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Evaluating the impact of Gamma radiation and synergistic factors on lenticel development of *Populus deltoides* stem cuttings

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Abstract
Three different age group of *P. Deltoides* stem cuttings, *viz*. 6 months (young age), 12 months (middle age), and 24 months (old age), have been selected to develop their regeneration potential with lenticel development on the stem cuttings, which were treated with various continuous and fractionated doses of gamma rays. Young age radiated cuttings were also presoaked with Kinetin 500 ppm for their synergistic effects. The treated cuttings were applied in rainy season. The number and area in all treated cuttings were more favorable at lower continuous and fractionated doses. Maximum lenticel induction number percentage (80%) have been observed under 2KR-F treatment in old age stem cuttings, which is higher than in middle and young age treated cuttings in all applied continuous and fractionated doses. Lower concentration 500R-C (52% in old age), 2KR-F (58% in young age) and 2KR-F with Kinetin 500ppm (49% in young age) showed better induction percentage in comparison of control. The area of lenticels was also found to be higher in the cuttings treated with low concentration of gamma rays, i.e., 2KR-C 69% at middle age, 2KR-F 57% at old age and 2KR-C 52% at young age which was higher than the control old age cuttings (22%).

Keywords: Mutagenesis, kilo-radiate, periderm, stomatous, fractionated doses, transpiration

Introduction

The density and distribution of lenticels can influence gas exchange rates and overall plant physiological process. When plants do not have leaves, lenticels play a significant important role in the transpiration proces. Lenticels facilitate controlled gas exchange**,** while the periderm inhibits water loss from the stem through a combination of suberin and waxes, believed to be analogous to the epidermis and cuticle in leaves (Lendzian, 2006) [13]. Some species, especially among angiosperms, have low hydraulic safety margins, closing their stomata quickly (Choat *et al.* 2012) [2].

It characterized by a loose arrangement of cells in stem and fruits with large air pores allowing for efficient gases exchange (Angyalossy *et al*. 2016) [1]. They form during secondary growth or fruit development, often replacing the epidermis. The primary skins of young fruit are often stomatous. A cuticle envelops the outer surfaces of all cells of the epidermis including of the stomatal apparatus where the inner surfaces of the guard cells and of those forming the substomatal chamber are also cuticular (Franke, 1967; Norris and Bukovac, 1968) [6, 16]. The periderm and, in particular, the lenticels are still among the least studied plant parts not withstanding their protective well as physiological role in plant functioning (Leite & Pereira 2017; Serra *et al*. 2022) [12, 22]. Moreover, it also has an influence on the susceptibility of bark and wood to insects and pathogenic fungi (Rosner & Führer 2002; Nemesio-Gorriz *et al*. 2019) [15, 19]. Physiologially, lenticels perform important biomechanical functions by causing the contraction and expansion of the stem axis to fluctuations in bark water content and subsequent changes in stem diameter (Ilek *et al*. 2021; Van Stan *et al*. 2021) [9, 27]. They allow oxygen to reach internal tissues for respiration and carbon dioxide to be released during photosynthesis. Gamma rays were selected as mutagen for present investigation as it is known to be extremely potent and effective in causing mutagenic effects. Mutation breeding has been developed rapidly to become a useful method for crop improvement (Stadler L. J., 1928) [25]. The effect of gamma rays on sprouting, survival percentage, leaf and growth traits were found to be significant (Dhillon, DS & Dhillon, GPS 2018) [3]. Present study aims to find out the effects of gamma radiation on the induction percentage and area of lenticels.

Materials and Methods

P. deltoides D-121 male clone, chromosomes no. 38, young age, 6-month (1.5 cm diameter), middle age, 12-month (2.0 cm dia.), and Old age, 18-month (2.5 cm dia.) stem cuttings (18.0 cm length), have been taken in rainy season from the mother plant (eight years old) growing at Magroun, Poukhal, District Tehri Garhwal, Uttarakhand. Gamma irradiation was done at Radio Isotope Laboratory, FRI, Dehradun. The cuttings were irradiated with acute gamma rays at 500R, 1KR, 2KR and 4KR levels. Irradiation was carried out in a gamma chamber having a dose rate of 100 rads/sec of a Cobalt 60 source with a strength of 4,000 curies. The doses were given in two different ways, i.e., continuous and fractionated treatments. Under continuous doses, the cuttings were irradiated continuously by giving the desired dose uninterruptedly. The fractionated treatment was done by fractionating the doses in two equal parts allowing an interval of 24 hrs between two equally fractionated doses. After irradiation of the cuttings with acute gamma rays 10 irradiated cuttings in four replicates for each set were planted in polypots to a depth of about 8 cm during first week of rainy season. Only two cuttings

were potted in each pot. The upper end of the cuttings was covered with moist cotton plugs to avoid drying. Untreated specimens were treated as controls. For observing the synergistic effects of gamma rays with Kinetin 500 ppm another set of experimentation has been maintained. After the gamma rays' treatments, the cuttings were presoaked in Kinetin 500 ppm solution for 72 hours at room temperature ^{0}C/^{0}F (29±2.0). For control, cuttings have been presoaked in distilled water for same duration. Observations were recorded on lenticels development and their induction percentage in treated cuttings during growth behaviour.

