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EFFECT OF GAMMA RADIATION ON SECONDARY SOMATIC EMBRYOGENESIS IN NUCELLUS CULTURES OF *CITRUS* CULTIVARS

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Abstract

Secondary somatic embryogenesis from 33 *Citrus* cultivars was studied with 0, 3, 6, 9 and 12 Kr doses of gamma radiation. Somatic embryogenesis and radiation sensitivity was cultivar dependent. Kinnow, Jaffa, Valencia, Blood red, Feutrell's early and Gada dehi showed good aptitude for embryo development while Chakotra, Tangerin, Eureka lemon, Kharna khatta, Chinese lemon, Gada dehi showed suppression of embryogenesis at 12 Kr radiation dose.

Introduction

Somatic embryos are formed by somatic cells except zygote either *in vitro* or *in vivo*. Adventive embryos are somatic embryos arising directly from other embryos or organs. Secondary somatic embryogenesis under *in vitro* conditions is the phenomenon where new somatic embryos are initiated from somatic embryos. It is associated with the loss of integrated group control of cells organized in the somatic embryos. Some cells break away from group control as pre- embryogenic masses and initiate new somatic embryos or globular embryos (Raemakers *et al.*, 1995). Advantage of secondary embryogenesis is high multiplication rate, independence of explant and repeatability.

In vitro selection of cyclic embryogenic nucellus culture lines are useful for *Citrus*. Since *Citrus* is highly heterozygous and has long juvenile period, the conventional breeding has led to relatively little improvement. The ability to alter a valuable cultivar for a single genetic trait has a great appeal when nucelli from various *Citrus* cultivars are radiated before *in vitro* culturing. The present report describes the effect of radiation on secondary somatic embryogenesis of nucellus tissue derived cultures of various *Citrus* cultivars that grow in the province of Punjab which is the major *Citrus* producing region of Pakistan with Kinnow mandarin as leading cultivar.

Materials and Methods

The experiment was conducted in Plant Tissue Culture Laboratory of the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad during 1997-2000. Fruits of 45 - 90 days after pollination (DAP) were obtained from NIAB orchard, AARI orchard, Post-graduate Agriculture Research Station University of Agriculture, Faisalabad and Horticultural Research Station, Sahiwal. After harvesting, the fruits were kept in a refrigerator until utilized for *in vitro* manipulation.

Citrus cultivars viz., Kinnow, Feutrell's early, Ponkan, Honey, Pixie, Tangerin, Orlando, Mosambi, Blood red, Pineapple, Valencia, Frost navel, Sanguinelli, Mediterranean, Succari, Ruby red, Washington navel, Moroblood, Jaffa, Hamlin, Tarocco, Seminole, Minneola, Sweet lime, Chinese lemon, Eureka lemon, Shamber, Foster, Marsh, Chakotra, Jatti Khatti, Kharna Khatta, Gada dehi were used in the study. Fruits were thoroughly washed with tap water and surface dried. The fruits were radiated at 0, 3, 6, 9, 12 Kr radiation doses in a gamma cell with Co^{60} source.

Table 1. Secondary embryogenesis in *Citrus* cultivars in response to gamma radiation.

	Radiation dose (Kr)				
Cultivar	**0**	**3**	**6**	**9**	**12**
Kinnow	7.90	8.83	8.39	8.00	7.00
Feutrell's early	5.85	5.00	4.85	4.20	4.15
Ponkan	3.80	3.68	3.48	3.00	2.85
Honey	4.10	3.90	3.85	3.00	2.60
Pixie	4.20	3.90	3.70	3.50	2.90
Tangerin	3.69	2.30	2.18	2.85	2.00
Orlando	5.20	4.28	4.10	3.90	2.80
Mosambi	5.30	4.60	4.20	3.20	2.90
Blood red	6.00	4.60	4.00	3.50	3.00
Pineapple	5.50	4.50	6.02	5.00	3.50
Valencia	6.50	6.30	6.00	5.50	4.00
Frost navel	5.00	4.50	3.50	3.00	2.67
Sanguinelli	4.50	3.50	2.87	2.83	2.50
Mediterranean	3.18	3.10	3.00	2.90	2.75
Succari	5.40	4.70	4.10	3.60	2.70
Ruby red	5.20	4.90	4.20	3.80	2.70
Washington navel	4.70	3.90	3.32	3.01	2.90
Moroblood	3.65	3.42	3.00	2.95	2.30
Jaffa	7.50	5.00	5.00	6.30	4.00
Hamlin	4.50	4.50	4.00	3.50	2.80
Tarocco	5.67	5.00	4.00	3.50	2.67
Seminole	4.80	4.75	4.01	3.89	2.50
Minneola	5.00	4.67	4.00	3.67	3.00
Sweet lime	5.38	5.20	5.00	4.00	3.00
Chinese lemon	4.00	3.50	3.00	3.00	2.33
Eureka lemon	3.50	3.00	3.67	2.67	2.00
Shamber	5.10	4.70	4.10	3.60	2.80
Foster	5.00	7.00	8.00	7.20	4.33
Marsh	4.00	4.60	3.89	3.30	3.29
Chakotra	3.10	3.00	3.10	3.00	1.95
Jatti Khatti	5.48	4.80	3.30	3.30	3.00
Kharna Khatta	4.33	4.30	4.00	3.00	2.00
Gada dehi	5.85	5.20	3.48	2.90	2.20

Before dissection the fruits were dipped in ethanol and flamed for surface sterilization. Healthy ovules were taken out in Petri plates and with sterile forceps and scalpel, the nucellus tissue was transferred onto MS medium (Murashige & Skoog, 1962) containing BA (0.5g/l) + Glutamine (5 mg/l) with 2% sucrose. The pH was adjusted at 5.5 - 5.8 and the medium with 1% agar was sterilized at 15 psi. Cultures were kept in low intensity light at 25 $\pm$ 2°C. After 2 months, embryogenic nucelli were recultured in the same fresh medium for further embryogenesis. The data for secondary embryos was taken after two months of first nucellus subculture from 0, 3, 6, 9 and 12 Kr radiation doses.

