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Growth and Development of Low Seeded Kinnow Mandarin Fruits in Dense Plantation

N. Altaf[1*], and A. R. Khan[1]

ABSTRACT

The work described here is unique since the low seeded (0-6 seeds per fruit) Kinnow (*Citrus reticulata*) germplasm was grown in dense plantation (P×P: 1foot and L×L: 2 feet) in the NIAB (Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan) field. Growth and development patterns of the fruits were studied. The low seeded germplasm contains 3,500 plants developed through natural and induced variability in Kinnow mandarin. The area is surrounded by other cross compatible *Citrus* varieties. The germplasm flower was hermaphrodite, off-white, the color of the anthers yellow, and the number of petals was five and all other flower characteristics were variable. In 2005 the fruiting was in 596 plants and the maximum fruit range was 21-40 in 99 plants followed by 41 to 75 in 98 plants. In 2006, 322 plants bore fruit and 1-5 fruits per plant were found in 93 plants followed by 6-10 fruits per plant in 61 plants. In 2007, 551 plants had first time bearing and the maximum fruit range per plant was 11-20 in 116 plants followed by 21-30 in 99 plants. It was found that the Kinnow fruit gains maximum weight during August-October whilst the volume of the fruit increases during August-November. Initially the height and diameter were identical in May giving the fruit a round shape and then the gradual increase in height slowed down, resulting in an oblate to subglobose shape of the fruit. In mid-January the average fruit weight was 192 grams and the average peel weight was 45 grams. Peel thickness was more at stalk end as compared to stylar end. All low seeded fruits had their navel at the stylar opening point. The maximum number of fruits had 11 segments but the segment range was 9-14 per fruit. In mid-January the juice pH, juice acidity and TSS had variable values in plants indicating variation in the fruit physiological maturity time within the germplasm. The growth of fruits was similar to that reported in low density orchards.

Keywords: Carpel polymorphism, Fruit shape, Growth of fruit, Kinnow germplasm, Low seeded.

INTRODUCTION

Citrus has an Indo-Chinese origin and was first mentioned in Chinese literature in 2200 BC. The entire scenario of Pakistani fruit production is dominated by *Citrus* both in terms of area and production, where Kinnow shares more than 60% of citriculture followed by oranges of which Mosambi is the leading variety, other mandarins among which Feuterell's early dominates and include limes, lemons and grapefruits. During 2006, the area under *Citrus* cultivation was 192.3 thousand hectares and production remained at 2,458.4 thousand tones (Anonymous, 2006). During 2006 Pakistan made the highest ever export of 200,000 tones of Kinnow to a number of countries (Syed, 2007). Kinnow has extreme variabilities in fruit quality and the seed number per fruit ranges from 0-54 in the cultivar. This is because the cultivar has mostly polyembryonic strains which have tendency to form apomictic seeds. The seedless/low seeded trait is found in the orchards of Punjab, Pakistan (Altaf and Iqbal, 2003). A low seed content tendency (0-10 seeds/fruit) also existed in a few trees in the surveys of Kinnow orchards

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(720.4 ha) in the Ferozepur region of Punjab, India (Sharma and Thind, 2005). Low seed content is highly desirable in fresh *Citrus* fruit like Kinnow. Overall this study is aimed at the selection of a low seeded/seedless Kinnow germplasm. The specific objective of this work was to study the growth and development of Kinnow fruits grown in dense plantations. The work is of a unique kind as fruit growth in seedless/low seeded strains of Kinnow mandarin in dense plantations have not been studied so far.