Results and discussion

Lenticels play a significant role in transpiration, especially when plants do not have leaves, eventually, the lenticels regulate and control physiological activities of plants. To investigate this process, the effect of gamma rays on *P. detoides* stem cuttings was observed. The average number (cm^2) and area (mm^2) of lenticels with different continuous and fractionated doses of gamma ray and their synergistic effect with kinetin are shown in Tables 1 & 2. In young age stem cuttings, maximum number of lenticels was recorded as 5 at 2KR-C and 4KR-F level before irradiation, and after gamma rays' treatments this number increased to 7±0.54, which was higher than control 4±0.21. Similarly, in middle-aged cuttings, maximum number of lenticels 6±0.50 was recorded at 2KR-F level, but it was 7±0.54 in 2KR-C and F level after gamma rays' treatment. In old aged stem cuttings, maximum number of lenticels was recorded as 9±0.68 at 2KR-F and 8±0.61 at 4KR-F level after gamma rays' treatment. 2KR-C and 4KR-F level with Kinetin 500ppm, was found more effective and 7±0.54 lenticels number was recorded, which was higher than control (Table-1). The lenticels induction percentage is shown in Fig.-1. Fractionated doses showed higher induction percentage in most of the doses, compared to continuous doses. Among all age group of cuttings, highest lenticels induction percentage, was recorded at 2KR-F (old age 80%) and 2KR-F (young age 58%) levels, which was higher than control (35%).

Before gamma ray's treatment, the area of lenticels in young stem cuttings was observed to be higher at 2KR-C (6.12±0.51 mm^2), 1KR-F (5.11±0.31 mm^2) and 2KR-F (5.78±0.34 mm^2) levels, whereas after gamma ray's treatment, the area of lenticels was recorded highest at 2KR-C level i.e. 9.35±0.68 mm^2 (Table-2). While in middle aged cuttings, the area of lenticels was recorded as 9.75±0.69 mm^2 at 2KR-C level and 9.25±0.69 mm^2 at 2KR-F level which was higher than the control 6.11±0.51 mm^2. Lenticels area induction percentage was also recorded highest at 69% in middle aged cuttings (Fig. 2).

After gamma ray's treatment lenticel area induction percentage at 2KR-F level was recorded to be 11.50% mm^2 in old stem cuttings, which was higher than that of continuous and fractionated & young aged stem cuttings treated with kinetin 500 ppm. Effects of gamma rays with their synergistic effects showed lower lenticel area induction percentage in young aged cuttings compared to other age groups of cuttings in different continuous and fractionated doses. Lenticels varied in size and shape, but their cellular interaction of cells directly influced by the morhological arrangements and atmosheric activities. These radial columns were similar to the phelloderm structure of lenticels in the mangroves *Avicennia germinans* and *Rhizophora mangle* **(**Yanez-Espinosa & Angeles, 2022) [28]. They act as pores, allowing oxygen and carbon dioxide to move in and out, supporting respiration and photosynthesis. Lenticels develop from the rupture of stomata during secondary growth or fruit enlargement. Germination and bud formation in different age group stem cuttings of *P. deltoides* after various continuous and fractionated doses of gamma rays showed that water and minerals are required for physiological growth, and lenticels present in the stem are essential for exchange of gaseous. Tan *et al*. (2013) [26] found that aquaporins in salt-secretion glands of *Avicennia officinalis* could selectively reabsorb water, leaving salt behind. In the absent of stomata, lenticel play an essential role, in controlling the respiration and transpiration by determining exchange of gases and loss of moisture during development of fruit and during ripening (Dietz *et al*., 1988; Khader *et al*., 1992) [4, 10]. The development of lateral roots and sprouting of buds in treated cuttings are the result of the activities of lenticels. Pfanz, H *et al*. (2002) [17] proposed an additional role for the lenticel; suggested that it facilitate sunlight permeance, resulting in effective corticular photosynthesis. Older treated cuttings were generally found to have larger lenticels than young and middle-aged cuttings, indicating that older cuttings had a greater potential for gaseous exchange relative to the diameter. The number of lenticels varied according to fruit species and individuals. Diffusion of gases across the fruit boundary and loss of water vapor from the fruit occur either through aqueous/waxy layers of the epidermis or through gaseous pores (stomata and lenticel) **(**Solomos, 1987) [24]. The interaction of internal tissues is also important for gaseous exchange in cuttings. It is believed that lenticular cells are formed due to lack of oxygen supply in the peridermal cells. While changes in the redox state and metabolite concentration in the cell are also another reason for the formation of lenticular cells. Lenticels facilitate controlled gas exchange (Rosner & Morris, 2022) [20], while the periderm inhibits water loss from the stem through a combination of suberin and waxes, believed to be analogous to the epidermis and cuticle in leaves (Graca, 2015) [8]. After gamma radiation, a change was also seen in the colour of lenticels. The provided text highlights the role of a particular biological process, implicated in both vascular development and the synthesis of secondary metabolites like indole, alkaloids, and anthocyanins, as described in the work by Duszka *et al*. (2009) [5].

