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Characterization and evaluation of conventional seeded Kinnow (*Citrus reticulata* Blanco) versus a novel less seeded Kinnow strain under Sahiwal climatic zone

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Abstract

Kinnow is the sole exportable entity among citrus fruits of Pakistan, but occurrence of large seed number is a big hindrance towards consumer acceptability and processing industry. Production of a less seeded/seedless Kinnow could boost the market value globally. The study described here was conducted at Horticultural Research Station Sahiwal, Punjab Pakistan during 2018-2020. Specific morphological traits (linked to tree, leaf, and fruit) and physico-biochemical traits (linked to fruits) were evaluated to make a comparison between conventionally growing Kinnow and a novel less seeded Kinnow strain. Most of the estimated characters varied in their performance and were comparatively better in seeded Kinnow but performance of less seeded Kinnow strain was also quite good and promising. It was concluded from the study that less seeded Kinnow strain is almost equally good in performance regarding physical and nutritional features and can prove to be a great contribution towards expanding the citrus industry to fetch higher market value both in domestic and international markets.

Key words: Seedless, Citrus, Sahiwal, Biochemical, Kinnow, Sugar contents, Vitamin C, Physical traits, Firmness

Introduction

Citrus fruits belonging to family Rutaceae, are economically and nutritionally very important and are cultivated in the tropical and subtropical zones of Pakistan (Hussain, *et al.*, 2004; Ghani et al., 2016). They are an excellent source of vitamins, minerals, fibers and carbohydrates (Hayat *et al*., 2017; Wu *et al.*, 2018). Pakistan fruit industry is driven by various citrus groups like mandarin, grapefruit, lemons and lime but the share of Kinnow (*Citrus reticulata* Blanco) is significant (i.e.>60%). Kinnow is an 'easy peel' citrus cultivar which has gained significant position in the market due to its elevated juice content, particular flavor and superb taste (Altaf, 2009). Kinnow evolved as a hybrid making by a cross between king and willow leaf mandarins (*Citrus nobilis* × *Citrus deliciosa*) and hence termed as Kinnow mandarin (Frost, 1935). Requirement of irrigation for Kinnow is low as compared to sweet orange and it is more convenient to grow in frost-free zones. Botanically, Kinnow is a large and vigorous plant, with evergreen and dense foliage producing highly juicy yellowish-orange colored fruit, with strong aroma (Singh, 2018). Pakistan's citrus industry has significant position globally with its leading export entity 'Kinnow" among which the share of Punjab province is 95–96%. Average area and production of citrus in Pakistan is 192,832 hectares and 2,395,550 tonnes respectively (FAO, 2019). Considerable variation (0-50) has been recorded in the number of seeds of Kinnow which might be due to its polyembryonic nature (Khan, 1992; Sharma and Thind, 2005; Altaf, 2009). Low seeded trait of Kinnow (0-10 seeds/fruit) was reported from the growing belt in Punjab province of Pakistan (Altaf and Iqbal, 2003) and in the Ferozpur region, India (Sharma and Thind, 2005). High number of seeds is a big issue specially for processing industry as seeds if present in juice cause bitterness (Shahid *et al*., 2016).

Universally, citrus breeding objectives encompass induction of low seeded or seedless trait, dwarf tree size, regular fruit bearing and insect-pest resistance. Seeedlessness is intimately linked with triploidy, whereas spontaneous polyploidy also exists in nature (Jaskani *et al*., 2005; Usman *et al*., 2008). Various methods to induce seeedlessness include natural selection, Chimeras, embryo rescue technique, crossing diploids with tetraploids (2n x 4n), crossing tetraploids with diploids (4n x 2n), spontaneous triploids, endosperm culture, irradiation and somatic hybridization (Hasnain Raza, 2003). Naturally occurring seedless citrus varieties include Satsuma mandarin, Mukakukishiu and Navel orange. 'Natural selection' is a simple and easy way to analyze genetic variation in Kinnow cultivars based on various morphological and biochemical features (Altaf *et al*., 2014). Although Pakistani Kinnow is unmatched in its taste and quality, but it has not been able to gain its real export potential in the prime European markets due to existence of large number of seeds and is therefore marketed at a low price. European citrus market is captured by USA, Spain, New Zealand and Morocco while Pakistani Kinnow market is limited to Middle East, Iran, Saudi Arabia, Russia and Uzbekistan. International market demand for less seeded/seedless Kinnow is raising and is above 62%, but no European market is currently selling it, so Pakistan can snatch massive export share by promoting and exporting seedless Kinnow. This study was planned to evaluate the performance of a less seeded Kinnow strain (introduced through selection) versus conventional seeded Kinnow under climatic conditions of Sahiwal, Punjab. Traits for physical performance and fruit quality were compared with seeded Kinnow which may help to recommend the commercial cultivation of less seeded Kinnow. After exploring research venues for seedless kinnow, its massive production can attract many western markets for fresh consumption as well as for processing. Seedless kinnow cultivation can attract massive foreign exchange and can create novel markets globally. It can become the unique export item of Pakistan and can turn into the largest customer attraction.

