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Effect of Gamma Radiation on Nucellar Embroyogenesis of Various Citrus Cultivars

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Abstract: Twenty *Citrus* cultivars were studied for various characteristics like fruit weight and diameter, ovule length, diameter and weight at the time of harvest for access of nucellus tissue for embryogenesis study in *in vitro* conditions. The maximum fruit weight, diameter, ovule weight, length and diameter was found in cultivar Shamber of Grape fruit which has minimum value of coefficient of nucellar regeneration (CNR). However, fruit characteristics studied have no relation with CNR values. Cultivars had variable behaviour for nucellar embryogenesis responses, perhaps they were studied in one medium MS + BA (0.5 mg/l) + 5 mg/l glutamine. The nutritional requirement of nucellus for embryogenesis can be different for different cultivars. The maximum CNR value in control was of Kinnow and the minimum CNR value was of Shamber. In radiation doses 3 to 12 Kr, the maximum CNR value was of cultivar Foster in 6 Kr and the minimum CNR value was in 12 Kr of cultivar Tangerine. The CNR values were different with 0, 3, 6, 9, 12 Kr within the same cultivar and among different cultivars. There were depression in embryogenesis above 9 Kr.

Key words: *Citrus* cultivars, nucellus, radiation, embryogenesis

Introduction

The genus *Citrus* has been recognized as one of the most important group of fruit plants in the world. Also *Citrus* is the major fruit of Pakistan both area and production wise (Anonymous, 1998). Punjab has main production with Kinnow as dominant cultivar. *Citrus* fruit production and market demand depends on complex of factors like health of fruit bearing plants and orchard in general, fruit bearing life of trees, fruit yield, harvesting period, quality of fruit including size, attractive colour of rind, less number of seeds or seedlessness, low acidity and better storage ability are important considerations. It is important to work on other cultivars to maintain *Citrus* production as different genotypes have different interactions towards environmental stresses.

Nucellus culture for embryo production has a key role in maintaining a specific clone genotype, because nucellus is the least differentiated tissue and it also provides virus free plants which is one of the important factor in *Citrus* decline (Raman *et al*., 1995). Also radiation of nucellus tissue prior to culture for embryogenesis provides solid mutants free of chimera. Plants have been regenerated from callus of *Citrus* species and related genera (Ling and Iwamasa, 1997). Nucellar embryos and plants have been regenerated from undeveloped ovules of cultivars belonging to Sweet orange navel group (Carimi *et al*., 1998). Similarly nucellar embryos and plants have been regenerated from ovules isolated from six weeks fruitlets of mandarin and mandarin hybrids by Perez *et al*. (1998).

Exposing the pollen of Clementine mandarin and Blood red orange to either 5 or 10 Kr, activated the pollen tube which penetrated the ovules within 5 days of pollination, increased fruit set and mature fruit weight (Aly, 1995). A seedless Nova tangelo mandarin (Nova SL.) was evolved by irradiating 30-75 Gy to the scion buds (Froneman *et al*., 1996). Gamma radiation has also been tried for studying radio sensitivity of Kinnow nucellus (Altaf and Ahmed, 1997). In this report, we are evaluating effects of gamma radiation on nucellus regeneration of different *Citrus* cultivars that are growing in Punjab province.

Materials and Methods

Fruits were collected from NIAB, Ayub Agricultural Research Institute (AARI), Post-graduate Agricultural Research Station (PARS), Faisalabad and Horticultural Research Station, Sahiwal. Fruits were harvested in a period 45-90 days after pollination (DAP) from the above orchards.