MATERIALS AND METHODS

Natural and induced variability was used to obtain the seedless/low seeded strain. The plants were developed by (i) natural selection of the low seeded trait from orchards in Punjab (ii) nucellar callus (iii) nucellus exposure to gamma radiation (30-120 Gray) prior to embryogenesis (iv) nucellus cultured on adenine sulphate (2-40 mg l^{-1}) and (v) soaking Kinnow seeds in 8-hydroxy quinoline (10-50 mg l^{-1} for 2-48 hours). It was a large experimental Kinnow germplasm (3,500 plants) and was studied in a dense plantation (P×P: 1foot and L×L: 2 feet) at the NIAB fields. There had been a mixed *Citrus* plantation such as grapefruits, oranges, lemons and other mandarins etc. around the Kinnow germplasm area that may provide unwanted foreign pollens for seed formation in Kinnow. Flowering and fruiting in the years 2005-2007 were studied. Undesirable material such as plants with seedy fruits, with rootstock incompatibility, plants with abscission character [fruit and leaf drop], and those with heat sensitivity etc. were continuously removed. The flowering characteristics of the Kinnow germplasm in the NIAB field were studied during the period 2005 to 2007 and the range of flower characteristics is given in Table 1. The flowering started in mid-February and the period lasted up to the first week of April. The number of fruits produced during 2005-2007 were recorded. The monthly weight and volume of the 100 fruits from the germplasm were recorded and average weight and volume were calculated. The height and diameter of these 100 fruits were noted and the average height and diameter of monthly growth was also recorded. Fruit diameter was measured at the equator of each fruit while height was measured between the stem and stylar end. From these values fruit shape can be assessed. The overall characteristics of 1,000 fruits in mid-January 2006 and 2007 were recorded. Peel thickness was measured with a vernier caliper by cutting the fruit into two halves at the equator and the peel thickness was calculated. The carpel polymorphism was also noted. The extracted juice was filtered through a muslin cloth to remove the aborted seeds and a few drops of the juice were put on the hand refrectometer. The reading was noted and was considered as Brix. For juice pH, the pH meter was calibrated with buffers and the pH of the juice was noted. For total acidity, five mls was taken from the filtered juice and, after adding two drops of phenolphthalein indicator, was titrated against 0.1N NaOH solution. The amount of total acids was expressed as gram citric acid/100mL of fruit juice.

RESULTS AND DISCUSSION

The low seeded plants were selected and flower and fruit characteristics were studied. Although, these plants were growing in dense plantings, the flower characteristics of the Kinnow germplasm were quite normal (Table 1). Cool dry periods of winter stimulate floral induction in Kinnow plants. There are slight differences in length of the stamens and stigma as the later is slightly larger in Kinnow plants without affecting fertility. In sterile Satsuma hybrids the stamen length was half of the style length (Ollitrault *et al.*, 2007) in addition to other abnormalities. Some flower characteristics are common in all Kinnow mandarin- for example, the flower type is always hermaphrodite, the color of the open flower is off-white, the color of the anther is yellow and the number

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Table 1. Flower Characteristics of Kinnow mandarin.

Sr.#	Character	Range
1	Pedicel length	2.5-3.0 (mm)
2	Calyx diameter	2.4-2.8 (cm)
3	Length of stamen	0.8-1.0 (cm)
4	Length of style+ovary	0.7-1.0 (cm)
5	Length of style+stigma	5-7 (mm)
6	Flower type	Hermaphrodite
7	Color of open flower	Off white
8	Color of anther	Yellow
9	No. of stamens	15-19
10	No. of petals	05
11	Petal length	1-1.5 (cm)
12	Petal width	5-6 (mm)

of petals is always five. All other flower characteristics have some variability.

The fruits produced in the Kinnow germ plasm are shown in Table 2 for the years 2005-2007. Since every year seedy plants were eliminated from the field, the germplasm was left with low seeded plants with more space in between. The embryonic plants normally have 1-10 fruits in the first year of bearing which subsequently increase in the following years. The seedy plants were producing more fruits compared to low seeded plants, perhaps the seed formation helps in a better fruit set. The fruit development behavior was quite normal looking, in a dense plantation where canopy is limited. In a well spaced orchard plantation the canopy is larger, and so is the yield. The maximum yield of mature Kinnow trees (aged above 10 years) is more than 4 tons acre^{-1} in the Sargodha district orchards of Pakistan (Ishfaq *et al*., 2004). The heavy crop often causes the complete inhibition of vegetative growth, depletion of nutritional sources and induction of alternate bearing. There should be an optimum number of fruits per plant in a germplasm, as more than fifty fruits is not good for plant health in dense plantation. The fruit setting rate in *Citrus* is a characteristic strongly influenced by the environment, and may also show variations depending upon the parents used (Filho *et al.*, 1995).

