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# Effect of Radiation on Nuclear Embryogenesis in Sweet Orange Cultivars

Nafees Altaf, Shahid Akbar Khalil, Ehsanullah Khan Marwat and Inksar A. Bhatti
Nuclear Institute for Agriculture and Biology (NIAB) Faisalabad

**Abstract:** All the Thirteen sweet orange cultivars which were tested for nuclellus embryos in MS + BA (0.5 mg/l) + Glutamine 5 rrigil responded to embryogenesis. After two months culture period, the maximum (7.0) coefficient of nuclellar regeneration (CNR) was of cultivar Mosambi, which has minimum ovvule weight (0.11 cm) and the minimum CNR was of Moro blood (2.0). The fruit characteristics like fruit weight, diameter, ovule weight, length and diameter have no relations with CNR. The maximum values of CNR were in Valencia (5.07), Succari (5.0), Mosambi (4.58) and in both Mosambi, Succari (3.5), for 3,6,9 and 12 Kr and minimum CNR values were 2.49, 1.66, 1.6, 1.12 in 3,6,9 and 12 Kr respectively.

**Key words:** Sweet orange, gamma radiation, nucellar embryogenesis

## Introduction

*Citrus* is on top of all fruits both production and area wise, about 38% of the total fruit area comes under *Citrus* constituting 41% of the total fruit production of Pakistan (Chaudhry *et al*.,1992). The major production is of mandarins in which Kinnow is the leading cultivar which occupies about 59% of the total *Citrus* area and 64% of the total *Citrus* production in the country. Sweet oranges *(Citrus sinensis* L. ❑sbeck) occupies second position after Mandarins and Mosmabi is the leading cultivar with 14.96% of total *Citrus* production and other sweet oranges are 21.7% of total *Citrus* production (Malik, 1992). Beside Mandarins and sweet oranges, the other Citrus fruits in Pakistan are sweet lime, Lemon, Sour/Kaghzi lime, Sour oranges, Grape fruit, Pummelo and some other Citrus fruits.

Broadening the base of genetic variability is needed for better adaptation to environmental stresses like virul diseasesof Citrus which have shown to be a major problem restraining vigour and productivity of orchards in all of the major Citrus producing regions of the world. Nuclear embryos arise from maternal nucellar tissue and are very useful for true to type genotype production and also for production of virus free plants (Raman *et al*., 1995). There are reports of nucellar tissue embryogenesis in Sweet orange as in cv. Mosambi by Das *et al*. (1995) and in cv. Valencia by Rey *et al*. (1995). Regeneration from salt tolerant limes of Shamouti orange nucellar callus by Spiegel-Roy and Saad (1997). Regeneration of plants from endosperm callus culture of sweet orange cv. Hongjiang by Chen *et al*. (1991). Sometic embryogenesis and plant regeneration from undeveloped ovules, stigma, style of sweet orange navel group hasbeen obtained by Carimi *et al*. (1998).

Four variant lines of superior fruit quality with large size, rind thicknessand early bearing were selected from nucellar population of "Navelate" sweet orange (Starrantino *et al*., 1990). There are natural changes like Qianyange seedless Dahong sport was selected with large size, juicy with an acid sweet flavour, high vitamin-C, good eating quality and storage ability for 3 months at room temperature (Chen *et al*., 1992). Similarly superior fruit quality Beipei Jincheng-447 orange was selected from cultivar Jincheng in 1980 (Li *et al*., 1992). Gamma rays have also been tried for inducing mutations in sweet orange cultivars (Hearn, 1994). Two selections namely Huaqing seedless 1 and Huaqing few seeded 2 were induced by gamma radiation of scion cv. Hongjiangcheng in 1978 (Xuan, 1994).