Materials and Methods

The present study was planned to evaluate the performance of seeded and less seeded Kinnow at Horticultural Research Station, Sahiwal, Punjab. This research station is situated in the Punjab province of Pakistan at Latitude: 31°58′28″ N and longitude: 72°19′51″ E, with elevation around 171 m. Topography of Sahiwal is flat with maximum elevation alteration of 43 feet. The average maximum temperature of Sahiwal in summer is 40-45°C whereas average minimum temperature in winter goes down to 8°C with average rainfall of about 230-260mm. All selected plants were of the same age (ten years), grafted on rough lemon rootstock and planted in square system at 20 x 20 ft. Data was collected for three consecutive years i.e. 2018 to 2020 and then the average was calculated. Experimental trees were subjected to standard agricultural practices i.e. NPK @ 1,000-500-500g along with 40 kg FYM per plant. Nitrogen was applied in three split doses i.e. first dose during January, second at mid April and third at the end of July. Irrigation was applied with canal water. The experiment was laid out in RCBD with 10 replicates and two plants for each treatment [i.e. seeded (T_1) and seedless (T_2)], hence making a total of 40 plants. Hundred fruits and leaves (five fruits and five leaves from each plant) were harvested for each treatment to collect and analyze data.

Morphological features for trees, leaves and fruits were estimated by following the citrus descriptor evolved by International Plant Genetic Research Institute (IPGRI,1999). Traits including tree height (m), yield (kg/tree), fruit weight (g/plant), fruit length (cm), fruit breadth (cm), fruit axis diameter (mm), juice

percentage (%), peel percentage (%), number of viable seeds per fruit, number of aborted seeds, total soluble solids %, acidity % were measured with recommended indicators i.e. meter rod, digital weighing balance, digital vernier caliper, refrectometer and acidity meter. Fruit firmness (kg) was computed by Penetrometer (Model B059M-01). High performance liquid chromatography (HPLC) was used to measure glucose, fructose and sucrose. Data for all investigated traits was collected for consecutive three years and average was taken to perform the analysis. Statistical software "Statistix 8.1" was used for computing both Analysis of variance (ANOVA) and least significant difference (LSD) test. Differences among means were compared at $p \leq 0.05$ probability level. Digital caliper (Model: KBD-MT 0014, Cingda Industry China) with an accuracy of 0.01cm was used to measure fruit dimensions whereas fruit weight was recorded by digital weighing balance (Model UniB1C., SHIMADZU, U x 320g, Min.0.02g, e=0.01 g and d=0.001 g).

Results and discussion

Kinnow tree has a single large cylindrical trunk with branches diverging at a height of five feet above ground level. Branching gives somewhat spherical shape to the crown. Leaves are arranged spirally around the stem and a general phyllotaxy of 3/8 is observed. A comparison of specific morphological traits for the tree, leaf and fruit are listed in Table 1.

Table. 1 Morphological features for tree, leaf and fruit for seeded and less seeded Kinnow