The colour of most of the cells changed to dark brown, which was light in colour before radiation. The appearance of lenticels on the surface of the fruit is highly variable, ranging in color from white to brown and in diameter from 2-3 mm (Khanal *et al*., 2020) [11]. Previous studies have suggested that lenticels act as channels for water movement and exchange of gases during vital activities of plants. Gil *et al*. (2000) [7] found that water was confined to lenticels and lenticular channels in cork cylinders soaked for 3 days, while a study on isolated periderm strips found lenticels to be far more permeable to liquid water under vacuum infiltration than the surrounding periderm (Schonherr & Ziegler, 1980) [21]. It is believed that lenticular cells are formed due to lack of oxygen supply in the peridermal cells. While changes in the redox state and metabolite concentration in the cell are also another reason for the formation of lenticular cells. However, water from storage tissue is discharged to the vascular tissue to support daily hydraulic function, and recharged following relaxation of

tension in the xylem vessels (Pfautsch, Holtta, *et al*., 2015) [18]. The size and spatial distribution of lenticels can affect gaseous exchange and internal activities of compact cells. so that it refers to formation and functional activities of lenticels. The number of lenticels in a single apple fruit changes remains constant or increases slightly during growth, while the density gradually decreases with the increase of fruit volume (Li *et al*., 2004) [14].

The clonal performance of 50 promising clones of *Populus deltoides* developed within and outside the country was tested for various six growth parameters in one-third commercial rotation period. Out of which selection of 10% i.e. 5 best clones, namely 40-N, UDH-9116, 25-N, 63-N and UDH-1002 gave an expected higher illiberality of 30.28% (Singh N B, *et al*., 2001) [23].

Table 1: *P. deltoides*- average number (cm^2) of lenticels with different doses of gamma rays and its synergistic effects with Kinetin 500ppm.

Dose/ Treatments	Young aged		Middle age		Old age		Young age (Kinetin500ppm)	
	A	B	A	B	A	B	A	B
Control	4±0.21	4±0.21	5±0.31	5±0.31	5±0.31	6±0.50	5±0.31	5±0.31
500R-C	5±0.31	5±0.31	5±0.31	5±0.31	4±0.21	6±0.50	5±0.31	6±0.50
1KR-C	5 ±0.31	6±0.50	5±0.31	6±0.50	5±0.31	7±0.54	4±0.21	5±0.31
2KR-C	5±0.31	7±0.54	4±0.21	7±0.54	6±0.50	8±0.61	5±0.31	7±0.54
4KR-C	5±0.31	6±0.50	4±0.21	6±0.50	5±0.31	7±0.54	5±0.31	6±0.50
500 R-F	4±0.21	4±0.21	4±0.21	5±0.30	4±0.21	6±0.50	4±0.21	6±0.50
1KR-F	5±0.31	6±0.50	5±0.31	6±0.50	6±0.50	8±0.61	5±0.31	5±0.31
2KR-F	4±0.21	7±0.54	6±0.50	7±0.54	5±0.30	9±0.68	5±0.31	7±0.54
4KR-F	5±0.31	6±0.50	5±0.31	6±0.50	6±0.50	8±0.61	5±0.31	6±0.50

SE ± of Means (A=Before treatment, B=After treatment)