## Materials and Methods

Fruits were collected between 45-90 days after pollination (DAP) from Nuclear Institute for Agriculture and Biology (NIAB) orchard, Ayub Agricultural Research Institute (AARI) orchard, Pot graduate Agricultural Research Station (PARS), Faisalabad and Horticultural Research Station, Sahiwal. Fruits were washed thoroughly and dried. They were exposed to gamma radiation prior to culturing in a cell with Co 60 sourc. Doses of radiation were 0, 3, 6, 9 and 12 Kr. Fruits were dipped in alcohol and Hammed before opening. The healthy °values were taken out and nuclellus tissue was cultured onto MS (Murashige and Skoog, 1962) medium with BA (0.5 WI) + glutamine (5 mg/l) with 1% agar. The medium pH was 5.5-5.8. Forty ml of culture medium was dispensed in 100 ml in each flask and were sterilized by autoclaving. Four nuclellus tissues were cultured in one flask.

Cultures were kept in a room with 1000 lux light /8 hours dark cycle with a temperature 25 ± 2°C, the data of nucellar embryogenesis was recorded after 8 weeks of culturing. The coeficient of nuclear regeneration (CNR) response was calculated by multiplying percent nuclellus responded to embryogenesis with the average number of embryos and the product divided by hundred.

## Results and Discussion

The fruit characteristics were recorded as fruit weigtht (gm), fruit diameter (cm), ovule weight (gm), ovule length and diameter (cm) (Table 1). The maximum fruit weight was 161.65 of cultivar Ruby red and minimum was 43.00 of cultivar valenica. The maximum fruit diameter was 7.8 of Frost navel with minimum value as 4.1 of Mediterranean. The maximum ovule weight was 0.48 of cultivar Blood red ofllowed by Hamlin 0.45. The minimum ovule weight was of Mosambi 0.11. The maximum ovule length was 0.85 of Blood red followed by 0.83 of Pineapple and the minimum ovule length was 0.72 in Mediterranean. The maximum ovule diamter was 0.6 of cultivars Ruby red, Succari, Frost navel and the minimum was in Mediterranean as 0.3.

It seems from the fruit characteristics data that there was no apparent relation of parameters like fruit weight and diameter, ovule weight, length and diameter with nucellus tissue embryogenesis regeneration responses. Mosambi has maximum coefficient of nucellus regeneration (CNR) and it has minimum ovule wieght as 0.11, but this relationis not true in other cases like cultivar Moro blood which has less ovule weight and this cultivar has minimum CNR value as 2. In

Table 1: Fruit characteristics of sweet orange cultivars

| Cultiar | Fruit weight (gm) | Fruit diameter (cm) | Ovule weight (gm) | Ovule length (cm) | Ovule diameter (cm) |
|---|---|---|---|---|---|
| Pineapple | 49.10 | 5.13 | 0.44 | 0.83 | 0.43 |
| Jaffa | 46.20 | 4.60 | 0.40 | 0.76 | 0.43 |
| Valencia | 43.00 | 4.22 | 0.39 | 0.73 | 0.36 |
| Blood red | 69.00 | 5.17 | 0.48 | 0.85 | 0.46 |
| Hamlin | 118.91 | 7.30 | 0.45 | 0.68 | 0.49 |
| Ruby red | 161.65 | 7.50 | 0.22 | 1.30 | 0.60 |
| Mosambi | 44.67 | 4.56 | 0.11 | 1.20 | 0.49 |
| Succari | 75.99 | 5.30 | 0.20 | 1.00 | 0.60 |
| Frost navel | 96.93 | 7.8 | 0.28 | 1.00 | 0.60 |
| Tarocco | 90.73 | 6.6 | 0.32 | 0.80 | 0.42 |
| Sanguinello | 104.91 | 6.9 | 0.90 | 0.75 | 0.42 |
| Moro blood | 106.84 | 6.0 | 0.15 | 1.50 | 0.40 |
| Mediterranean | 48.30 | 4.1 | 0.60 | 0.72 | 0.30 |

