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Original Article

Radiosensitivity of *Albizia chinensis* (osbeck) Merr at Early Seedling Stage and Its Role in Vigor and Xylogenesis Enhancement

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ABSTRACT

The radiosensitivity of *Albizia chinensis* was determined by exposing its seeds to various gamma radiations doses. Air-dried seeds were subjected to numerous gamma irradiation doses using a 60Co gamma source (10 KR [kilorad], 20 KR, 40 KR, and 8.0 KR). The gamma irradiations were given in continuous and fractionated pattern. For analyzing the protagonistic and antagonistic effects of plant growth regulators with gamma radiations on xylogenesis development, GA3 and STIK were used for the experiment. The germination and vigor parameters were stimulated significantly ($p \leq .05$) by all fractionated doses. In contrast, the lower continuous doses (10 KRC [Continuous kilorad] and 20 KRC) increased the germination and vigor, while the higher irradiation inhibited the germination and vigor significantly. The growth and biomass attributes of *A. Chinensis* seedlings were altered by the irradiations in a dose-dependent manner. In this study, we observed that the gamma irradiation and combination treatments were favorable in enhancing the xylogenesis mechanism than the pure gamma irradiations. The overall data of this study suggest that the morphogenesis, growth, anatomy, and unknown cellular events in *A. chinensis* are influenced by gamma irradiations. The current study using *A. chinensis* as a case study reports crucial cues for selecting a specific irradiation type or dose depending on the need to maintain or improve a specific germination, growth, or xylogenesis property.

Keywords: *Albizia chinensis*, combination effects (gamma rays + GA3 and STIK), gamma irradiations, germination, vigor, xylogenesis, KRC and KRF(KiloRad - Continous and fractionated dose)

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Introduction

Albizia chinensis is a medium-sized armed deciduous tree (Family: Fabaceae) and it occurs naturally in India, Thailand, China, Java, and Myanmar. It has been grown extensively in tea plantations to provide shade and improving fertility of the soil (Nabil et al., 2020) and sometimes the tree is planted for stabilization of the slope also. Seeds of *A. chinensis* exhibit an adaptive dormancy with hard seed coat cover that ensure its survival under different climatic condition. The cultivation of *A. chinensis* with dormant seeds becomes a problem which delays germination and seedling growth development. The seed coat impermeable to water is a major cause of dormancy in this species. Gamma rays irradiation to seeds before germination can help in breakup seed coat dormancy and enhancing the germination growth and physiological and biochemical processes at its lower dose levels (Beyaz et al., 2016; Beyaz 2003; Saadati et al., 2022). *A. chinensis* tree has much economic importance, but has not got much attention for its conservation and genetic improvement. Rapid advancements in a variety of forest tree improvement fields have given rise to sophisticated techniques for developing forest tree species with adaptive responses (radioresistance). Gamma-induced mutagenesis has received the most attention of all the techniques due to its capacity to enhance a number of plant traits (Wi et al., 2007). Modification of growth and development of organs of higher plants may be induced by exposure to appropriate doses of irradiations. These modifications are due to damage to cytological, genetical, or physiological processes in cells and tissues (Gunckel 1957; Gunkel & Sparrow, 1954; Guncked & Sparrow 1961; Witherspoon, 1965).

Gamma rays relate to ionizing radiation and are the biggest energetic form of those kinds of electromagnetic radiation having an energy stage from round in 10 kiloelectron volts (keV) to many hundred keV. Accordingly, they have better penetrating power than various types of radiations, like alpha and beta rays (Kovacs & Keresztes, 2002), this is gamma rays' most practical and efficient quality, which has expanded the range of applications for them in the development of forest tree species. Gamma rays cause a variety of variations in physiological and economic characteristics (Fu et al., 2008; Matsukura et al., 2007). Gamma-induced mutagenesis has received a

lot of attention globally due to the way it works and how much it can enhance a variety of plant traits (Wi et al., 2007). These variations include changes in expression, growth and reproductive ability (Sumira et al., 2012), physiological variations like dilation of thylakoid membrane, modify in photosynthesis, variation of anti-oxidative system, concentration of anthocyanin, and initiation of trichome structure (Kim et al., 2004; Kovacs and Keresztes, 2002).