Traits		Seeded Kinnow	Less Seeded Kinnow
Tree	Tree shape	Obloid	Ellipsoid
	Tree growth habit	Spreading	Erect
	Branching habit	Spreading	Erect
	Density of branches	Dense	Medium
Leaf	Leaf lamina shape	Ovate	Elliptic
	Leaf division	Simple	Simple
	Vegetative life cycle	Evergreen	Evergreen
	Leaf color variegation	Absent	Absent
	Leaf laminia attachment	Brevipetiolate	Brevipetiolate
	Petiole wing	Absent	Absent
	Leaf apex	Acute	Acute
	Leaf base	Obtuse	Obtuse
	Leaf color upper	Dark green	Light green
	Leaf color lower	Light green	Light green
	Leaf petiole color	Dark green	Light green
	Leaf margins	Acuminate	Acuminate
	Leaf texture	Medium	Soft
	Foliage density	Medium	Sparse
Fruit	Fruit shape	Spheroid	Spheroid/oblique
	Fruit base shape	Truncate	Truncate
	Fruit apex shape	Truncate	Truncate
	Fruit epicarp colour	Dark orange	Light to dark orange
	Fruit surface texture	Smooth	Slightly smooth
	Adherence of albedo to pulp	Week	Week
	Nature of oil glands	Conspicuous	Conspicuous
	Absence/presence of areola	Absent	Absent

Evaluation of seeded and less seeded Kinnow depicted significant and non significant differences in various traits. These variations might be induced due to their genetic differences (Bernier *et al.,* 1993). Average fruit weight (151.55g) was significantly higher in seeded Kinnow and lower in less seeded Kinnow (147.30g). No significant difference was noted for fruit length between seeded and less seeded Kinnow. Fruit breadth was higher (6.1cm) in seeded Kinnow as compared to less seeded Kinnow (5.7 cm). Diameter of fruit axis was higher (12.88mm) in less seeded Kinnow as compare to seeded Kinnow (9.39mm). Fruit firmness was higher (2.48Kg) in seeded kinnow as compare to less seeded kinnow (1.96Kg). Peel thickness was bigger (2.8cm) in less seeded Kinnow and lower in seeded Kinnow (2.1cm). Juice percentage was elevated (43.18%) in seeded Kinnow and lower (41.6%) in less seeded Kinnow. Peel percentage was more (22.6%) in less seeded Kinnow and less (19.6 %) in seeded Kinnow. Number of viable seeds (17) and number of aborted seeds (10) was surpassed in seeded Kinnow as compare to less seeded Kinnow (Table 2).

Table 2. Physical characteristics of seeded and less seeded Kinnow fruit

Treatments	Fruit weight (g)	Fruit length (cm)	Fruit breadth (cm)	Fruit axis diameter (mm)	Fruit firmness (Kg)	Peel Thickness (mm)	Juice %	Peel %	Number of Viable Seeds	Number of Aborted Seeds
Seeded Kinnow	151.55a	6.4a	6.1a	9.39b	2.48a	2.1b	43.18a	19.6b	17a	10a
Less Seeded Kinnow	147.30b	6.3a	5.7b	12.88a	1.96b	2.8a	41.6b	22.6a	3b	5b

Values with same alphabetic letter are not statistically significant; values with different alphabets are statistically significant at $p \leq 0.05$.

Both seeded and less seeded Kinnow had significant variation in their vegetative performance. Seeded Kinnow trees had more height (4.1 m) as compare to less seeded Kinnow trees (3.3m) (Figure 1). Fruit yield was also significantly higher (524.9 kg/tree) in seeded trees as compared to less seeded trees (511.5 kg/tree) (Figure 2).

