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Full Length Research Paper

Development of sparse-seeded mutant kinnow (Citrus reticulata Blanco) through budwood irradiation

Shahid Akbar Khalil[1,2]*, Abdus Sattar[1] and Roshan Zamir[2]

[1]**Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan.**
[2]Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan.

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Kinnow is the major fruit of Pakistan and has a high export potential due to its excellent fruit and juice quality. However, high number of seeds (25 ± 5) per fruit is limiting its export on a large scale. Benefiting from the induced mutations for selectivity, especially in the vegetatively propagated fruit crops like citrus, induced mutation for seedlessness in Kinnow with gamma irradiation of dormant bud which was attempted at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. Dormant bud irradiation-cum-grafting technique was employed, using the *Citrus jambhiri* rootstock for propagation of the scion. A sparse seeded (5 ± 3 seeds/fruit) mutant was evolved from an exposure dose of 20 Gy. The mutant was put to the conventional propagation up to mV_5, for confirmation of the continuity of the mutation. The sparse seeded mutation was found to be a solid and stable one. The quality parameters in fruit and juice of the mutant were comparable with its parent.

Key words: Kinnow, seedlessness, dormant bud, gamma irradiation, mutant kinnow.

INTRODUCTION

In Pakistan, citrus fruits are cultivated on 199.9 thousand hectares producing 2132.2 thousand tonnes of fruits annually, whereas in Punjab, the area under citrus fruits cultivation is 189.8 thousand hectares which produced 2059.5 thousand tonnes of fruits (Agricultural Statistics of Pakistan, 2009). The ecozones of the central and southern Punjab Province are very conducive for the optimum quality potential expression of Kinnow (*Citrus reticulata* Blanco). From the juice and fruit quality stand point, it is an unmatchable mandarin in the national and international markets. Currently, a striking quantity of Kinnow is exported against a reasonable amount of foreign exchange. High number of seeds per fruit in Kinnow is the only constraint which limits its export beyond a certain level. Induced mutations in the somatic tissue(s) of the vegetatively propagated crops, especially the fruit crops, is the method of choice for creating genetic variability because it is more economical in terms of time and space than the conventional cross breeding method. Induced mutations can be used to create useful genetic variations in terms of great economic importance without disturbing the useful traits of a genotype. This facilitates the easy marketing of the mutant variety along with its parent as the consumer is already well aware of its quality attributes. Many researchers got achievements over the past few decades in seedless breeding techniques with fruits such as citrus, grape (*Vitis vinifera* L.), litchi (*Litchi chinensis* Sonn.), loquat (*Eriobotrya japonica* Lindl. Cv. Jiefangzhong), mango (*Mangifera indica* L.) and wampee (*Clausena lansium* Lour.) (Ye et al., 2009; Raza et al., 2003). Many seedless citrus cultivars, especially seedless mandarin cultivars, originate from the bud mutation of seedy cultivars (Shen, 1997). Some morphological variations in kinnow seedlings caused by gamma irradiation of seeds have been reported (Ahmed et al., 1992; Varoquaux et al., 2000). Bermejo et al. (2011) concluded that budwood irradiation is a suitable technique to improve cultivars, and produce seedless cultivars. Addressing the problem of high number of seeds in Kinnow, a project was initiated at the Nuclear Institute for Agriculture and Biology (NIAB) to develop a seedless kinnow variety through gamma irradiation induced mutation; the major results of this project is reported in this paper.

MATERIALS AND METHODS

Dormant buds of Kinnow (*C. reticulate* Blanco) were subjected to

*Corresponding author. E-mail: shahidakhalil@yahoo.com.

Table 1. Physical characteristics of kinnow and its seedless mutant (range and mean value).

