Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2013, 3(2):266-270

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Effect of gamma irradiation on germination growth and biochemical parameters of *Pterocarpus santalinus*, an endangered species of Eastern Ghats

Akshatha and K. R. Chandrashekar*

Department of Applied Botany, Mangalore University, Mangalagangothri, Karnataka, India

---

ABSTRACT

Seeds of highly medicinal plant,P.santalinuswere subjected to different doses(0, 10, 25, 50, 100, 150, 200, 250 and 300 Gy) of gamma radiation using ^{60}Cosource and observed for germination, growth and biochemical attributes. Germination percentage was found to be highest at 50 Gy whereas, speed of germination increased up to 100 Gy. A threefold increase in vigor and growth in terms of dry mass was observed at 50 Gy and 100 Gyrespectively. An enhanced production of chlorophyll pigments like Ch-a, Ch-b, and total chlorophyll in leaf was observed at a dose of 50 Gy along with the total carbohydrate. An increase in phenolic content was observed at 25 Gy andmost of the treatments showed an enhanced radical scavenging activity compared to unirradiatedcontrol. Results obtained in the present research showed that low dose of gamma irradiation could be useful in enhancing growth and germination of economically important and endangered P. santalinus plant.

Key words: *Pterocarpus santalinus*, Gamma irradiation, Germination, Growth, Biochemical.

INTRODUCTION

*Pterocarpus santalinus*L.f. (Red Sanders) is an endemic andhighly endangered species largely confined to the southern portion of the Eastern Ghats with low regeneration capacity [1]. Heart wood of this plant is used inthe treatment of diabetes, and also in industries such as furniture, carvings, and musical instruments. Red pigment "santalin"is used as a coloring agent in cosmetics, pharmaceutical preparation, food stuffs, paper pulpwood, leather and in textile industries[2].

Radiation exposure of plants has both direct and indirect effects on seed germination, plant growth and reproduction by changing cellular and tissue structure or genetic aberration leading to different phenotypic development[3]. Irradiation with gamma rays iscurrently used as a tool in mutation breeding technology for enhancing the production of plant secondary metabolites like alkaloidsor to increase biomass production in medicinally valuable plants [4]. Even though, inhibitory effect of gamma irradiation was observed in plants like *Pisumsativum*[5]stimulation of seed germination, plant growth by gamma irradiation had been observed in most of the agricultural crops and also in three amaranth varieties[6], [7],[8].

Gamma rays are the electromagnetic radiation with highest form of energy having energy level ranging from 10 keV to several hundred keV, with higher penetration capacity [9]. Low dose gamma irradiation on plants are helpful in

Enhancing growth, chlorophyll pigment along with yield in okra [10].Although, lot of work has been carried out on the beneficial effects of ionizing radiation in improving the crop by reducing time of germination and accelerate the growth, insufficient work on radiation hormesisin forest tree species was performed due to their longer life span compared to crop plants. Till now, there is no report informingabout impact of gamma irradiation on enhancement of physiological characteristics of*P.santalinus*, where results of such experiments on highly endangered and endemic plant could be used to lay a foundation for the improvement of regeneration capacity and higher production of biomass in forest trees, where as radiation sensitivity of crop plants like Rice varieties were reported from earlier reports [11].Hence, the present work aiming at investigating the biological effects of presowing irradiation treatment on germination, growth and production of plant metabolites in *P. santalinus*has been designed.

MATERIALS AND METHODS

Mature seeds of *P. santalinus* were collected from the Kadaparegion of Eastern Ghats during the month of June and moisture content was found to be 6.558±0.141(mean±SD). Clean seeds were packed in polyethylene bags and subjected to different doses (10, 25, 50, 100, 150, 200, 250 and 300Gy) of gamma irradiation using gamma chamberwith ^{60} Cogamma source at dose rate of 1.386kGy/hr at BRIT (Board of Research in Radiation & Isotope Technology, Mumbai). Control sets were maintained without any treatment. Each treatmentwas replicated 5 times with 10 seeds in completely randomized block design. Along with control all treated seeds were kept for germination on sand bed for 70 days in green house of Applied Botany department, Mangalore University.