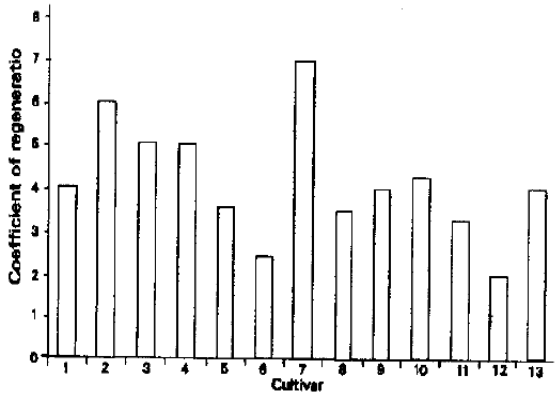


Fig. 1: Nuceilar regeneratin in response to control

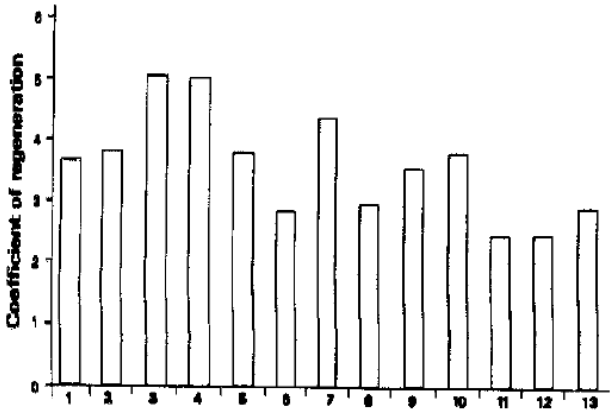


Fig. 2: Nucellar Regeneration in response to 3 Kr gamma radiation

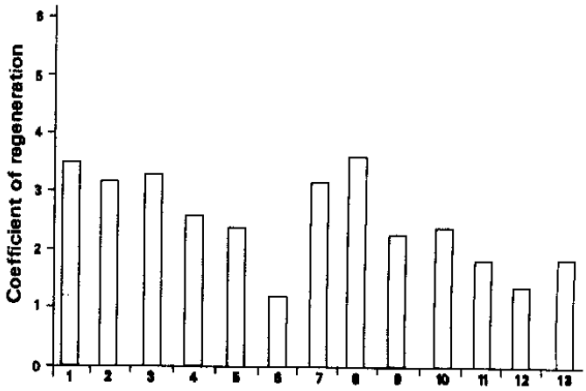


Fig. 3: Nucellar regeneration in response to 6 Kr gamma radiation

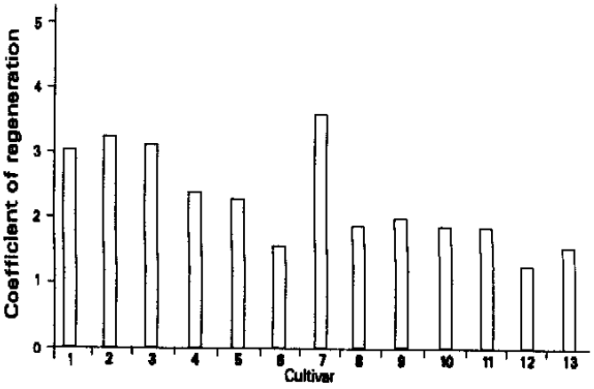


Fig. 4: Nucellar regeneration in response to 9 Kr gamma radiation

control conditions (0 Kr), the CNR was maximum for Mosambi and minimum for, Moro blood as given in Fig. 1. The average value ofCNR tested for sweet orange cultivars used in the study for control (0 Kr) is 4.17 with a gradual decrease in CNR is 3.52, 3.49, 2.89 and 2.35 for 3, 6, 9 and 12 Kr respectively which Is 15.59, 16.31, 30.70 and 43.65 % decrease in CNR for 3 to 12 Kr doses.

