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Review of Seedless Kinnow Project

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Citrus is major fruit of Pakistan, which covered 193.3 thousand hectares with 1472.4 thousand tones production during 2006-07 (Anonymous, 2006). The Punjab is the most populated province; with fertile, irrigated land mostly plaines. In Punjab *Citrus* orchards capture 182.1 thousand hectares with a major share in national production, 2385.1 thousand tones [1]. It is important for *Citrus* industry to have superior cultivars adaptable to different climatic and soil conditions and capable to meet the increasing demand of national and international market. The merits of Kinnow tree are its vigorous growth, high yield potential, large and attractive fruit size, good blend of sugars and acids, deep orange color, adaptation of Kinnow with soil and climatic conditions of Punjab. Demerits are alternate bearing, high number of seeds per fruit, bitterness if seeds crushed in juice, acidity, fruit and leaf abscission and short fruit shelf life. Kinnow mandarin has extreme variability because of its hybrid nature. It reverts to changes via chance genetic combinations causing genetic variation. Somatic mutations and large scale propagations mostly without proper bud wood selections is also important in creating variability in Kinnow mandarin. Variability in seed number [8, 9] per Kinnow fruit was 0-52. We found 0 -3, 2-6 developed seeded fruit branches. The seedless trait (Fig. 1 a, b) was 0-6 seeds and the most common was 14-32 in seedy background. The developed seeds were of different shapes (Fig. 2) and sizes. Variability in fruit characteristics itself has a negative economic impact in fresh fruit market.

Genetic improvement within Kinnow cultivar is by selection and vegetative multiplication of natural mutant branches. Importance of nucellar embryony for isolation of somatic cell lines having desirable fruit characteristics cannot be ignored. Kinnow plants [2] were developed through natural selections, nucellar embryogenesis [4] with and without callusing, immature fruit gamma radiation (30-120 Gray) exposure, the nucellus culture (Fig. 3), seed soaking in 8-hydroxyqinoline, seed culture of low seeded fruits, culture of small and large size seeds and gamma radiation (10-100 Gray) exposure of buds. Kinnow materials were grafted on rough lemon seedlings [6]. Low seeded/seedless Kinnow fruits were screened on the basis of stylar ring and narrow, new emerging leaves of the sprouts and shoots. The natural change of narrowness of new emerging leaves is linked to low ovule viability. Besides seed number variability, fruits also have variable characteristics in size, color, shapes, peel thickness with its tightness or loose nature, aroma, acidity, sweetness and juice contents etc. The seedless trait was also found in normal fruits. Parthenocarpy is

(a) (b)

Fig. 1:

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Fig. 2:

Fig. 3:

Fig. 4:

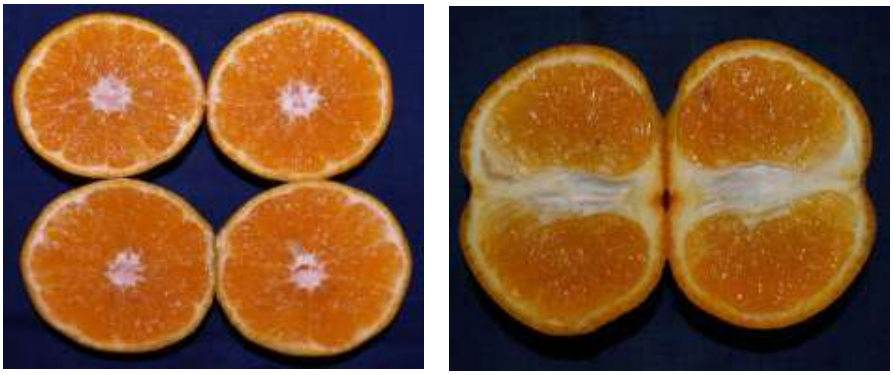
absent in Kinnow mandarin, although in 0.4% selected marker fruits, ovules aborted at the stage that it was difficult to recognize between juice vesicle and ovule. It was observed that narrow new emerging leaves of embryos have similarity to new emerging leaves of the branches from where the low seeded fruits were harvested. Narrow embryonic leaves are related to over 20 seed shapes and in small seed size. However the intensity of leaf narrowness varied in different seeds.