The monthly fruit growth was taken as weight of fruit in grams (Figure 1) and volume of fruit as milliliters (Figure 2). Both the weight and volume rapidly increased up to November and there was slight comparative increase until February which is the normal physiological maturity period of Kinnow. The height and diameter of the fruit

Table 2. Fruits produced in Kinnow germplasm.

Years 2005	2006	2007
# Fruits in (plants)	#Fruits in (plants)	#Fruits in (plants)
01(22)	1-5(93)	1-5(35)
02(42)	6-10(61)	6-10(56)
3-4(37)	11-15(42)	11-20(116)
5-7(69)	16-20(43)	21-30(99)
8-12(74)	21-25(27)	31-40(82)
13-20(59)	26-30(13)	41-50(54)
21-40(99)	31-40(17)	51-60(38)
41-75(98)	41-50(12)	61-70(33)
76-100(31)	51-70(07)	71-80(18)
>101(65)	>70 (07)	>81 (20)
Total Fruits(596)	(322)	(551)

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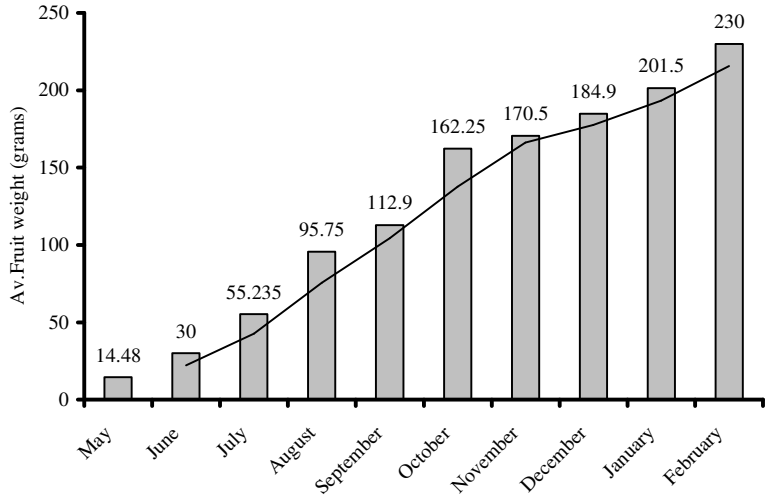

Figure 1. Weight gain in Kinnow Fruit.

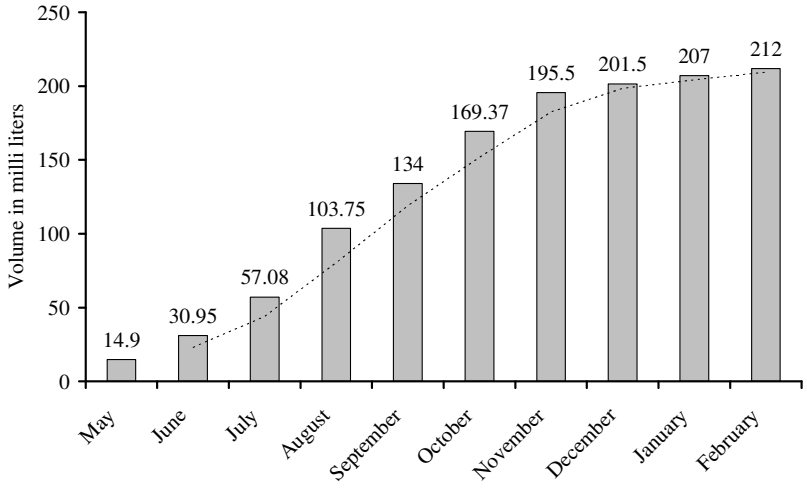

Figure 2. Volume gain in Kinnow fruit.

