Pak. J. Bot., 39(6): 2003-2008, 2007.

THE SEEDLESS TRAIT IN KINNOW FRUIT

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Abstract

The seedless trait in Kinnow mandarin (*Citrus reticulata*) is desirable because of natural factors like ovule, pollen and pollen self incompatibility. Various degrees of ovule sterility are present in Kinnow plants. The collapse of the developed ovules as rudiments of seeds in fruits is because of embryo abortion. The *In vitro* pollen germination on agar medium is around 18% in selected low seeded plants. Some plants have pollen self incompatibility. The fruit-set in start of May was 23, 9 and 43 percent in self pollination (SP) with Gibberellic Acid (GA) spray, SP without GA spray and in open pollination (OP) respectively. The fruit set in end of July was reduced to 8, 2 and 22 % respectively. The fruit abscission was highest (82%) in SP without GA spray and the minimum fruit abscission was 48% in OP in these plants. The June fruit drop was variable in Kinnow germplasm in high density plantation. The seedless trait (0-6 seeds/fruit) was found in all three types of plants originated from bud radiation, sprouts and embryonic seedlings.

Introduction

Citrus has number one position in area and production of fruits in Pakistan. It is grown over an area of 192.3 thousand hectares with the production of 2458.4 thousand tonnes (Anon., 2006). The province of Punjab is the key share holder (97.02%) in national *Citrus* production and area (94.64%) under *Citrus* grooves. Kinnow mandarin is the leading cultivar and has extreme variability in fruit quality. The seed number per fruit ranges from 0-54, so low seeded germplasm was collected from orchards of Punjab. The seed number in fruit depends upon several factors like mono and poly embryony, degree of pollen and ovule sterility, pollen self incompatibility, pollen load, bee population, mixed *Citrus* plantings and the level of natural parthenocarpy in Kinnow strains. Plants have developed self incompatibility response to prevent fertilization by their own pollen. Scientists have identified a receptor-like cytoplasm Kinase as a crucial player in the self-incompatibility response (Goring & Walker, 2004). Pollination increases Gibberellins levels as the developing ovules produces GA which helps in development of ovary. Natural parthenocarpy does the same for the development of fruit. Gibberellic Acid spray in seedless cultivars increases the fruit set and reduces the fruit abscission. In this study the natural June drop in Kinnow was also noted in dense plantation (Graph-1). Since seedless trait is dependent on several factors in Kinnow mandarin, we studied (1) the fruit set and seed number in self and in open pollination of the selected clones on the basis of low seeded trait from orchards. The pollen germination was also studied. 2).The fruit seed number was recorded from plants developed by bud radiation (0-100 Gray), by sprout selections from orchards and plants developed by grafting embryos on rough lemon.

Materials and Methods

Fruit set and abscission in self and in open pollination: Eight plants with low seeded trait (0-10 seeds per fruit) were selected from Kinnow germplasm growing under high density plantation system. Branches from these plants were used for self pollination with GA (8-10 mg l^{-1}) spray. The self pollination was assured by covering the inflorescence by fine muslin cloth bags and GA was sprayed at petal fall stage. Some selected branches

were used for self pollination without GA spray and the remaining twigs were left for natural open pollination. The data regarding fruit set and fruit abscission was recorded in (Table 1). The June drop was noted in the high density plantation system (plant to plant distance 1 foot) by counting number of fruits per plant in the last week of May and then in the first week of July.

Seed number per fruit in plants originated from bud radiations, sprouts and embryonic seedlings: The Kinnow buds were taken from four plants. The range of seed number per fruit was 2-38 in parent plants whose bud sticks were radiated with 0-100 Gray using Gamma cell 220 (^{60}Co Gamma-source) in air medium at 25±2°C. The radiated scions were grafted on to one year old rough lemon rootstock seedlings. Seed number of fruits was recorded in (Table 2A). The seeds of marker fruits which have stylar splitting, were cultured in MS basal medium (Murashige & Skoog, 1962) and embryos were grafted on two months old rough lemon seedlings. The sprouts were selected from Kinnow plants having variable seed number per fruit. The sprouts were grafted on to healthy rough lemon seedlings. The seed number of fruits was studied for three years and the data is given in (Table 2B).

Pollen germination: The low seeded Kinnow germ plasm was selected from Punjab orchards. The culture medium used for pollen germination was Agar (10g l^{-1}), Boric Acid (200mg l^{-1}), Sucrose (100g l^{-1}) and Calcium Nitrate (800mg l^{-1}). The pH of medium was adjusted to 6.5 prior to adding agar. The medium was sterilized by autoclaving. The pollen from open flowers of low seeded plant were dusted on the culture medium in laminar air flow cabinet. The germinated pollens were studied after one week using microscope under 1000X magnification. The data is mentioned in Table 3.