Results and Discussion

Secondary embryogenesis of nucellus tissue in response to various radiation doses (0,3,6,9,12 Kr) is presented in Table 1. Out of 33 cultivars examined maximum embryos per nucellus (7.90) in 0 Kr were found in Kinnow followed by Jaffa (7.50) and Valencia late (6.50), while minimum embryos 3.10 were in Chakotra. In radiation dose of 3 Kr Kinnow produced maximum number of embryos per nucellus (8.83) followed by Foster and Valencia late with 7.00 and 6.30 embryos respectively, while minimum response of 2.30 was in Tangerin.

Kinnow's response for embryo formation per nucellus was highest (8.39) in radiation dose (6 Kr) followed by Foster (8.00) and Pineapple (6.02) and minimum (2.18) in Tangerin. In radiation dose of 9 Kr maximum embryos (8.00) were recorded in Kinnow followed by Foster (7.20) and Jaffa (6.30), while minimum embryos per nucellus were noted in Eureka lemon (2.67). Maximum number of embryos were produced in Kinnow (7.00) in 12 Kr followed by Foster (4.33) and Fewtrell's early (4.15) and minimum embryos (1.95) in Chakotra.

In radiation dose (0 Kr) more than 5 embryos/nucellus were recorded in cultivars Kinnow, Feutrell's early, Orlando, Mosambi, Blood red, Pineapple, Valencia, Succarai, Ruby red, Jaffa, Tarocco, Sweet lime, Shamber, Jatti khatti and Gada dehi, while 5 embryos/nucellus in cultivars Frost navel, Minneola, Foster and less than 5 embryos in remaining cultivars. Five cultivars produced more than 5 embryos per nucellus, 3 cultivars 5 and the rest of the cultivars less than 5 in radiation dose of 3 Kr. In 6 Kr radiation dose, more than 5 embryos/nucellus were recorded in 4 cultivars, 5 embryos in 2 and in rest of the cultivars the response was less than 5. In radiation dose (9 Kr), 4 cultivars produced more than 5 embryos and 5 embryos in cv. Pineapple and less than 5 in remaining cultivars, while in 12 Kr radiation dose all cultivars except Kinnow produced less than 5 embryos per nucellus.

The embryogenic response of maximum cultivars except Kinnow, Tangerin, Pineapple, Jaffa, Eureka lemon, Marsh and Chakotra was in descending order with the increasing doses of radiation. The response of all Sweet orange cultivars for embryogenesis was excellent in control and the radiation doses was excellent as compared to other *Citrus* cultivars and nucellus can be exposed to comparatively high doses (6-12 Kr) of radiation for inducing mutations. Kinnow, Valencia, Jaffa had good aptitude for secondary somatic embryos as compared to Chakotra, Mediterranean and Eureka lemon. A specific *Citrus* clone or mutant multiplication can be benefited by this procedure.

Plant regeneration from callus somatic embryogenesis has been obtained from *Citrus* and its relatives (Jumin,1995) as for example nucellar embryo derived callus embryogenesis in

Citrus sinensis var. Mosambi (Tapati *et al.*,1995) which is known as secondary embryogenesis. The process of primary nucellar embryogenesis is divided into three parts (i) formation of primordium cell of the nucellar embryo (ii) division of these primordium cells (iii) development of nucellar embryos. Kobayashi *et al.*, (1979) reported that fertilization and pollination were not essential for the first step of nucellar embryogenesis. As for the third step, it is considered that pollination and fertilization are necessary for formation of endosperm whose nutrition helps the development of nucellar embryo. For the second step, the coditions are not clear. However, the pollinated ovaries showed a peak in endogenous cytokinin activity at anthesis in contrast to unpollinated ovaries (Francisca *et al.*,1990). Once started, *Citrus* nucellar embryogenesis process is always continued with subcultures as the production of adventitious embryos from nucellar embryos and nucellar cell derived callus which happens as inherent ability. Secondary embryogenesis is the next step of primary embryogenesis. However, if not properly maintained embryogenesis is gradually decreased or lost in subsequent generations of cultures. Exposure of nucellus (45-90 DAP) pre- embryogenic cells to gamma radiation and subsequent multiplication by subculturing of cell masses with embryogenic potential can help in high survival of mutant embryos.

References

Francisca, M., M. Hernandez and E. Primo-Millo. 1990. Studies on endogenous cytokinins in *Citrus*. *J. Hort. Sci.*, 65: 595-601.

Jumin, H. B. 1995. Plant regeneration via somatic embryogenesis in *Citrus* and its relatives. *Phytomorphology*, 45: 1-8.

Kobayashi, S., I. Isami and N. Muneichi. 1979. Studies of nucellar embryogenesis in *Citrus*. *J. Japan. Soc. Hort. Sci.*, 48: 179-185.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. plant.*, 15: 473 - 497.

Raemakers, C. J. J. M., E. Jacobsen and R. G. F. Visser. 1995. Secondary somatic embryogenesis and applications in plant breeding. *Euphytica*, 81: 93-10.

Tapati, D., G. C. Mitra and A. Chatterjee. 1995. Micropropagation of *Citrus sinensis* var. Mosambi - an important scion. *Phytomorphology*, 45: 57-64.

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