Table 1: Fruit characteristics of *Citrus* species

Cultivar	Fruit weight (g)	Fruit diameter (cm)	Ovlue weight (g)	Ovule length (cm)	Ovule diameter (cm)
Kinnow	41.80	4.90	0.13	1.00	0.40
Fewtrell's early	22.02	3.45	0.06	0.72	0.30
Honey mandarin	23.60	3.50	0.18	0.43	0.21
Pixie	27.40	3.60	0.04	0.08	0.03
Ponkan	71.44	5.00	0.07	0.90	0.30
Foster	116.21	6.65	0.10	0.70	0.50
Shamber	302.80	9.50	0.53	1.50	0.80
Marsh	109.80	5.90	0.09	0.70	0.52
Chinese lemon	7.20	2.50	0.05	0.80	0.50
Eureka lemon	106.34	3.50	0.14	1.30	0.50
Minneola	49.13	4.45	0.29	0.63	0.28
Seminole	100.44	5.50	0.08	0.70	0.40
Jatti Khatti	72.94	4.80	0.10	1.00	0.40
Gada dehi	76.10	4.50	0.09	1.00	0.50
Kharna Khatta	196.73	7.30	0.14	1.00	0.50
Chakotra	189.00	9.23	0.52	0.85	0.48
Mitha	33.80	4.50	0.09	1.40	0.60
Tangerine	68.00	5.07	0.31	0.65	0.25
Orlando	102.42	5.00	0.15	0.90	0.50
Mosambi	39.70	3.90	0.08	0.83	0.35

The cultivars and their fruit and ovule characteristics studied are given in Table 1. Before utilizing these fruits for nucellus culture, they were exposed to gamma radiation at 0,3, 6, 9 and 12 Kr doses in a cell with Co^{60} as gamma source.

Fruits were washed thoroughly and surface sterilized by ethyl alcohol and flamed. Fruits were dissected in laminar air flow and normal ovules were picked. Both the ovule integuments were removed and nucellus tissues were cultured in flasks containing MS (Murashige and Skoog, 1962) medium supplemented with 0.5 mg/l BA and 5 mg/l glutamine. This medium was solidified with 1% agar and pH of medium was adjusted at 5.5-5.8, prior to autoclaving. The cultures were kept in 600 lux with 10 hours dark cycle. Temperature of growth room was 25 ± 2°C.

The data of percent nucellus responded to regeneration and the average number of embryos per nucellus was recorded after two months of culture period.

The coefficient of nucellar regeneration response was calculated by multiplying per cent nucellus responded to embryogenesis with the average number of embryos and the product divided by 100.

Table 2: Percent increase (+) or decrease (-) in coefficient of nucellar embryogenesis in radiated cultures as compared to control

Cultivar	3 Kr %	6 Kr %	9 Kr %	12 Kr %
Kinnow	8.00-	8.41-	8.68-	20.41-
Fewtrell's early	27.11-	41.34-	49.23-	32.69-
Honey mandarin	25.28-	32.67-	33.52-	50.28-
Pixie	29.68-	39.06-	37.50-	46.56-
Ponkan	24.72+	16.47-	45.31-	31.46-
Foster	49.78+	93.53+	41.29+	21.42-
Shamber	20.47-	10.47-	1.91+	44.28-
Marsh	38.74+	14.62+	3.95+	21.42-
Chinese lemon	11.00-	33.33-	11.00-	46.66-
Eureka lemon	12.73-	24.72+	25.09-	37.45-
Minneola	21.41-	24.62-	38.54-	56.31-
Seminole	7.00+	12.00-	28.25-	54.25-
Jatti Khatti	28.69-	42.82-	42.82-	57.17-
Gada dehi	20.00-	46.60-	60.00-	66.60-
Kharna Khatta	38.89+	15.63+	19.09-	44.44-
Chakotra	28.08-	0.00	31.46-	56.55-
Mitha	3.34-	10.71-	38.61-	54.46-
Tangerine	47.88-	41.31-	15.02+	15.17-
Orlando	3.52_	23.17-	33.75-	57.93-
Mosambi	14.70-	0.26+	59.89-	46.52-

Results

The immature fruits harvested for access of nucellus tissue was studied for their characteristics like fruit weight (g) and diameter (cm), ovule length, diameter (cm) and weight (g) as described in Table 1. The maximum fruit weight, diameter, ovule weight, length and diameter was found in cultivar Shamber of Grape fruit followed by fruit weight of cultivar Kharna khatta, diameter and ovule weight of Chakotra and ovule length and diameter of cultivar Mitha. The minimum fruit weight and diameter was of Chinese lemon and ovule weight, length and diameter was of Pixie.