Previous research has demonstrated that low-dose gamma irradiation of plants increases their stress tolerance, germination rate, cell growth, enzyme activity, and crop yield (Charbaji & Nabulsi, 1999; Kim et al., 2012). Additionally, it has been reported that the growth, maturation, and disease resistance have all increased as well as the development of reproductive structures (Luckey, 1998). Plants exposed to gamma irradiation have a significant effect on their growth and development by inducing cytological, biochemical, genetic, physiological, and morphogenetic changes in the cells/tissues depending on the irradiation regime (Kim et al., 2004; Wani et al., 2018; Wi et al., 2005). But, across all these studies, only acute pattern of irradiations have been used. So there is also a need to alter the pattern of irradiations, which can generate new protocols and directions in the improvement of forest trees. Change at anatomical level in leaves and stem should often leads to likely function of significances of this radiation-induced structural changes. Differential radiation sensitivity of zones within shoot meristems have been observed by Pratt et al. (1959). After irradiation, both activation or inhibition of mitotic processes and induced physiological changes may occur.

In actuality, a lack of elite and well-established *A. chinensis* stands is caused by issues with germination and early seedling establishment. Thus the current study has been conducted to observe the reaction of *A. chinensis* toward diverse doses and sequence of gamma irradiations, which will support the development of earlier conservation and improvement strategies for this species.

Methods

The air-dried seeds have been used for the present experimentation. The moisture percent of the seeds of *A. chinensis* at the time of irradiation was 29.4%. The seeds were irradiated with gamma rays at various dose levels (10 KR, 20 KR, 40 KR, and 80 KR). The irradiation was carried out in gamma chamber at Forest Research Institute, Dehradun. The gamma chamber has a dose rate of 100 rad/s of CO^{60} source with a strength of 4000 curies (model 4000 A BARC, India). For continuous doses, the seeds have been irradiated continuously by giving the desired dose uninterruptedly at only one time. When using fractionated doses, it was done by dividing the dose into two equal portions and allowing 48 hours to pass between each dose. The seeds which were pre-treated by a desired dose, after 48 hours, these previously irradiated doses were irradiated with the same dose again.

The experiment was carried out in petridishes to look into seed germination and early seedling development. Both irradiated and non-irradiated seeds were scarred with sharp-edged blades to aid in absorption. Unirradiated seeds were taken as a control set. The treated and untreated seeds were then soaked in distilled water for 24 hours at room temperature (22˚C ± 1˚C). They were then washed with distilled water and put into petridishes. In petridishes, Whatman filter paper No. 1 has been used. The emergence of radicle has been treated as the germination criteria. Four replicates of 100 seeds each have been maintained for each treatment and planned in a randomized block design.

For analyzing the protagonistic and antagonistic effects of plant growth regulators with gamma radiations on the anatomy of hypocotyl and radicle, another set of experiment was conducted. GA3 and STIK [a new synthetic plant growth regulator supplied by an American company which has naphthalene acetic acid and sodium salts as main components] were used for this experiment. Two types of experimentation had been carried out for studying the interaction effects (protagonistic and antagonistic) of plant growth regulators and gamma rays on the anatomy of radicle and hypocotyl. The combinations were selected on the basis of previous work (Singh & Sujata, 2004; Singh & Vandana, 2008). The scarred irradiated seeds were soaked in 100 ppm solution of GA3 and STIK with different combination of irradiated seeds and growth regulators. The seeds were transferred to petridishes after a 24-hour pre-soaking period and were then cleaned with distilled water.

Germination was described by the presence of a radicle at least 2 mm long by naked eye (Mackay et al., 1995). From the beginning of the experiment until its conclusion, daily counts of seed germinations were made, and the germination percentage was then calculated (ISTA, 1999).