Speed of germination was determined using Eq (1) [12].

$$S= (N_1x1) + (N_2- N_1) \times1/2+ (N_3-N_2) x1/3……..+ (N_n-N_{n-1}) \times1/n. \quad (1)$$

Where $N1_{,}$ $N_{2,}$ N3…… N_{n-1}, $N_{n.}$ =Proportion of germinated seeds observed at 1, 2, 3……up-to n-1 and n^{th} day. Germination percentage (G) was calculated at the end using the Eq (2).

$$G= \frac{\text{Number of germinated seeds after nth day}}{\text{Total number of seeds kept for germiation}} \times 100. \quad (2)$$

Vigor was determined using the Eq (3), where V=Vigor index, %G=germination percentage, ASL=Average shoot length and ARL=Average root length.

$$V=\%G\times (ASL+ARL) \quad (3)$$

Shoot length, root length and number of leaves were measured on the day of harvesting. Relative growth rate was measured in terms of dry weight by keeping the plants in an oven at 103^{o}C for 18 hours.Total chlorophyll content and chl-a and chl-b in the leaves were determinedArnon method[13], proline content by Bates et. al[14], total carbohydrates by Anthrone method [15], phenolic content of ethanol extract of plants were determined using Gallic acid as standard[16]. DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined following Mensoretal. [17]and percentage radical scavenging activity was determined using the equation (4), ethanol with DPPH was used as control.

$$\%\text{Radical scavenging activity} = \frac{\text{Ab.of control} - \text{Ab.of sample}}{\text{Ab.of control}} \times 100 \quad (4)$$

The data were analyzed using IBM SPSS20 statistical software (SPSSInc. Chicago, IL, USA) in completely randomized block design. Mean values were compared using Duncan's Multiple Range Test (DMRT) at 0.05% level of probability.

RESULTS

3.1 Radiation effects on germination and growth

There was asignificant increase in the germination percentage of seeds treated with gamma rays compared to the control (Table. 1). The highest germination percentage of 51% was observed in the seeds treated with 50 Gy which was almost two fold higher thanthe control (23%). Gamma rays imposed a significant increase in the germination speed upto 100 Gy compared to control, the highest being 0.95 in the seeds treated with 25 Gy. Irradiation treatment

did not affect total shoot length but higher dose of 300 Gy declined the shoot growth.Similar to shoot,there was no significant difference in root length.

A gradual increase in vigor index was noticed in the seedlings up to50 Gy and further increase was notconsistent, the highest being in 50 Gy treated ones which wastwo fold higher than the control. Irradiation did not show any impact on leaf number. Asignificanttwo fold increase in dry weight of the plants at 10 Gy, 50 Gy and 100 Gywas noticed.

Table1. Effect of different doses of gamma irradiation on germination and growth parameters of *P.santalinus*.Means within a column followed by the same letter are not significantly different (P: 0.05). The data shown are means of five replicates ±SD.

SL. No	Dose	Germination%	Germination speed	Shoot length(cm)	Root length(cm)	Vigor index(V)	No.of leaves	Dry weight(g)
1	Control	23.5±1.5 ^{e}	0.605±0.2^{de}	14.75±3.15 ^{a}	25.45±9.3^{ab}	784.3±259.1^{c}	7.16±1.47^{a}	0.2±0.12^{d}
2	10gy	34.3±2.5 ^{c}	0.904±0.011^{ab}	12.57 ±2.27^{abc}	23.14±5.05^{abc}	1073.6±317.5^{bc}	5.96±1.5^{ab}	0.4±0.1^{ab}
3	25Gy	36.6±2.08^{bc}	0.95±0.015^{a}	12.09±1.72^{bc}	26.2±5.5^{ab}	1294.5±509.3^{b}	6.92±1.2^{a}	0.26±0.028 ^{cd}
4	50Gy	51 ±2.64 ^{a}	0.803±0.009^{abc}	12.29±1.7^{bc}	27.4±7.26^{a}	1827.18±820^{a}	7.23±1.165^{a}	0.39±0.13^{abc}
5	100Gy	30.6±1.5^{d}	0.876±0.019^{ab}	13.66±2.3^{ab}	25.18±10.14^{ab}	966.9±504.3^{c}	7±2.16^{a}	0.42±0.134 ^{a}
6	150Gy	30.3±1.5^{d}	0.654±0.01^{cde}	13.88±1.127^{ab}	21.94±6.14^{abc}	890±355.83^{c}	6.4±1.8^{a}	0.329±.0.029^{bcd}
7	200Gy	35.3±1.5^{bc}	0.754±0.011^{bcd}	12.88±2.08^{abc}	19.78±6.14^{bc}	1051.0±309^{bc}	7±1.7^{a}	0.3±0.1^{bcd}
8	250Gy	29.6±0.015^{d}	0.582±1.2^{e}	11.28±2.05 ^{cd}	19.5±6.29^{bc}	901.3±215.7 ^{c}	6.3±1.03^{ab}	0.21±0.04 ^{d}
9	300Gy	38.6±1.5^{b}	0.78±0.023^{abc}	9.827±1.86 ^{d}	17.76±4.95^{c}	1037.7±321^{bc}	5.3±1.6^{b}	0.23±0.075^{d}