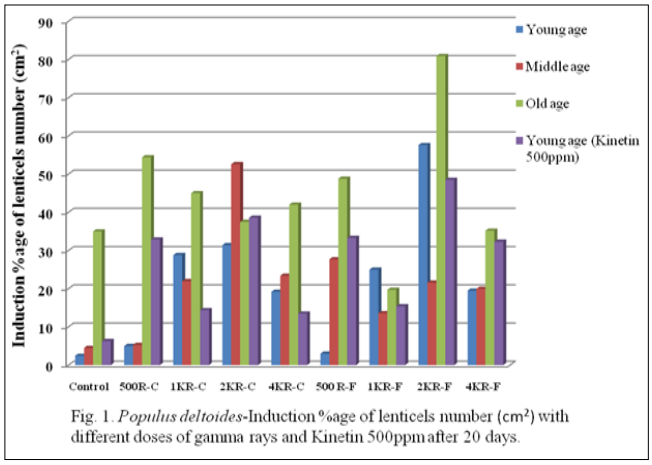

Fig. 1. *Populus deltoides*-Induction %age of lenticels number (cm^2) with different doses of gamma rays and Kinetin 500ppm after 20 days.

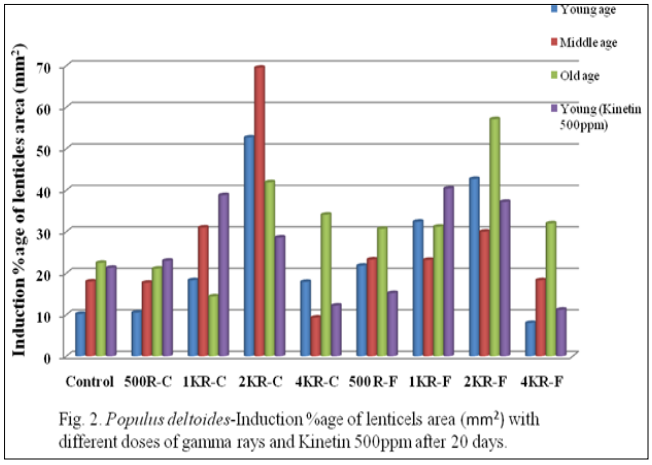

Fig. 2. *Populus deltoides*-Induction %age of lenticels area (mm^2) with different doses of gamma rays and Kinetin 500ppm after 20 days.

Table 2: *P. deltoides*- average area (mm^2) of lenticels with different doses of gamma rays and its synergistic effects Kinetin 500ppm.

Dose/ Treatments	Young aged		Middle age		Old age		Young age (Kinetin500ppm)	
	A	B	A	B	A	B	A	B
Control	5.25±0.33	5.78±0.34	6.11±0.51	7.21±0.50	6.0±0.50	7.35±0.53	5.15±0.31	6.25±0.51
500R-C	4.75±0.22	5.25±0.32	6.75±0.52	7.95±0.53	6.81±0.54	8.25±0.62	4.60±0.21	6.12±0.50
1KR-C	5.28±0.32	6.25±0.52	6.32±0.53	8.28±0.62	6.25±0.51	7.15±0.50	5.40±0.34	7.50±0.50
2KR-C	6.12±0.51	9.35±0.68	5.75±0.34	9.75±0.69	7.12±0.63	10.11±0.80	6.10±0.51	7.85±0.63
4KR-C	5.75±0.34	6.78±0.52	7.50±0.54	8.20±0.62	6.30±0.53	8.45±0.63	6.10±0.51	6.85±0.54
500 R-F	4.35±0.22	5.30±0.32	7.20±0.50	8.88±0.63	6.25±0.52	8.17±0.62	6.25±0.51	7.20±0.63
1KR-F	5.11±0.31	6.77±0.52	6.98±0.52	8.60±0.62	7.1±0.50	9.32±0.64	4.95±0.23	6.95±0.52
2KR-F	5.78±0.34	8.25±0.62	7.11±0.50	9.25±0.69	7.32±0.50	11.50±0.91	6.01±0.50	8.25±0.62
4KR-F	4.95±0.23	5.35±0.33	6.85±0.52	8.11±0.60	6.25±0.52	8.25±0.62	6.25±0.51	6.95±0.53

SE ± of Means (A=Before treatment, B=After treatment)

Conclusion

Gamma rays are used as a biological tool to observe mutagenic effects in various crops, i.e., when a specific dose of mutagen is given to the cells, the plant cells produce new mutagens. These mutagens are formed in plants during secondary growth or fruit development, often replacing the epidermis. The above study concludes that after gamma ray treatments the lenticels and the spaces between them increase the gas exchange rate during transpiration. This research investigates the interactions between various pathogens and lenticels, as well as the factors influencing disease susceptibility, the cellular and molecular processes involved in their formation including cell division, differentiation and activation of specific metabolic pathways.

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