The germination energy index was determined using the daily germination record (GEI). Germination data were considered for GEI up until the day when germination became constant for consecutive days. On the same day, the GEI was calculated beginning with the following equation (1).

$$\mathrm{GEI}=\frac{A_1+(A_1+A_2)+(A_1+A_2+A_3)+(A_1+A_2+A_3+\cdots+A_n)}{Y\times N}\times 100 \quad (1)$$

where A_1, A_2, A_3, …, A_n is the number of seeds newly germinated on 1, 2, 3…, and nth days, respectively. N is the total number seeds used for the treatment, Y represents the number of days for each observation.

The germination value (GV) was determined from the daily germination records by applying Equation (2).

$$\mathrm{GV}=\mathrm{MDG}\times\mathrm{PV} \quad (2)$$

where MDG is mean daily germination and PV is the peak value.

Vigor was calculated (Akshatha, 2014) by Equation (3)

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

where V is the vigor index, %G is the germination percentage, ASL is the average shoot length, and ARL is the average root length.

Measurements of morphological traits like shoot length, radicle length, and area of cotyledonary leaves were made from the daily observed data record. The relative growth rate of the plants was calculated in terms of their dry mass by placing the plants in an oven set at 80˚C for 24 hours.

Histological changes in different parts of seedlings by the exposure of gamma irradiation treatment were studied. Radicle and hypocotyl of the treated and control group were cut and left in the FAA solution for more than 2 days and then preserved in 70% ethyl alcohol. The stored materials in 70% alcohol were dehydrated by passing them in ethanol-xylene series. While dehydration, the infiltration was carried out by adding paraffin wax to the ethanol-xylene mixture. By this process, the

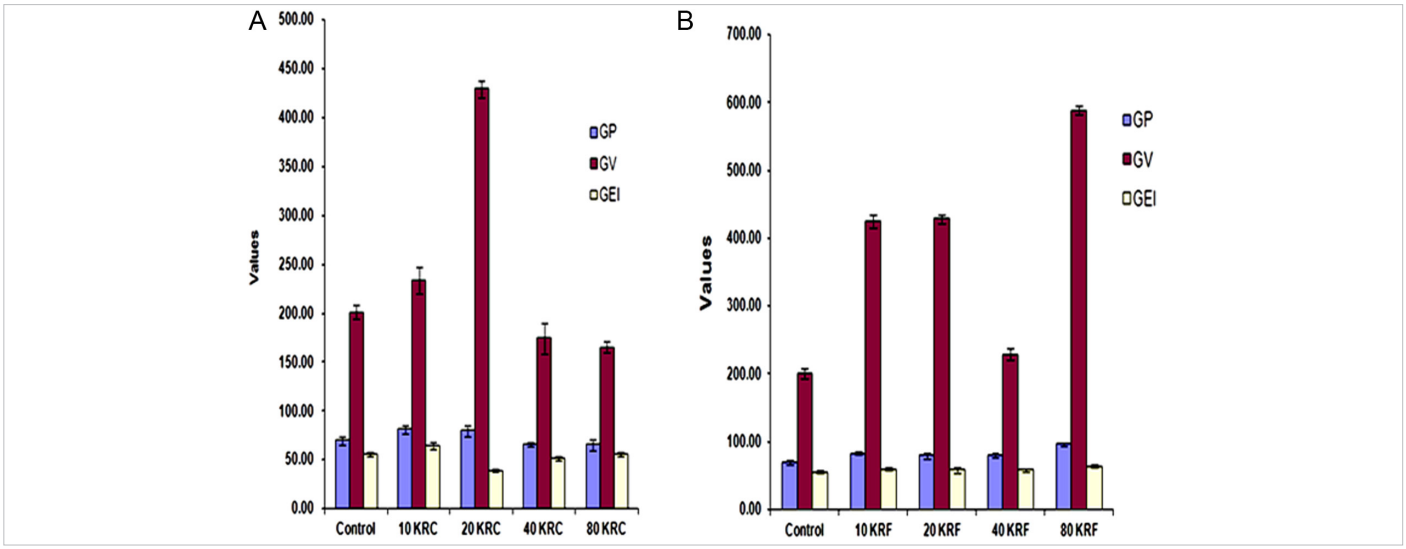

Figure 1.
Effect of Different Continuous (A) and Fractionated (B) doses of Gamma Irradiation on Germination Percentage (GP), Germination Value (GV), and Germination Energy Index (GEI) in A. chinensis After 1 Month. The data shown are mean ± SE. Bars indicate standard error; n = *4.*

