. Storey. 2007. Irrigation management and rootstock effects on navel orange [*Citrus sinensis* (L.) Osbeck] fruit quality. *Agric. Water Manage.* 91: 24–32. <https://doi.org/10.1016/j.agwat.2007.04.002>
- Usman, M., B. Fatima, K.A. Gillani, M.S. Khan and M.M. Khan. 2008. Exploitation of potential target tissues to develop polyploids in citrus. *Pak. J. Bot.*, 40: 1755–1766.
- Yamamoto, M., R. Malsumoto, N. Okudai and Y. Yamada. 1977. Aborted anthers of citrus resulted from gene cytoplasmic male sterility. *Sci. Hortic.*, 70: 914–920.