Crop year	Fruit size diameter (mm)	Juice yield (%)	*Seeds/fruit	Peel thickness (mm)	**Organoleptic score (1-10)
Percent (mV_0)					
1987/1988	65-80 (74.3)	47-50 (48.33)	20-30 (23.50)	2.3-2.5 (2.47)	7.5-8.2 (7.88)
2003/2004	64-81 (73.6)	46-50 (48.04)	19-28 (23.00)	2.2-2.4 (2.34)	7.4-8.00 (7.75)
Mutant (mV_1)					
1987/1988	63-78 (70.2)	45-47 (45.48)	3-7 (4.67)	3.0-3.5 (3.28)	7.2-8.5 (7.61)
2003/2004	65-79 (72.4)	46-48 (46.47)	2-8 (4.39)	2.90-.4 (2.34)	7.4-8.00 (7.75)
Mutant (mV_2)					
1992/1993	65-79 (71.79)	45-48 (46.21)	3-7 (5.00)	3.1-3.4 (3.20)	7.5-8.2 (7.86)
2003/2004	61-82 (70.78)	45-47 (46.20)	3-7 (4.89)	3.3-3.5 (3.34)	7.4-8.3 (7.79)
Mutant (mV_3)					
1996/1997	65-82 (72.78)	45-48 (45.99)	3-8 (5.11)	2.9-3-5 (3.18)	7.8-8.3 (7.91)
2003/2004	63-81 (79.5)	46-48 (46.00)	3-7 (4.95)	3.2-3.4 (3.05)	7.4-8.2 (7.75)
Mutant (mV_4)					
2000/2001	60-78 (76.2)	46-48 (45.72)	2-8 (5.04)	3.0-3.4 (3.17)	7.0-8.0 (9.92)
2003/2004	62-79 (76.5)	45-48 (46.20)	3-7 (4.72)	3.1-3.3 (3.11)	7.2-7.5 (7.30)
Mutant (mV_5)					
2003/2004	60-80 (75.7)	45-47 (46.31)	2 – 7 (4.91)	3.2-3.4 (3.35)	7.0-8.0 (7.71)

*Total number of ovules in the parent Kinnow = 50; ovule + seed abortion in parent Kinnow = 20%; ovule + seed abortion in the seedless Kinnow mutant = 80%. ** Organoleptic quality was based on fruit size, appearance, color, taste and flavour of juice. 1 to 10 rating scale was used for the evaluation of each quality character.

gamma irradiation, using Gamma Cell 220 (^{60}Co, -Source). Radiation doses of 20, 40, 60, 80 and 120 Gy were applied. The irradiated bud scions were grafted onto *Citrus jambhiri* rootstock, using the side-graft technique. After a growth period of three to four years, all the plants entered the flowering stage. The fruit from these plants were analyzed for the number of seeds per fruit and other quality attributes. One of the mV_1 (20 Gy) plants bore fruit with significantly reduced seeds against the highly seeded parent kinnow fruit. The seedless mV_1 material was used for successive vegetative propagations up to mV_5. The continuity of the seedless mutation has been confirmed at each stage of propagation.

For the physicochemical analyses and the organoleptic quality evaluation of the fruit, nine fruits per plant from three different plants in each generation were taken during the first week of January. In case of physical characters, number of seeds per fruit, fruit size, peel thickness and juice yield were determined. As regards chemical characteristics, vitamin C, total soluble solids (TSS) and acidity were determined according to the standard AOAC methods and TSS : acid ratio was calculated (AOAC 1996). Sensory quality of the parent as well as the mutant Kinnow was tested by a panel of ten trained judges and the data was analyzed statistically (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Data regarding the fruit physical parameters like fruit size, juice yield, peel thickness and number of seed per fruit and juice chemical constituents such as vitamin C acidity, total soluble solid (TSS) and TSS : acid ratio and organoleptic quality of the parent kinnow (mV_0) and its seedless mutant are given in Tables 1 and 2. In the sparse seeded mutant of kinnow (Figure 1), only the seed number per fruit was reduced to a significantly low level (averaged at 4.00), while the rest physical and chemical characters remained almost unchanged.

Normally, Kinnow enters the flowering stage after a growth period of three years of shoot from the grafted bud. In mutant Kinnow (mV_1, mV_2, mV_3, mV_4 and mV_5), fruiting to the shoots from the grafted buds also occurred within three to four years. Sparsely seeded mutant Kinnow was identified from 20 Gy plants. The number of seeds per fruit in the mutant kinnow was lower (2 to 8) than its parent kinnow (20 to 30). The induced sparse seediness was retained by the successive vegetative generations of the mutant. Retention of the induced character in the successive vegetative progenies showed the induced mutation to be a solid and stable one (Tables 1 and 2). Lower pollen viability and increased ovule sterility resulting in lower seed number in citrus has been reported (Hensz, 1971; Hearn, 1984, 1986; Spiegel-Roy and Aliza, 1989). Irradiation with 25 Gy of Perlette grape cuttings caused sterile ovules and pollen, resulting in

Table 2. Biochemical constituents of juice of Kinnow and its seedless mutant (range and mean value).

