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A NEW KINNOW STRAIN WITH 0-4 SEEDS PER FRUIT

Nafees Altaf
Nuclear Institute for Agriculture & Biology (NIAB), Faisalabad
Email: nafeesdcs@yahoo.com

Abstract

The objective of the study was to know how natural variations in Kinnow mandarin can contribute to seedless or very low-seed number per fruit. Variability was observed in fruits of Kinnow and careful selections of new improved superior clones were identified for low-seeded/seedless fruit trait. Most of the mutations in Kinnow resulted in deterioration of fruit quality. Many fruit forms may have arisen from natural mutant branches. Seedless trait was cloned by sprout/shoot apex and embryo grafts. Presence of leaf abscission in 50% sprouts and in embryos indicated that the trait is of genetic origin as the embryos are derived from cell lines present in the vegetative tissues. Two percent sprouts have leaf retention under graft stress. The clones retained stability of selected seedless trait and destroy their seeds early in development. These clones produce seedless fruits by undergoing post fertilization embryo abortion. The plants were also developed by nucellar regenerations with and without callusing, gamma radiation (30-120 gray) exposure to nucellus prior to embryogenesis, nucellus cultured on Adenine Sulfate (2-40 mg l^{-1}), by soaking Kinnow seeds in 8 hydroxy quinoline (10-50 mg l^{-1}) for 2-48 hours and small and large size seed culture from low seeded fruits. Radiation of Kinnow buds to 1-10 Kr gamma radiation. The seedless trait was found as was present in nature. It was not the result of a particular experiment. In all, 5 monoembryonic plants with 0-4 seeded trait were selected. Seedy plants were eliminated from field. The seedless trait is because of mono embryony and post fertilization ovule degeneration which can be observed as empty seed coats in fruits. Around 20% pollen germination was noted in culture medium. These plants require GA_3 spray for fruit set in self pollination. The seeds were 0-3 per fruit under self pollination.

DESCRIPTION OF DEVELOPMENT OF KINNOW STRAIN

Citrus is the major fruit of Pakistan with Kinnow mandarin as dominant cultivar because the plant has heat tolerance and adaptable to climate and soil conditions of Punjab province. Citrus area in Punjab during 2005-06 was 182.1 thousand hectares with 2385.1 thousand tonnes production (MINFAL, 2005-06). During the year 2006 Pakistan made the highest ever export of around 200,000 tonnes of Kinnow to a number of countries including Middle East and the South East Asia (Syed, 2007). Kinnow is highly variable in all characters. The seed number per fruit is 0-54. Natural parthenocarpy is lacking in Kinnow mandarin as there was no branch per tree in orchards with all seedless fruits. The seedless/low seeded fruits had some aborted, under

developed ovules (Altaf & Iqbal, 2003) which mean post fertilization ovule abortion. The developing ovules provide Gibberellins which is necessary for ovary to develop into fruit (Ben Chiekh et al., 1997). The trees with low seeded trait (0-10/fruit) were also found in Indian Kinnow orchards (Sharma & Thind, 2005). The embryony status of Kinnow plants is variable i.e. can be mono or poly embryonic. The later is dominant as it usually has heat tolerance and hence survives in hot seasons of Punjab. Polyembryonic plants form apomictic seeds. These are parental characters as Willow leaf is monoembryonic and King is poly embryonic, may be phenomenon of cellular endo-reduplication and chromosome shedding is working in Kinnow population. Kinnow is liked by its growers for its juice and good fruit yield, often borne in clusters. Fruits are good for fresh squeezed juice in winter. The objective of the study was the development of quality seedless Kinnow fruit plants from the exiting variability in orchards, preferably seedless, easy peel, juicy and sweet fruits with seasonality.

Kinnow plants were developed through natural selections of seedless/low seeded trait, nucellar embryogenesis with and without callusing, immature green fruit exposure to 3-12 Kr gamma radiation and then its nucellus culture, seed germination after soaking in 8-hydroxy quinoline, seed culture of low seeded fruits, culture of developed and underdeveloped seeds. Radiation of Kinnow buds 1-10 Kr of Gamma radiation (Altaf et al., 2004). All the Kinnow materials obtained from above experiments were grafted on six months to one year old rough lemon seedlings.

The initial Kinnow germplasm in field comprised of 2500 plants, planted at plant to plant 1 ft and row to row 3ft distance. The initial removal from field was of 693 plants because of poor growth responses, necrosis in twigs, heat sensitivity, tree breakage during violent wind storm, defoliation, curling and yellowing of leaves, root stock failure etc. A total of 671 plants were eliminated from the field because of seedy fruits during 2005-2007. There are 1136 plants in field. Five strains were selected out of 386 low seeded plants in 2004-07. One plant was selected out of 57 plants in 2006-07 which need further confirmation in next fruiting season as this was its first fruiting year. So far we have studied fruits of 443 low seeded plants. This year 485 new plants have flowering in February-March, 2007. A total of 208 plants are still in juvenile phase. The five selected strains have normal flowers. The rate of pollen germination is 18-20% in *in vitro* conditions. Fruit set in Kinnow strains is different in self (8%) and in open pollinations (23%)The seedless/low seeded trait was similar as found in natural existing variability.