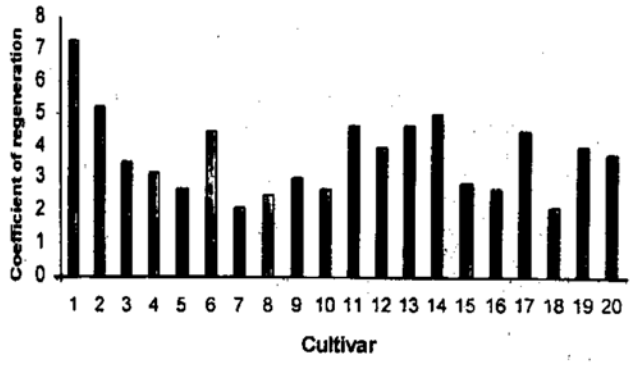

Fig. 1: Nucellar regeneration in reponse to control

Twenty cultivars showed variable behaviour for nucellar embryogenesis response and radiation sensitivity. The maximum coefficient of nucellar embryogenesis responses (CNR) in 0, 3, 9, 12 Kr doses were 7.25, 7.83, 6.62, 5.77, respectively for Kinnow (Fig. 1, 2, 4, 5) and 8.67 in 6 Kr for Foster of Grapefruit (Fig. 3).

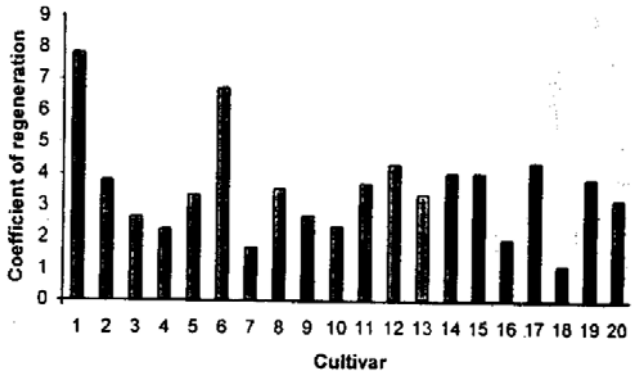

Fig. 2: Nucellar regeneration in response to 3 Kr gamma radiation

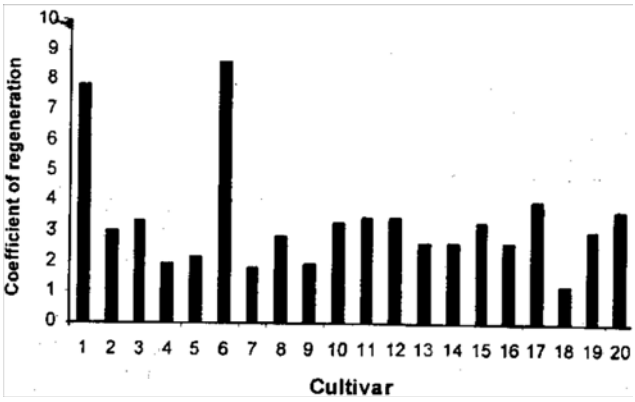

Fig. 3: Nucellar regeneration in response to 6 Kr gamma radiation

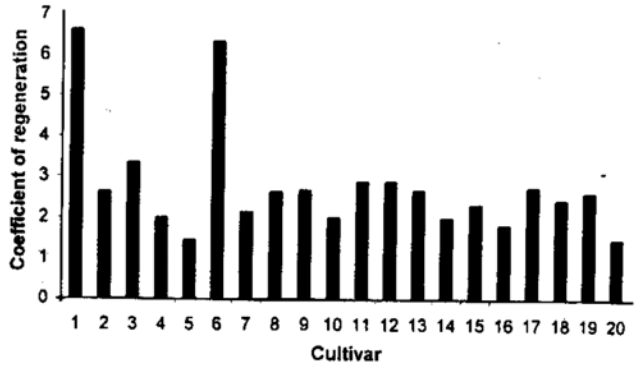

Fig. 4: Nucellar regeneration in response to 9 Kr gamma radiation

In 0Kr the minimum CNR value was 2.10 for cultivar Shamber (Fig. 1) which has maximum fruit weight and diameter, ovule weight, length and diameter, while 1.11, 1.25, 1.04 were in 3, 6, 12 Kr doses respectively for cultivar Tangerin (Fig. 2, 3, 5) and 1.46 was in 9 Kr for cultivar Ponkan (Fig. 4). However, coefficient of nucellar regeneration responses (CNR) of various *Citrus* cultivars seems to have no relation with the fruit characteristics data.

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

In 3 Kr dose of radiation, the increase in CNR value as compared to control (0 Kr) was noticed in cultivars Ponkan, Foster, Marsh, Seminole and Kharna khatta. The maximum increase in CNR was in cultivar Foster of Grape fruit followed by Kharna khatta and Marsh of Grape fruit. The maximum decrease in CNR was in cultivar Tangerine (Table 2).

In 6 Kr radiated nucellus cultures, the increase in CNR was observed in cultivars Kinnow, Foster, Marsh, Eureka lemon, Kharna khatta and Mosambi. The maximum increase in CNR value was in Foster and the minimum reduction in CNR was in rootstock cultivar Gada dehi (Table 2).

In 9 Kr radiation dose, the increase in CNR value was observed in Foster, Shamber, Marsh and Tangerine. The maximum increase in CNR value in 9 Kr was of cultivar Foster. The minimum decrease in CNR was in cultivar Gada dehi (Table 2).

In 12 Kr radiated nucellar cultures, all the cultivars had decreased CNR value as compared to control. The minimum decrease was in cultivar Marsh of Grape fruit and the maximum decrease in rootstock cultivar Gada dehi (Table 2).