were also recorded in centimeters (Figure 3). In May, the height and diameter were equal, giving Kinnow fruit a round shape. Then, gradually, the increase in equatorial diameter was greater than the longitudinal diameter, giving Kinnow a mandarin shape. The bio-mass gain in fruits is rapid until October-November and there is slow growth onwards up to February. The fruit of the hybrids is oblate to subglobose and usually presents an open styler disrupted by a navel formed by the floral meristem, which is conserved near the apex of the fruit. In the non-prone-to-split Clementines as well as in Owari Satsuma, the floral axis protrudes inside the style, and the flower meristem is lost through abscission shortly after petal fall. The abaxial side of the carpels fuses with the flower axis forming at the styler end of the fruit, a solid tissue, which externally has a small scar at the point of the style abscission. Most fruits in these cultivars have no navel (Garcia-Luis *et al.*, 2001) since navel formation is influenced by cul-

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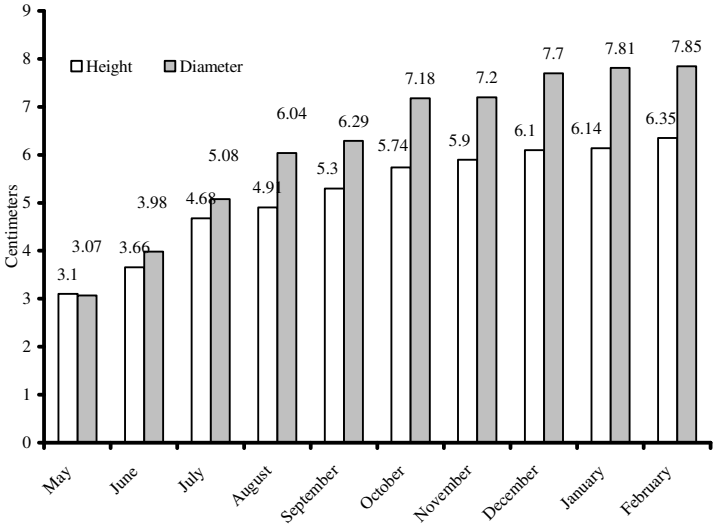
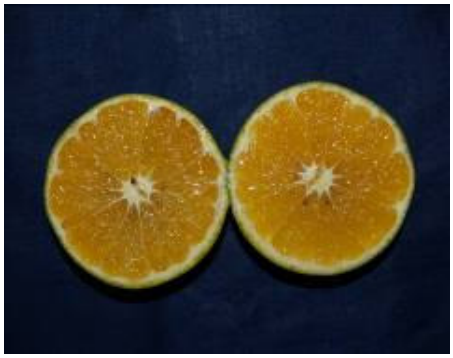


Figure 3. Height and diameter of fruit

tural practices and the environment. Fruit height and diameter determine the shape of the fruit. Low seeded fruits in Kinnow mandarin varied marginally in shape, had a prominent central hollow core and a few seeds (Sharma and Thind, 2005).

Kinnow fruit is the ripened ovary with a cover of rind, inside which is juicy pulp and sweet flesh. The fruit characteristics were studied in mid-January and the average figures are noted in Table 3. In Kinnow mandarin, the peel weight is higher compared with other mandarins as also is the peel thickness (Singh and Singh, 2004). Kinnow mandarin has peel thickness inherited from the parental cultivar King. The maximum peel thickness is 2.9 mm at the stalk end and the minimum (2.47 mm) is at the styler end. The navel exhibits characteristics of fruits which have stylar splitting due to the hybrid nature of the plant's the navel is the stylar opening point. In the low seeded Kinnow germplasm, all the fruits have a navel with an average of 2.73 mm. The segment size very much depends on fruit weight- the larger the fruit, the larger the segment and the number of segments per fruit. Segments are the most important part of the fruit as it contains mesocarp or pulp which is the edible portion of the fruit.