dissolved paraffin accumulates into the radicle and hypocotyl. "After saturation," the radicle and hypocotyl were embedded in paraffin wax. The paraffin-embedded specimens were sectioned with rotary microtome. Sections were cleared of sodium hypochlorite and stained with aqueous safranin to visualize the important tissues and stained sections that were mounted on gelatin. After well-stained sections were photographed under a compound microscope, the number of cell layers in the xylem, cortex, and sclerenchyma, as well as the typical size of the xylem and cortex cells, were measured and noted.

Data Analysis

The data were tabulated and statistically analyzed with analysis of variance, and the treatment means were compared with the Duncan multiple range test (Duncan, 1965) at $p \leq .05$ level of significance.

Results, Discussion, and Conclusion

The separate doses of gamma radiations showed an increase in the germination percentage, germination energy index, and germination value (see Figure 1A). A twofold enhancement in the germination value was observed in all fractionated doses except 40 KR fractionated dose. 10 KR and 20 KR continuous dose levels showed an increase in germination percentage and germination value, but the 40 KR and 80 KR continuous doses showed reduction of germination over the control set (see Figure 1A). Across all continuous and fractionated doses, the maximum germination percentage (97.14%) and germination value (589.20) have been recorded under the 80KR fractionated treatment than the control (GP-70%,GV-200), and highest germination energy index (64.57) was obtained under 10 KR continuous dose than the control (55.71), respectively. The increased germination brought on by various gamma irradiations may result from the activation of RNA or protein synthesis (Waterworth et al., 2019) which has occurred during the early stage of germination after the seeds were exposed to gamma irradiation. This potential for fractionated doses over continuous in *A. chinensis* seed germination can be linked to a potential increase in the enzymatic activation system and subsequently the awakening of the young embryo, which increases the rate of cell division and consequently raises the germination percentage (Sjodin, 2009). In *Robinia pseudoacacia* and *Albizia julibriss* in the fractionated doses increased the germination value, germination percentage, and germination index than the continuous doses (Singh & Sujata, 2004, Singh & Vandana, 2008). On the other hand, the lower continuous doses also show an ability in the improvement of germination. In *Pterocarpus santalinus*, a lower continuous dose of 25 Gy and 50 Gy significantly increased the germination speed and vigor compared to the control (Akshatha, 2013). Both *Terminalia tomentosa* and *Pinus* (Thapa, 2004) have reported lower germination percentages with increasing gamma radiation doses (Singh & Wani, 2017).

Our results also reveal that the gamma irradiation above 20 KR continuous dose decreased the percentage of germination, germination energy index and germination value in *A. chinensis*. The increased production of reactive radicals that result in seed lethality and the lower germination caused by higher gamma irradiation doses may