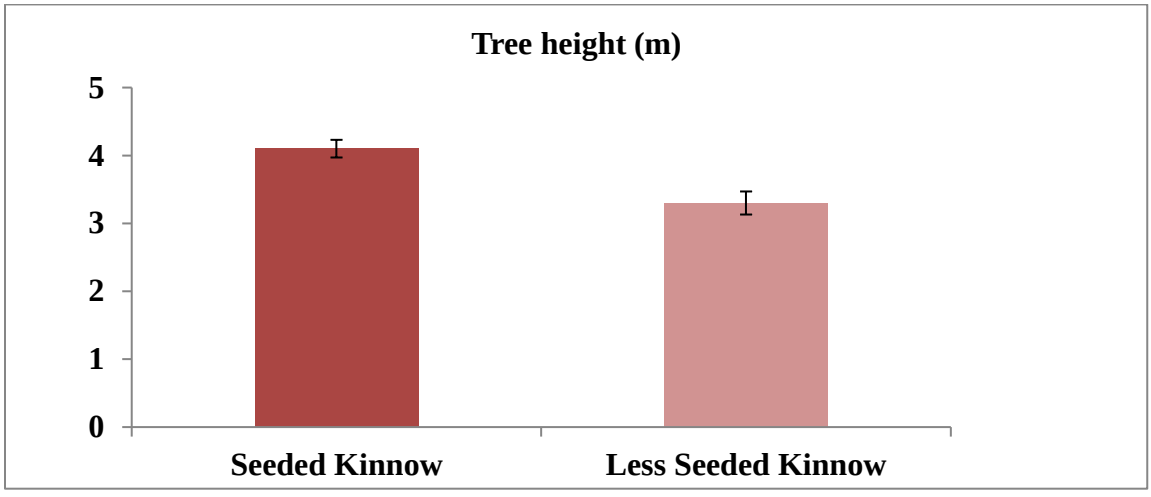

Figure 1. Response of seeded and less seeded Kinnow varieties for tree height (m) under Sahiwal climatic conditions.

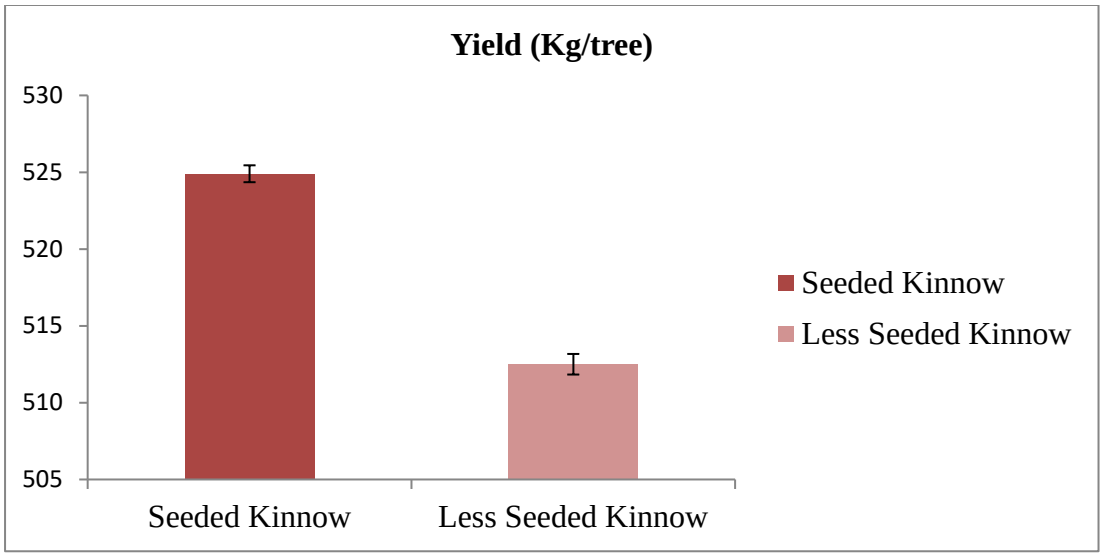

Figure 2. Response of seeded and less seeded Kinnow varieties for yield (kg/tree) under Sahiwal climatic conditions.

Table 3. Biochemical characteristics of seeded and less seeded Kinnow fruit

Treatments	Total soluble solids (TSS) %	Titrateable acidity %	Vitamin-C (mg/100g)	Glucose (g/L)	Fructose (g/L)	Sucrose (g/L)
Seeded Kinnow	11.4a	0.65a	18.56a	48.08a	25.07a	4.56a
Less Seeded Kinnow	9.8 b	0.63a	18.08a	43.32b	19.6b	6.34b

Values with same alphabets are not statistically significant; values with different alphabets are statistically significant at $p \leq 0.05$.

Similar studies with varied fruit and seed quantitative (fruit length, fruit width, fruit weight and seed number) characters were reported by Khan *et al.* (1992) for seeded and seedless Kinnow. The findings are also parallel to Gillaspy (1993) and Nitsch (1970) who described that developing embryos in seeds trigger the speed of cell division in surrounding tissues.

These results are in accordance with the findings of Altaf *et al*. (2008) who investigated those different strains of Kinnow had a higher level of variation with respect to their fruit weight, length, and breadth. Differences in peel thickness were also observed by Singh and Singh (2004) who measured high peel thickness (4.59 mm) for seedless Kinnow along with elevated peel weight in different Kinnow strains. In our findings, number of viable seeds and aborted seeds were more in seeded Kinnow as compared to less seeded Kinnow. Altaf *et al.* (2008; 2009) and Fatima *et al.* (2010) also counted more number of seeds (healthy and aborted) in seeded Kinnow as compare to less seeded kinnow. The results for fruit firmness are verified by the findings of Khalid *et al.* (2018), who concluded that a greater level of variation exists in Kinnow mandarin with fruit firmness varying from 2.1 kg to 2.3 kg.