3.2 Biochemical attributes

Effect of different doses of gamma irradiation on total chlorophyll content, total carbohydrate, proline, phenolics and DPPH radical scavenging activity is shown in Table 2. All the treatments showed enhanced Ch-a content compared to control. Two fold increase in Chl-b content was observed at 25 Gy and 100 Gy respectively. There was an increase in total chlorophyll content with increased dose of radiation upto 50 Gy, the highest being in 50 Gy which is three fold higher compared to control.

Seed treatment with gamma irradiation imposed a significant impact on total carbohydrate content where treatment with 50 Gy showed78.48% elevation in total carbohydrate content compared to control plants(Table 2).A slight increase in proline content was observed which is on par with the control plants.The application of gamma irradiation at a dose of 25 Gyand 200 Gy significantly increased the phenolic content compared to control ones. DPPH free radical scavenging activity increased with increasing dose up to 100 Gy.

Table 2. Effect of different doses of gamma irradiation on chlorophyll, total carbohydrate, proline, phenolics content and DPPH radical scavenging activity of *P.santalinus*.Means within a column followed by the same letter are not significantly different (P≤ 0.05).

Sl. No	Dose	Chlorophyll content (mg/g FW) a	b	Total	Total carbohydrate (mg/g DW)	Proline Mg/gFW	Phenolics mg/mg extract	DPPH scavenging activity (%)
1	Control	0.96±0.16^{c}	4.97±0.34 ^{b}	5.94±0.5 ^{d}	94.39±0.57 ^{cd}	0.0591±0.015^{ab}	0.114±0.0056^{c}	63.36±5.31 ^{e}
2	10Gy	1.93±0.093^{ab}	5.87±1.77 ^{b}	7.76±1.69^{bcd}	137.60±9.15^{b}	0.086±0.026 ^{a}	0.11675±0.002^{bc}	71.533±5.06^{d}
3	25Gy	2.13±0.13 ^{a}	10.45±0.71 ^{a}	11.03±1.79 ^{b}	128.1±10.1^{bc}	0.078±0.036^{a}	0.1483±0.0038^{a}	74.83±0.68 ^{cd}
4	50Gy	2.11±0.043 ^{a}	12.36 ±2.28 ^{a}	14.45±2.23 ^{a}	168.4±7.9 ^{a}	0.032±0.005 ^{b}	0.1195±0.0007^{bc}	86.166±0.5 ^{a}
5	100Gy	2.07±0.65 ^{a}	5.54±0.128 ^{b}	6.98±0.1^{cd}	77.3±7.03^{d}	0.0286±0.025 ^{b}	0.1049±0.0011^{d}	86.533±2.3 ^{a}
6	150Gy	1.54±0.29 ^{b}	7.64±1.26 ^{b}	9.21±1.67^{bcd}	72.76±6.5^{d}	0.029±0.013 ^{b}	0.10493±0.0029^{d}	83.873±1.97^{ab}
7	200Gy	1.97.1±0.113^{ab}	7.43±0.7 ^{b}	9.40±0.8^{bcd}	72.50±4.64^{d}	0.0512±0.039^{ab}	0.1244±0.0062^{b}	82.943±0.6^{ab}
8	250Gy	1.7±0.073^{ab}	6.64±0.07 ^{b}	9.47±1.95^{bcd}	79.80±10.7^{d}	0.023±0.0043 ^{b}	0.105±0.0038^{d}	61.433±3.72^{e}
9	300Gy	2.13±0.136 ^{a}	7.54±0.944 ^{b}	10.05±1.2^{bc}	94.61±7.23^{cd}	0.0783±0.016 ^{a}	0.125±0.00049^{b}	78.466±3.9^{bc}