In 3 Kr dose (Fig. 2). The cultivars Hamlin, Ruby red and Moro blood has 8.39, 17.14 and 30.00% increase in CNR, while Valencia has the same CNR as the of control. The cultivar Mosambi has maximum decreasein CNR in 3 Kr among the sweet orange cultivars tested. In 6 Kr radiated cultures (Fig. 3), cultivars pineapple and Succari has 21.85 and 42.86% increase in CNR as compared to control conditions and again a maximum decrease in CNR in Mosambi as 37.14%. The minimum decrease is in Moro blood as 4.50 over control. In 9 Kr exposed nucellus tissue (Fig. 4) all the cultivars have decreased CNR as compared to control. The maximum decreaseis in Mediterranean while minimum

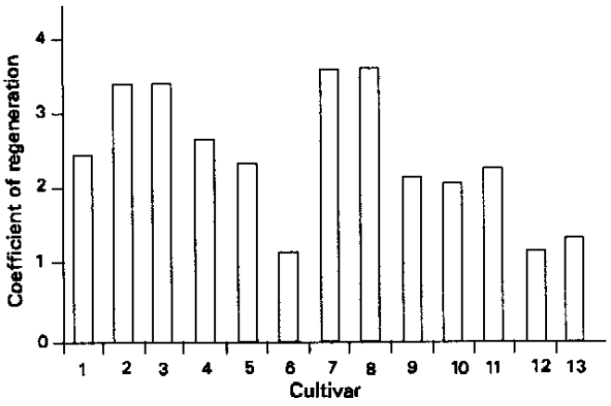


Fig. 5: Nucellar regeneration in response to 12 Kr gamma radiation

decreasein cultivar Pineapple as 4.25% over control (0 Kr). In case of 12 Kr (Fig. 5), the cultivar susccarri has the same CNR value as that of control. The maximum reduction is 33.33% over control. The cultivars above 50% CNR reduction are Hamlim, Ruby red and Tarocco.

*Citrus* can be improved with mutations. In *Citrus* genus, somatichromosome number is diploid (2n) but tetraploids and triploids occur in small frequencies in all major groups of triploids occur in small frequencies in all major groupsof cultivars, Geneticstudies and hybridization is complicated becuase of parthenocarpy, nucellar embryony, heterozygosity, self-incompatibility, sterility and long generation cycle (Gmitter *et al*., 1992). It is relatively easy to produce new varieties of mandarin by hybridization, but it is relatively very difficult to improve in the same way sweet oranges.

Natural mutations occur frequently in *Citrus* and valuable mutations have been found in Sweet oranges (Xin, 1999). Mutation breeding in *Citrus* is possibel (Xuan, 1994) as improved cultivars have originated through spontaneous natural mutations. Mutation have originated through spontaneous natural mutations. Mutation is an event in a single cell and the adjacent celsin the bud or seed remained unchanged. This forms a chimera. Depending upon the proportion and size of the mutated cell lines in an organ such as a shoot forming lateral buds is prone tounstability and due to loss to vigour caused by mutation, the mutated cell lines tend to be out grown by normal tissues and never come to expression.

So it is logic to mutate pre-embryogenic cultures of nuclellus tissue with ionizing radiations like gamma rays. Genetic changes like rich colour and flavour, sweetness and more juice contents, reducednumber of seeds or no seeds etc, in sweet orange scion varieties can be introduced through induced mutations. Ernbryogenic ultures of *Citrus* species including sweet oranges have been made by De Pasquale *et al*. (1999) and nicrografts are used toresuce tissue ulture regenerants in Citrus (Perez *et al*., 1998).

It seems no relation of fruit weight, fruit diameter, ovule weight, ovule length and ovule diameter to nucellar embryogenesis responses. However, it is reported in Washington navel, the growth of albedo calk was dependent on the 20% juice of the specific age of fruit with diameter 3045 mm (Amo-Marco and Picazo, 1994). In fruit species *My icaria cauliflora*, ovule diameter have 64% explant callusing and ovule diameter (0.8 cm) have 31% embryogenic cultures (Litz, 1984). The cultivars generally have high NCR value in control conditions, while the radiation doses 3-12 Kr gradually decreased NCR with few exceptions as in Fig. 1-5. However, different sweet orange cultivars haveembryogenesis potential in the same media and in the same radiation dose. The embryosare growing in cultures and the developing plantlets will be grafted onto seedling later on.

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