September Fruit

January Fruit

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Table 3. Fruit characteristics in mid-January.

Sr.#	Fruit Characteristics	Magnitude
1	Fruit Weight	191.70 gm
2	Peel Weight	44.70 gm
3	F Wt/P Weight	4.34
4	Segment Weight	14.10 gm
5	Segment Length	5.25 cm
6	Segment Breadth	2.68 cm
7	Segment L/B	1.97
8	Fruit Weight/Segment Wt	13.80
9	Peel Thickness at Styler end	2.47 mm
10	Peel Thickness at Centre end	2.79 mm
11	Peel Thickness at Stalk end	2.90 mm
12	Navel Diameter	2.73 mm

Citrus fruit is a berry known as hesperidium, which is divided internally into segments; a segment is a piece contained by two walls. Segments are also referred to as locules or fused carpels. Each *Citrus* species and cultivar in the Aurantioideae subfamily exhibits a distinct range of carpel (segment) numbers within their fruits. The observed range of segments for fruit within any species or cultivar can be influenced by branch location on a tree. The carpel number for all fruits produced on a single tree may have a symmetrical, unimodal distribution as well as a positively or negatively skewed unimodal distributation (Tisserat *et al.*, 1990). Fruit geographical orientation on the tree does not affect the segment number. Abscission of fruit does not select for any particular segment number. Crossing parents with dissimilar fruit segment numbers produces progeny with a range of segment numbers (Tisserat *et al*., 1990). Carpel polymorphism in Kinnow fruit was studied in 1,010 fruits. The segment range was 9-14 (Table 4). The majority of the fruits had 11 segments followed by 10 and 12 segments. However, 9 and 14 segments were rare and the maximum number of segments per fruit was 12 in Kinnow in *Citrus* cultivars studied under the mid hills altitude of Meghalaya (Singh and Govind, 2002).

Fruit maturity was studied in mid January as juice pH, acidity by titration against NaOH and Brix using a refrectometer. The results are given in Table 5 which clearly

demonstrates that the germplasm has different maturity times. Molecular and genetic analysis of fruit development and, especially, the ripening of fleshy fruits indicated that ethylene bio-synthesis and its response, cell wall metabolism and environmental factors, all have an impact on ripening (Giovannoni, 2001). The relationship between the ratios of the transverse diameter to the longitudinal (D/L ratio) and titerable acidity in the axial direction was determined at harvest in 14 *Citrus* cultivars and species in Japan with different fruit shapes: Nichinan No.1, Okitsu, Nankan No.20, Kuno, Aoshima satsuma mandarin, Miyauchi, Iyo Amakusa, Yoshida, Ponkan Shiranui, Valencia, Delta orange, Marsh grapefruit, lemon and Hebezu. The D/L ratio was positively correlated with the

Table 4. Carpel polymorphism in Kinnow fruits.

Sr.#	No. of fruits	No. of segments
1	01	09
2	351	10
3	572	11
4	83	12
5	02	13
6	01	14
Total Fruits Studied	1010	Segment Range: 9-14

Table 5. Fruit maturity in mid-January.

Sr. #	Juice Characteristics	Range
1	Juice pH	3.5-4.3
2	Juice Acidity	0.7-1
3	Brix.	8.5-14.5

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mid-section/stylar end ratio of acid content (r= 0.76). It seems that the more depressed globular the fruit shape, the higher the acidity at the mid-section. Acidity decreased with foliar application of Zinc, alone and in combination with Fe and Mn in comparison to the control in Kinnow mandarin (Monga and Josan, 2000), which indicated that proper plant nutrition can improve Kinnow fruit quality. Fruit production, seediness and sugar content of 'Ellenor' mandarin can be improved by interplanting with 'Murcott' (Wallace and Lee, 1999). This concludes that a cross planting plan with suitable *Citrus* cultivars can improve the yield and sugar contents of fruit.