The cultivar Foster of Grape fruit has increased CNR values in 3, 6 and 9 Kr radiation doses respectively as compared to 0 Kr, while rootstock cultivar Gada dehi has maximum decrease in 6, 9 and 12 Kr radiation doses.

Discussion

Citrus somatic embryogenesis has long been recognized as inherent ability of ovular tissues and also it is known that it has been affected by different media (Takayanagi *et al*., 1991).

Low cytokinin containing media seems to accelerate somatic embryogenesis as BA (0.1 mg/l) + GA (0.1 mg/l) gave the highest frequency of somatic embryogenesis from immature ovules of dangyooza (*Citrus grandis* Osbeck). In this study embryoid production was promoted by low cytokinin and was inhibited by auxin (Song *et al*., 1991). Age of fruit as days after pollination and the radiation doses have influence on embryogenesis (Altaf and Ahmad, 1997).

The fruit weight and diameter, ovule weight, length and diameter were studied to assess if these have any relation to nucellar embryogenesis potential in MS + BA (0.5 mg/l) + glutamine (5 mg/l). The results clearly demonstrated that fruit characteristics data have no responses which varied considerably among the genotypes studied. May be different genotypes require different culture media and environment for optimum responses. It is clear from figures that different radiation doses have different coefficient of nucellar regeneration values within the same cultivar and among different cultivars. It is true that nucellar embryogenesis differ within the nucelli cultured in the same flask because of endogenous differences in the tissues. The increase in radiation dose gradually have depression on embryogenic potential and some regenerants are weak and slow growing as compared to control regenerants.

These regenerants will be grafted onto one to two years plants because the radiated tissue derived embryos are difficult to survive in soil (Altaf and Ahmad, 1997). Grafting not only survive and grow mutant seedlings but this is the most efficient artificial method, which can reduce juvenility by two to four years (Ligeng *et al*., 1995).

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