TSS was higher (11.4%) in seeded Kinnow and lower (9.8 %) in less seeded Kinnow. No significant differences existed for fruit acidity percentage. Differences in titrateable acidity % and Vitamin-C contents (mg/100g) were non-

significant among seeded and less seeded Kinnow strains. Variation was also observed for glucose, fructose and sucrose contents between seeded and less seeded Kinnow strains and it was significantly higher in seeded Kinnow i.e. 48.08 g/L glucose, 25.07 g/L fructose and 4.56 sucrose while lower in less seeded kinnow i.e. 43.32 glucose, 19.6 fructose and 6.34 sucrose (Table 3)

Altaf *et al*. (2014) reported no significant differences in the biochemical parameters of seeded and seedless Kinnow. These results are supported by the findings of Altaf *et al.* (2008) who concluded that A greater level of variation existed in studied seeded Kinnow mandarin strains. These results were in accordance with the findings of Altaf *et al*. (2008) who also reported that a greater level of variation i.e. 9.7° to 11.00°Brix in studied Kinnow strains. These results were further verified 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 are inducted might be 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. Similar results were also reported by Altaf and Khan (2009) with titrateable acidity ranging from 0.7% to 1.0% in Kinnow mandarin. These results were in accordance 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 67 mg/100g to 56.8 mg/100g in studied Kinnow strains. Our results were supported by the findings of Qureshi *et al.* (2021), who evaluated sugar contents glucose (6.5 g/L), fructose (20.2 g/L) and sucrose (37.9 g/L) of Kinnow mandarin on different rootstocks.

Conclusion

Diversification of Pakistan citrus industry with less seeded Kinnow strains will be highly valuable to boost the trade. The less seeded strain evaluated in this study showed good physico-chemical attributes. It was governed with non-significant titrateable acidity, vitamin C and fruit weight versus seeded Kinnow strain. Results also showed that juice obtained from seeded strain is of good quality and could be valuable source of health promoting constituents. However, many investigated features were better for the seeded Kinnow strain and detailed research studies are required for future recommendations of less seeded Kinnow.

References

Altaf, N., & Khan A.R. (2009). Growth and development of low seeded Kinnow mandarin fruit in dense plantation. Journal of Agricultural Science and Technology,**11**:191-198.

Altaf, N., & Iqbal, M.M. (2003). Towards a Seedless Cultivar of Kinnow Mandarin III. Variability of Developed and Undeveloped Seed Number in Seedless/Low Seeded Fruits. Pakistan Journal of Botany,**35**: 339-342.

Altaf, N., Khan A.R., & Hussain, J.(2008). Fruit variability in Kinnow mandarin. Pakistan Journal of Botany, **40**:599-604.

Altaf, S., Khan, M.M., Jaskani, M.J., Khan, I.A., Usman, M., Sadia, B., Awan, F.S., Ali A., & A.I. Khan. (2014). Morphogenetic characterization of seeded and seedless varieties of Kinnow Mandarin (*Citrus reticulata* Blanco). Australian Journal of Crop Sciences, **8:**1542-1549.

Bernier, G., Havelange, A., Houssa, C., Petitjean, A.& Lejeune, P. (1993). Physiological signals that induce flowering. Plant Cell, **5**: 1147-1155.

FAO, 2019. Food and agricultural organization, Available online with updates at http://www.fao.org/faostat/en/#data/QC

Fatima, B., Usman, M., Khan, I.A., Khan, M.S., & Khan, M.M. (2010). Exploring citrus cultivars for underdeveloped and shriveled seeds: A valuable resource for spontaneous polyploidy. Pakistan Journal of Botany,**42**: 189-200.

Frost, H.B. (1935). Four new citrus varieties-the Kara, Kinnow and Wilking mandarins and the Trovita

orange. University of California, Agricultural Experimental Station Bulletin, 597.