CONCLUSION

It is concluded that dense plantation fruits were similar to those in normal plantations as mentioned by Ishfaq *et al*. (2004). In general, 200 plants of Kinnow mandarin are grown per hectare. This number of plants per hectare can be enhanced without affecting the fruit quality but increasing the average yield by several times.

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رشد و نمو میوه‌های کم بذر نارنگی رقم Kinnow در کشت متراکم

ن. الطاف و ا. ر. خان

چکیده

این بررسی برای اولین‌بار روی نارنگی رقم کینو (*Citrus retieulata* var: Kinnow) که ژرم پلاسم آن به‌صورت کشت متراکم (P*P: 1 foot, L*L: 2foot) در مزرعه انستیتوی اتمی ویژه کشاورزی و بیولوژی فیصل آباد پاکستان کاشته شده بود، اجرا شد. در این بررسی الگوی رشد و نمو میوه‌ها مورد مطالعه قرار گرفت. ژرم پلاسم نارنگی کم بذر رقم کینو شامل ۳۵۰۰ نهال معمولی و متغیرهای القاء شده بود. منطقه مورد مطالعه به‌وسیله ارقام سازگار Citrus احاطه شده بود. گلهای ژرم پلاسم هرمافرودیت، سفید رنگ، بساک زرد رنگ و تعداد گلبرگ‌ها پنج عدد بود، در صورتی‌که سایر خصوصیات گل متفاوت بود. در سال ۲۰۰۵ میوه‌دهی در ۵۹۶ درخت که حداکثر میوه‌دهی معادل ۴۰-۲۱ میوه در ۹۹ درخت و ۷۵-۴۱ میوه در ۹۸ درخت بود ثبت شد. در سال ۲۰۰۶ تعداد ۳۲۲ درخت به بار نشستند و بازای هر درخت ۳-۱ میوه در ۹۳ درخت و ۱۰-۶ میوه بازای هر درخت در ۶۱ درخت ثبت شد. در سال ۲۰۰۷ تعداد ۵۵۱ درخت برای اولین‌بار میوه دادند و در ۱۱۶ درخت و بازای هر درخت ۲۰-۱۱ میوه و در ۹۹ درخت بازای هر درخت ۳۰-۲۱ میوه شمارش شد. بررسی‌ها نشان داد که در نارنگی رقم کینو حداکثر وزن میوه در طی ماههای اوگوست و اکتبر حاصل می‌شود در حالی‌که حجم میوه در طول ماههای اوگوست و نوامبر افزایش می‌یابد. در ابتدای بررسیها طول و قطر میوه در طول ماههای اوگوست و نوامبر افزایش می‌یابد. در ابتدای بررسی‌ها طول و قطر میوه‌ها یکسان و در ماه مه شکل میوه گرد بوده و پس از آن بتدریج این ویژگی افزایش یافت در صورتی‌که وزن میوه کاهش نشان داد که منجر به پهن شدگی و گردشدگی میوه گردید. در اواسط ژون وزن میوه ۱۹۲ گرم و متوسط وزن پوست میوه ۴۵ گرم بود. ضخامت پوست میوه در منطقه دم میوه در مقایسه با بخش منطقه خامه میوه بیشتر بود. کلیه میوه‌های کم بذر در منطقه خامه شکل نامنظم داشتند. حداکثر میوه‌ها ۱۱ برچه‌ای بوده و دامنه تغییرات برچه‌ها بین ۱۴-۹ برچه بازای هر میوه مشاهده شد. در اواسط جون pH و اسیدیته افشره میوه و همچنین TSS میوه بیشتر بود که بیانگر تغییرات فیزیولوژیکی زمان رسیدگی میوه در داخل ژرم پلاسم می‌باشد. رشد میوه‌ها مشابه میوه باغات کم تراکم گزارش شده است.

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