DISCUSSION

Energy required for initial growth is already available in the seeds but low dose of gamma irradiation may increase the enzymatic activation which stimulates the rate of cell division along with vegetative growth [18][19]. The stimulation of germination may be due to the activation of RNA and protein synthesis which occurs during the early stages of germination [20]. Boosting of germination speed and percentage at lower doses of 20 and 30 Gy induram wheatwas observed [21].Similar results were observed in the present study on *P. santalinus*which showed low dose hormesis as explained by [22].Irradiation dose with 50 Gy enhanced the germination percentage whereas speed of seedling emergence was significantly higher in all doses compared to control.

Tangible support for the usefulness of low dose gamma irradiation at 0.03 to 0.07kGy in improving plant vigor in wheat was observed [23]. In okra, irradiation treatment at 10 and 20 Krad improved seedling vigor compared to control [24]. Similar observations were made on*P. santalinus*in the present study. In snap beans,plant vegetative growth parameters such as height, leaf number and dry weight recorded highest values with 30 Gy [25]. It was found that irradiation treatment with 400 Gy to 500 Gy enhanced the dry weight of the plant[26]. An increase in the dry weight at 100 Gy was observed in *P. santalinus* which is similar to the results obtained in *Vignaunguiculata*where,plant height and total number of leaves per plant was increased with a dose of 25 Krad [27].

Exposing the *Lactuca sativa var. capitata*seeds at doses ranging from 2-30 Gy enhanced the photosynthetic pigments such as chlorophyll a and chlorophyll b content. Interaction of gamma rays with okra seeds enhanced its chlorophyll content at 300 Gy and 400 Gy [11]. Several morphological and chlorophyll mutant varieties were obtained in Vignamungoby gamma irradiation[28].In the present study also, similar results were observed where irradiation treatment with 50 Gy was effective in enhancing the ch-a, Ch-b and total chlorophyll content in the seedlings obtained from gamma irradiated seeds.

Concurrent to enhanced pigment content in *P.santalinus* seedlings there was an enhancement in the carbohydrate content at 50 Gy.In chamomile seeds irradiation with 0.0 to 10 k-rad showed gradual increase in total soluble sugars with increase in dose[29]. In *Erucavesicaria*pre-sowing γ-irradiation treatment at the dose of 20 Gy enhanced the total soluble sugars [30]. From the present study and previous reports it is clear that the irradiation treatment with 50 Gy is effective in enhancing the chlorophyll content and also the synthesis of carbohydrates in *P.santalinus*.

Increase of phenolic acid was due to the free radicals formation during irradiation [31]. In *Eryngiumfoetidum*, treatment with gamma irradiation at 40 Gy enhanced the total phenolic content[20]. In *P.santalinus*an enhancement in phenolic production was observed at 25 Gy and 200 Gy.

Although no conclusive explanations for the stimulatory effects of low-dose gamma radiation on *P. santalinus*are available until now, it may be concluded on the basis of the results of the present study that the low dose of gamma irradiation treatment may help in triggering the germination and further growth of seedlings.

CONCLUSION

Irradiation of*P. santalinus* seeds showed anincrease in theproduction of chl-pigments and carbohydrates and other metabolites at lower dose may help in increased growth of plant. Phytochemical analysis revealed the production of higher concentration of metabolite likephenolics which act as a defense system for external stimuli. Enhanced phenolicsand other metabolites in plants are likely to enhance the capacity of scavenging the free radical formed during irradiation. Hence, lower irradiation could be useful in enhancing germination growth and production of metabolites in *P.santalinus*.

Acknowledgement
Authors are grateful to BRNS (Board of Research in Nuclear Sciences) for the financial support, Mangalore University for providing research facility, Dr. N. Sivaprasad, K.Kalpana and Dr.Vijay Kadwad,BRIT (Board of Radiation and Radioisotope technology) Mumbai for their help in irradiating the sample.