Fig. 5:

Fruit and leaf drop have same genetic mechanisms. The sprout grafts (Fig. 4) helped screening against abscission. If the branch has fruit drop tendency, its leaves and petioles fell within 72-75 h under graft stress. The detachment of leaves and petioles is from abscission zones. The stems regenerate new leaves after 2-3 months of graft union with rootstock. Such grafts were eliminated because they have leaf fall tendency in harsh weather conditions. The dropped fruits had 13% embryos without root system [3], which upon grafting had 100% plants with leaf abscission trait without any stress conditions. This indicated that the trait is of genetic origin as the embryos are derived from cell lines present in the vegetative parts.

In Pakistan the temperature range during summer is 35-51°C. It was continuously observed that plants with dominant characteristics of cultivar willow leaf cannot survive hot temperatures (above 40°C). Only 0.5% of these plants had heat tolerance. While a higher proportion of plants (80%) with dominant characteristics of cultivar king can survive harsh hot field temperatures. The embryogenic plants have extreme low frequency (0.05%) of heat tolerance. They are sensitive to summer heat even if the plants were transferred to field at the age of 2 years.

Seedlessness is because of pollen self incompatibility, weak parthenocarpy, defective ovules and embryo abortion. Monoembryonic pollen self-incompatible clones have seedless potential if the plants were kept isolated from other pollen sources and bees during flowering time. The polyembryonic clones have tendency of apomictic seed formation. However, in these strains were low seeded if ovule sterility is high. Seed formation in pollen self-incompatible clones is because of type of *Citrus* pollinator in mixed plantings and the bee population around at flowering time because if the Kinnow material taken for grafting



(a) (b)

Fig. 6:

from any orchard and if it has ovule fertility, the developed plant in NIAB orchard has different seed number as compared to its parent orchard, depending upon the compatibility of the available pollen in the two orchards.

Nearly 200 plants have 0-6 seeds per fruit. Early maturing and less acidic strains will be selected for commercialization. The commercial plantings will be isolated from other *Citrus* cultivars as pollen sources because these are auto sterile. Kinnow farmers will enjoy new cultivars having better retention, heat tolerance, low seeded and less acidic fruits with different physiological maturity times.

CONCLUSIONS

- Kinnow cultivar is highly variable, with 0-52 seeds per fruit.
- The parental characters appear in Kinnow population.
- Clones having seedless trait have different vegetative characteristics.
- Seedless trait can be cloned into plant by sprout/shoot apex/embryo grafts.
- The seedless trait is highly associated with fruit abscission since fruit and leaf drop have same genetic mechanism. The final selections were made on the basis of leaf retention under graft stress.
- The clones retained stability of selected seedless trait.
- The use of undeveloped ovules with optimum culture conditions can avoid loss of potentially valuable genes because high or complete degeneration of ovules occur in seedless mutants despite selection of healthy ovules for culture.
- Spontaneous triploid and tetraploid plants were obtained from *in vitro* culture of ovules.
- A unique seedless plant was obtained from nucellus callus embryogenesis.
- The monoembryonic clones have seedless potential while polyembryonic clone have low seeded trait if with high ovule sterility. Two clones have different fruit shapes.
- Reversion of delayed style abscission to normal abscission by invigourization of plant was made. This explains the presence of marker fruit branches and non-marker fruit branches on the same tree in orchards.
- Significant achievement is the discovery of mono embryony coupled with pollen self incompatibility [5, 7], which is responsible for low seeded/seedless trait in Kinnow mandarin (Fig.6 a, b). The seedy trait is because of poly embryony which forms apomictic seeds.

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