Ghani, A., Ikram, M., Hussain, M., Ahmad, I., & Iftikhar, M., (2016). Comparative Analysis of Ascorbic Acid Concentration in Citrus Reticulata (Kinnow) Collected From Different Tehsils of Sargodha. Indian Journal of Fundamental and Applied Sciences, **6**: 43-47.

Gillaspy, G. (1993). Fruits: a developmental perspective. Plant Cell, **5**:1439-1451.

Hayat, F., Khan, M.N., Zafar, S.A., Balal, R.M., Nawaz, M.A., Malik, A.U., & Saleem, B.A. (2017). Surface coating and modified atmosphere packaging enhances storage life and quality of 'Kaghzi lime'. Journal of Agricultural Science and Technology, **7**:1151-1160.

Hussain, I., Asif, M., Ahmed, M., Khan, M., & Shakir, I. (2004). Effect of Uni-Packaging on the Post Harvest Behavior of Citrus Fruits in N.W.F.P. Pakistan Journal of Nutrition **3**:336-339.

IPGRI. 1999. Descriptors for Citrus. International Plant Genetic Resources Institute, Rome, Italy.

Jaskani, M.J., Kwon, S.W., & Kim, D.H. (2005). Comparative study on vegetative, reproductive and qualitative traits of seven diploid and tetraploid watermelon lines. Euphytica, **145**: 259-268.

Khalid, S., Malik, A.U., Singh, Z., Ullah, S., Saleem, B.A., & Malik, O.H. (2018). Tree age influences nutritional, pectin, and anatomical changes in developing 'Kinnow' mandarin (*Citrus nobilis* Lour× *Citrus deliciosa* Tenora) fruit. Journal of Plant Nutrition, **41**:1786-1797.

Khan, I.A. (1992). "The Kinnow Culture." In Proceedings of the first international seminar on citriculture in Pakistan, pp. 2-5.

Nitsch, J.P. (1970). Hormonal factors in growth development. In: Hulme AC (ed). The biotechnology of fruits and their products. Vol. 2. Academic press, London, p. 427-472.

Qureshi, M.A., Jaskani, M.J., Khan, A.S., Haider, M.S., Shafqat, W., Asif, M., & A. Mehmood. (2021). Influence of different rootstocks on physico-chemical quality attributes of Kinnow Mandarin. Pakistan Journal of Agriculture Sciences, **7**:346-365.

Raza, H., Khan, M.M., & Khan, A.A. (2003). Seeedlessness in citrus (review). International Journal of Agriculture and Biology,**7**:1560-8530.

Riaz, M., Zamir, T., Rashid, N., Jamil, N., Masood, Z., Jabeen, U., Mandokhel, F., Behlil, F., Mengal, F., & 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. American-Eurasian Journal of Toxicology and Science, 7:203-208.

Shahid, A.K., Abdus, S., & Roshan, Z. (2016). Development of sparse-seeded mutant kinnow (*Citrus reticulata* Blanco) through budwood irradiation. African Journal of Biotechnology. **10**:14562-14565.

Sharma, J.N., & Thind, S.K. (2005). An approach for the establishment of seedlessness in Kinnow mandarin. Indian Journal of Horticulture,**62**: 8-11.

Singh, S.K. (2018). Characterization of Kinnow mandarin fruit juice stored under incubator. Annals of Biology,**31**: 126-129.

Singh, I.P., & Singh, S. (2004). Evaluation of seedless cultivar of mandarin (Citrus species) for export. Indian Journal of Agricultural Sciences, **74**:376-378.

Treeby, M.T., Henriod, R.E., Bevington, K.B., Milne, D.J., & Storey, R. (2007). Irrigation management and rootstock effects on navel orange [*Citrus sinensis* (L.) Osbeck] fruit quality. Journal of Agriculture and Water Management, **91**: 24-32.

Usman, M., Fatima, B., Gillani, K.A., Khan, M.S., & Khan, M.M. (2008). Exploitation of potential target tissues to develop polyploids in citrus. Pakistan Journal of Botany, **40**: 1755-1766.

Wu, G.A., Terol, J., Ibanez, V., López-García, A., Pérez-Román, E., Borredá, C., Domingo, C., Tadeo,. FR., Carbonell-Caballero, J., Alonso, R., & Curk, F. (2018). Genomics of the origin and evolution of Citrus. Nature, **554**: 311-316.