REFERENCES

[1] V. Rajeshwari, KailashPaliwal. *Indian journal Boitechnol*.,**2008**, 7, 541-546.
[2] T.T Mulliken, Petra Crofton. Review of the Status, Harvest, Trade and Management of Seven Asian CITES-listed Medicinal and Aromatic Plant Species BN federal agency for nature conservation.,**2008**, 77-92.
[3] De Micco, A. Carmen , P. Diana , M.Durante. *Environ. Biophys.*, **2011**, 50, 1-19.
[4] J.R.Sharma, R.K, Lal, H.O, Misra, M.M, Gupta, R.S, Ram. *Ram Euphytica* 1989, 40, 2 5 3 -258.
[5] R. Zaka, C. Chenal, M.T.Misset. *Science of the Total Environment*.,**2004,** 320, 121–129.
[6] Abd El-Hameed Abo HamadSh , M. Abu ElyazeedAbdElhaak, Mahfouz Ghareeb K. Saad-Allah.*IJPP*.,**2012**, 2 (3), 435 – 443.
[7] M.Melki, A.Marouni. *Environ. Chem. Lett.*,**2010**, 8 (4), 307-310.
[8] A.Aynehband, K.Afsharinafar *Euro. J. Exp. Bio*., 2(4), **2012,** 995-999

[9] E. Kovacs, A.Keresztes. *Micron.*,**2002,** 33 , 199-210.
[10] A.Z Hegazi, N.J.Hamideldin. *Hortic. For*., **2010**, 2(3) 038-051.
[11] D. Pavan Kumar, AnuragChaturvedi, M. Sreedhar,M. Aparna ,P. Venu-Babu, R. K. Singha*Asian J. Plant Sci. Res.*,**2013**, 3(1), 54-68.
[12] G.Chiapuso, A.M. Sanchez, M.J.Reigosa, L. Gonzalez, F. Pellissier. *J Chem. Ecol*., **1997**, 23, 2445–2453.
[13] D.L. Arnon. *Plant Physiol.*,**1949**, 24, 1-15.
[14] L. S Bates *Plant soil.*,**1973**, 39, 205-207.
[15] J.E.Hegde, B.T.Hofreiter Carbohydrate chemistry seventeenth ed. Academic Press, New York., **1962**
[16] M.S Taga, E.E Miller,D.E Pratt.*J. Am. Oil Chem. Soc.***1984,** 61, 928-931.
[17] L.I.Mensor, F.S.Menezes, G.G.Leitao, A.S Reis, T. Dos Santos, C.S Coube, S.G Leitao*Phytother. Res*., **2001**, 15, 127-130.
[18] J .Sjodin. *Hereditas.*,**1962**, 48, 565–573.
[19] T.M Shah, B.M Atta, M.A Haq, J.I Mirza*Pak. J. Bot.*,**2012**, 44(2), 631-634.
[20] A.A.Aly. *Tom*., XVII 2, **2010**, 356-361.
[21] M.Melki, T.H.Dahmani. *Pak.J.Biol.Sci*.,**2009**, 12(23), 1531-1534.
[22] S.Szarek . Part II. *EJPAU Serie Econ*., **2005,** 8, 61-61.
[23] B. Singh, P.S Datta. *Radiat. Phys. Chem*. 79, **2010,**139-143.
[24] Arvind-Kumar, M.N, Mishra. Adv. Plant Sci., **2004**, 17 (1) 295-297.
[25] A. Abou El-Yazied. *Aust. J. Basic & Appl. Sci*., **2011,** 5(11), 30-42.
[26] A. Z. Hegazi, N. Hamideldin *J. Hortic. For.* **2010,**2(3), 038–051.
[27] S.Gnanamurthy, S.Mariyammal, D.Dhanavel, T.Bharathi. *Int J Plant Sci*.,**2012**, 2(2), 39-42.
[28] U. P. Bhosale and B. V. Hallale. *Asian J. Plant Sci. Res*., **2011**, 1(2), 96-100.
[29] A.H.Nassar, M.F.Hashim, N.S. Hassan, H. Abo-Zaid. *Int. J. Agri. Biol***2004**, 6(5),776-780.
[30] H.R Moussa. *Russ Plant Physiol*., **2006**, 53, 193–197.
[31] X Fan, P.M.A Toivonen, K.T Rajkowski, K.J.BSokorai. *J. AgricFood Chem*., **2003**, 51, 1231-1236.