Results and Discussions

Seedlessness in *Citrus* is highly desirable trait and is requirement of fresh fruit market and juice industry. The seeds cause bitterness and physical hindrance in consumption and it is more civic to utilize seedless fruit. The seedlessness in *Citrus* varies among accessions and some times in different environmental conditions. The number of seeds in almost all accessions is less than the number of ovules; therefore it is probable that most of the commonly cultivated accessions have some degree of ovule or pollen sterility. Strong sterility coupled with parthenocarpy is necessary for the production of stable seedless fruits. Sterility can be divided into female sterility, male sterility and pollen self incompatibility (Ollitrault *et al*., 2007).

Kinnow mandarin has various degree of embryony and seeds can be apomictic. Mono and poly embryony is expressed in different strains Fig. 2. It is well known that degree of polyembryony of a *Citrus* variety is influenced by the environment, variations being observed from seed to seed, from sector to sector in the same plant and from year to year (Filho *et al.*, 1995). Although apomixis is a complex process, it is inherited as a simple Mendelian trait controlled by a few master regulatory genes (Eckardt, 2003). Kinnow is a hybrid of Willowleaf (monoembryonic) and King (polyembryonic) cultivars. In Kinnow mandarin, the ovule fertility and sterility is variable. Some ovules die at very early stage so they are difficult to distinguish from the juice vesicles. Some seeds abort at later stages after pollination because of embryo death which appears as compressed seed coats. The *In vitro* pollen germination on agar medium in low seeded plants is given in (Table 3).

Table 1. Fruit set and fruit drop in low seeded plants.

Treatments	Number of flowers	Fruits in first week of May (Fruit set %)	Fruits in July end (Fruit set %)	Fruit abscission (% Fruit drop)
Self pollination with GA spray	1388	315 (23)	113 (8)	202 (64)
Self pollination without GA spray	758	71 (9)	13 (2)	58 (82)
Open pollination	1138	492 (43)	256 (22)	236 (48)

Table 2A. Fruit seed number of bud radiated, embryonic and sprout selections of Kinnow.

Bud radiated plants		
No. of plants	**Dose**	**Seed range**
03	0	15-20
05	10	9-22
12	20	11-22
06	30	7-24
07	40	4-24
08	50	7-16
02	60	12-20
06	70	10-20
03	80	2-16
01	90	5-7
03	100	7-13

Table 2B.

Embryonic plants		Sprout selections	
No. of plants	**Seed range**	**No. of plants**	**Seed range**
10	6-32	34	4-29

Table 3. Pollen germination of selected low seeded Kinnow strains.

Embryony status	No. of plants	% Germinated pollen (Range)	Type of pollen morphology
Monoembryony	05	14-18	Normal Pollen, Distorted pollen, germinating plugs from pollen pores, pollen with elongated tubes
Polyembryony	03	19-29	

Monoembryonic plants have more pollen sterility as compared to polyembryonic strains. Both the types have some pollen with normal tube germination which indicates that these strains have capability of forming seeds in other plants as reported in grape fruits (Chacoff and Aizen, 2007). Five plants used in self and open pollination were monoembryonic and the remaining three plants had 2-4 embryos per seed in culture. The monoembryonic strains were completely sterile in SP without GA sprays. However 1-2 GA sprays at petal fall stage produced some fruits in SP. The self Pollination had 0-3 rudimentary seeds as compared to 1-7 in open pollinations (Fig. 1).

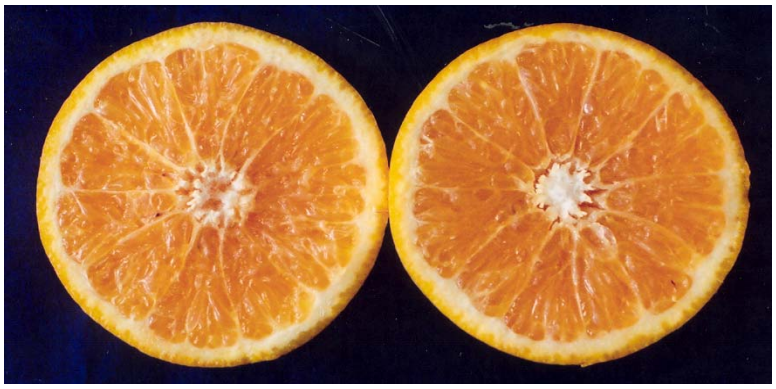
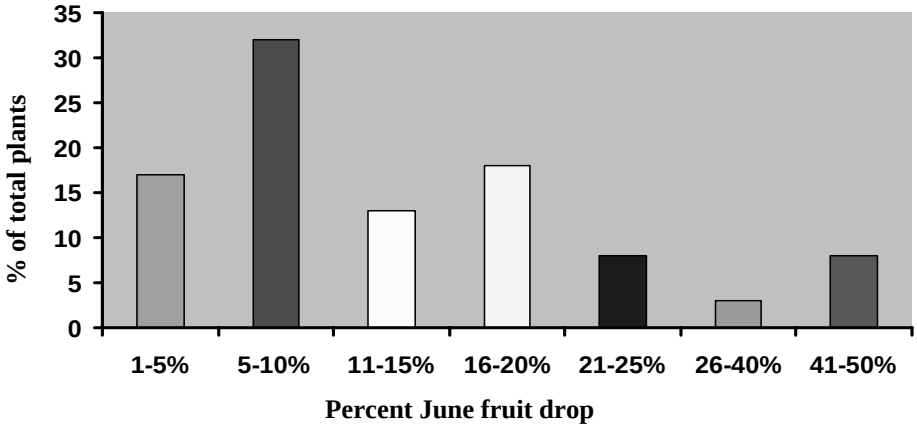