Crop/year	TSS (%)	Acidity	Vitzmin C (mg/100 g of pulp)	TSS/acid (ratio)
Parent (mV_0)				
1987/1988	10.5-11.2 (11.0)	0.58-0.62 (0.59)	17.2-20.7 (19.24)	17.5-19.20 (18.58)
2003/2004	10.4-11.5 (11.2)	0.60-0.62 (0.60)	18.5-21.7 (20.41)	17.7-19.76 (18.87)
Mutant (mV_1)				
1987/1988	9.95-10.7 (10.41)	0.61-0.62 (0.62)	19.2-22.6 (20.45)	16.1-16.9 (16.55)
2003/2004	10.3-10.8 (10.62)	0.60-0.62 (0.62)	18.5-21.7 (20.41)	16.5-17.1 (16.71)
Mutant (mV_2)				
1992/1993	10.6-11.1 (10.69)	0.60-0.63 (0.62)	19.5-21.3 (20.19)	16.8-17.5 (17.21)
2003/2004	10.5-11.4 (10.75)	0.62-0.62(0.62)	18.8-20.7 (19.90)	16.5-17.3 (17.8)
Mutant (mV_3)				
1996/1997	10.3-11.9 (10.48)	0.59-0.62 (0.61)	18.4-20.5 (19.37)	16.8-18.10 (17.5)
2003/2004	9.8-11.5 (10.70)	0.61-0.62 (0.61)	19.6-22.0 (20.50)	17.1-18.4 (17.4)
Mutant (mV_4)				
2000/2001	10.5-11.7 (11.0)	0.62-0.65 (0.63)	18.7-21.8 (19.60)	17.5-19.7 (18.2)
2003/2004	10.7-11.6(11.2)	0.60-0.63(0.60)	17.8-22.5 (19.82)	17.3-21.0 (19.72)
Mutant (mV_5)				
2003/2004	10.4-10.7 (10.5)	0.62-0.66 (0.63)	19.2-21.8 (20.31)	16.5-18.4 (17.75)

Figure 1. Mutant kinnow plant and its sparse seeded fruit.

poor fruit set (Hearn 1984). Russo et al. (1981) carried out induced mutations with gamma rays in seeded 'Monreal' Clementine mandarin (*C. reticulate* Blanco) and produced mutants with fewer seeds per fruit. Seed production in citrus fruit is affected both by the genetic and environmental factors. Some varieties of oranges rarely produce any seed under ordinary culture conditions because they entirely lack viable pollen. Due to pollen sterility, Satsuma mandarin of Japan has become seedless without losing its production potential due to the enhanced parthenocarpic fruit formation. However, with cross pollination due to compatible citrus varieties, some seeds may be formed even in the absence of viable selfing potentials in some citrus varieties.

The seedlessness in Kinnow, in essence, is caused by the abortion of both ovules and seeds. Certain chemical factors would have played some role in the discontinuation of the seed development process, at various stages of its growth. In the present case, the reduction of seeds in the mutant Kinnow to a lower level could be due to sterility, as the pollen in the parent kinnow is highly potent (approximately 80% viability). The sparse seeded mutant was identified from a small population of mV_1 (80 plants). In our studies, the sparse seed may be due to the recessive gene involvement. These points to a low number of recessive genes involvements in the induction of seedlessness in kinnow. Furthermore, the presence of seedless characters up to mV_5 generation indicates that very low genes are involved in seedlessness.

The sparse seeded mutant (5 ± 3) of highly seeded parent Kinnow (25 ± 5) was developed through irradiation of dormant bud. Sparse seeded character was retained up to mV_5. The seedless mutant Kinnow is ready for distribution to growers.

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