After fertilization, the embryo, endosperm, integuments and carpel develop. Endogenous gibberellins play a role in regulating expansion and promoting cell divisions in carpels in citrus. Fruit is the ripened ovary and seed is the developed ovule. The developing seeds produce gibberellins that help in development of fruit in citrus (Talon et al., 1990) and (Guardiola et al., 1993). Ovule sterility results in embryo abortion. Ovule sterility may be because of defective embryo sac development. The defective ovules are because of sterility genes. The occurrence of empty seed coats in Kinnow fruits suggests embryo abortion. Early embryo abortion is preferred or low level of fertile ovules is required in fruits. Sometimes aborted ovules inside the mature fruit appear like tiny black dots. Embryo may fail to develop resulting in sterile ovules especially in monoembryonic plants. The polyembryonic plants can have apomictic seeds.

Five strains were selected in 2004-07 on the basis of 0-6 seeded trait with good quality fruit. One plant consistently had 0-4 seeds for three years. In 2006-07, one more plant was selected with 50% seedless fruits which need further confirmation as this was its first fruiting year.

The fruit maturity time ranges from December to March in the selected strains. The fruits should be clipped off from the tree to avoid post harvest infection as the stem areas tear easily with hand picking. Fruit is at its peak for 4-6 weeks when skin has fully colored. External color of fruit is orange just like Kinnow. The rind texture is slightly pitted. The rind is quite easy to peel when fruits are mature, can be adherent when fruits are immature. The cultivar Kinnow mandarin has variability in fruit shape, size and colour. The fruit shape of selected strains is similar to Kinnow. The fruit is oblate. The flesh is bright orange, quite juicy with good blend of sugars and acids and

has a rich distinctive flavor. The fruit quality decreases when the fruit is held on the tree past maturity for weeks. The new 0-4 seeds per fruit Kinnow strain had stability of seedless trait and heat tolerance above 45°C which is usually lacking in seedless mandarin cultivars of the world. The plant has low spine density. The flowering is in March and the fruit maturity time is mid to late February. The strain is a result of natural genetic changes within Kinnow genome in orchards of Punjab (Figure 1, 2, 3). All the low seeded material obtained is a result of natural genetic change/s, already present in the experimental materials.

The true fruit quality and yield of this strain can be fully assessed after studying the tree characteristics and field performance on different rootstocks, management spacing, pollinator requirements, nutrition for good plant health, physiological fruit drop and factors affecting fruit yield. The strain will be registered and multiplied for commercial plantings. The fruit characteristics of the 0-4 seeded strain are given in table below.

Table: Fruit characteristics of new Kinnow strain

Fruit Characteristics	2004-05	2005-06	2006-07
Total fruits	5	44	18
Fruits studied	5	19	15
Seed range	0-4	0-4	0-4
Average seed number	2	2	1.53
Fruit weight (g)		120-150	146-160
Fruit volume (ml)		130-165	135-170
Fruit height (cm)		5.2-5.5	5.3-5.6
Fruit diameter (cm)		6.5-7.0	6.3-7.1
Navel diameter (mm)		2.3-3	2.1-2.9
Number of segments		10-11	10-11
Peel weight (g)		41-52	49-60
Peel thickness (mm)		3-3.5	2.8-3.7
Juice pH on January 01, 2006 and January 26, 2007 Juice pH on March 03, 2006		3.9-4.2 4.3-4.6	4.2-4.4
TSS (°Brix) on 26th January		13-18	11-13
Acidity (%) on 26th January		0.6	0.67

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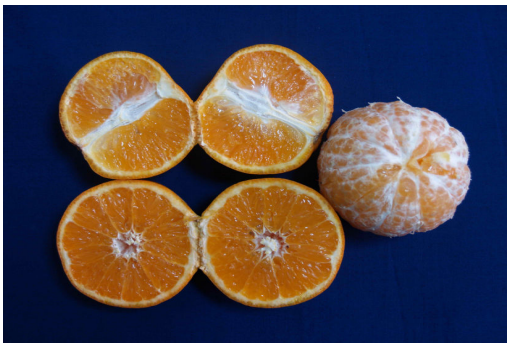

Figure 1: Fruits of 0-4 seeded strain

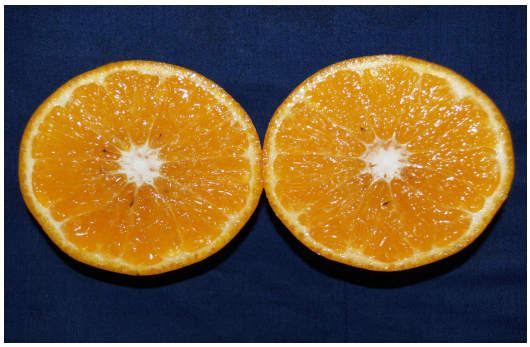

Figure 2: Black spots of dead ovules

Figure 3: Fruit clusters of new strain