Fig. 1. 0-3 Seeded fruit of Kinnow mandarin.

Fig. 2A. (A) Polyembryony (B) Monoembryony in Kinnow seeds.



Graph 1. June drop in dense plantation of Kinnow mandarin

These results clearly indicated that self incompatibility was present in strains and GA enhances fruit development. The low seeded strains require GA for seedless fruit set. Low seeded Kinnow strains have low parthenocarpy because of low endogenous gibberellin levels. The fruit set can be enhanced and fruit abscission can be minimized by more GA sprays in self pollinations (Table 1). Clementine growers apply GA_3 during bloom and post-bloom to enhance fruit set and fruit yield. (Chao & Lovatt, 2006).

In this case we only had single spray at petal fall stage. Endogenous gibberellins play a role in regulating cell expansion and promoting cell divisions in carpels (Smith & Koltunow, 1999). This study shown tells that seedless fruits in Kinnow mandarin can be obtained by growing Kinnow in solitary blocks, excluding cross pollination by controlling bee hives and spraying GA for induced parthenocarpy.

It is widely accepted that reactivation of fruit development requires synthesis and action of growth regulators such as GA's and auxins and it is well documented that pollination increases Gibberellins levels in developing ovaries of *Citrus* (Ben-Cheikh *et al.,* 1997). At petal fall, fruitlets of Satsuma and Clementine contains 65 and 13 picograms of GA_1 respectively. Levels of GA_9 were very low in both species (Talon *et al.,* 1992). Endogenous free polyamines may act as a nitrogen source rather than a regulator of fruit set in low and high parthenocarpic ability citrus cultivars (Arias *et al*., 2005). Since Kinnow has variable seed number, fruit set, fruit abscission and retention. A variable behavior was observed in June fruit drop (Graph-1). Fruit drop, leaf drop and flower drop have same genetic mechanisms in *Citrus* and it occurs in well defined areas called abscission zones (Burns, 1998). Abscission in *Citrus* is always in stress conditions like high heat in field during the month of June. This is also called physiological drop.

Fruit drop in Kinnow is inherited from the parental cultivar Willow Leaf as this phenomenon is genetically controlled and is more in high density plantation system in which our plants are growing. Influence of rootstock on fruit drop in Kinnow in high density plantings in India has been studied. The highest fruit drop was observed in Sohsarkar (86.41%) and lowest (69.79%) was found in Troyer Citrange (Sharma *et al*., 1999). The low seeded trait was obtained in plants radiated with 40, 80 and 90 Gray (Table 2A). Overall seed number range was 2-24 per fruit in 10-100 Gray.

In embryonic plants, the seed range was 6-32 per fruit. The sprout selections had seed range as 4-29 (Table 2B). Since seedless trait depends on multiple factors, only natural mutations can contribute to seedlessness in Kinnow mandarin. Mutations are the ultimate fuel for evolution and the deleterious fitness effects are, on average, magnified in stressful environments (Elena & Visser, 2003). Mutations can be linked to changes in DNA sequences for some plant traits and to establish molecular maps in structural and functional genomics of crop plants (Ahloowalia & Maluszynski, 2001). The seedless character in Kinnow cultivar accompanies valuable mutations in addition to heat tolerance trait which Kinnow has inherited from parental cultivar King, which is a Tangor.

Acknowledgement

Scientific assistance of Mr. Anees Ahmad, Liaqat Ali and Inkisar Ahmad is gratefully acknowledged. Thanks to Mrs. Nasim for Kinnow bud radiations. The work was partially sponsored by PARC, ALP, Project No.01-02-01-10.

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(Received for publication 5 September 2007)