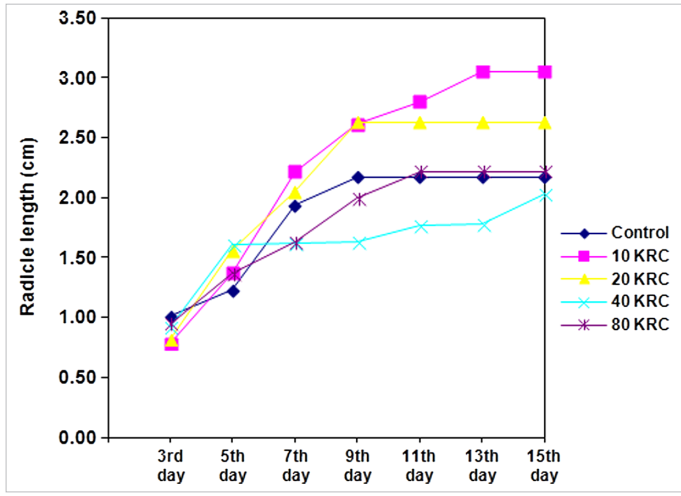

Figure 2.
Influence of Continuous Doses of Gamma Radiation on Radicle Growth (cm) of A. chinensis. The data shown are mean ± SE of four replicates (error bars = ±SE).

be caused by direct DNA or membrane damage (Maity et al., 2009). The same outcomes applied to *Tectona grandis* (Bhargava & Khalatkar, 1987).

In the earlier stage, there was no significant variation in radicle length between the treated and control groups. However, after the 15th day, it was observed that the lower continuous and fractionated doses recorded a higher radicle growth rate than the control. Except 40KR continuous doses, all the continuous and fractionated doses enhanced the radicle length than the control after 15 days of germination (see Figures 2 and 3). Across all the gamma irradiation doses, 10 KR continuous dose record a highest radicle length (3.05 mm) than control (2.17 mm) after 15 days. The hypocotyl growth in the seedlings of *A. chinensis* did not differ significantly between the treatment and control groups. Data pertaining to the hypocotyl growth depicts a radioresistance of hypocotyl growth toward all the continuous (see Figure 4) and fractionated (see Figure 5) gamma irradiation doses. The stimulation of cell division or cell elongation, along with the alteration of metabolic processes that affect the synthesis of phytohormones or nucleic acids, may be the causes of the seedling growth stimulation by lower doses of gamma irradiation (Riviello-Flores et al., 2022).

In *A. lebbek*, the lower continuous and fractionated doses increased the rate of radicle and hypocotyl growth, however the higher continuous doses caused reduction in these parameters (Singh & Paliwal, 1987). When the seeds were pre-exposed to various doses of gamma irradiations, an expansion of the shoot and root length in two species of *Pinus* was observed (Thapa, 2004). The enhancement of the early growth of the seedlings can be correlated with its faster seedling establishment. Seedling establishment of forest trees is a controversial issue for different plantation programs. The results of the present study can be used to overcome these problems in *A. chinensis* specific and other forest trees in general.

Fresh weight of the radicle was significantly stimulated in all the continuous and fractionated treatments (see Tables 1 and 2). 20 KR to 80 KR continuous doses recorded a twofold increase in the fresh weight of radicle than the control (see Table 1); in contrast, except 20 KR, the

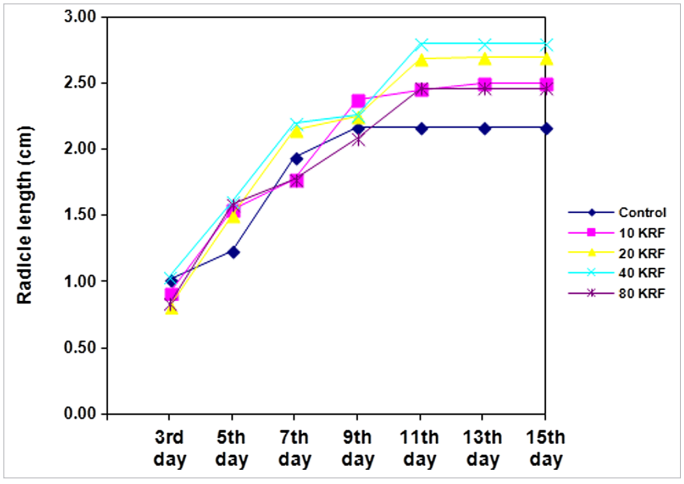

Figure 3.
Influence of Fractionated Doses of Gamma Radiation on Radicle Growth (cm) of A. chinensis. The data shown are mean ± SE of four replicates (error bars = ±SE).

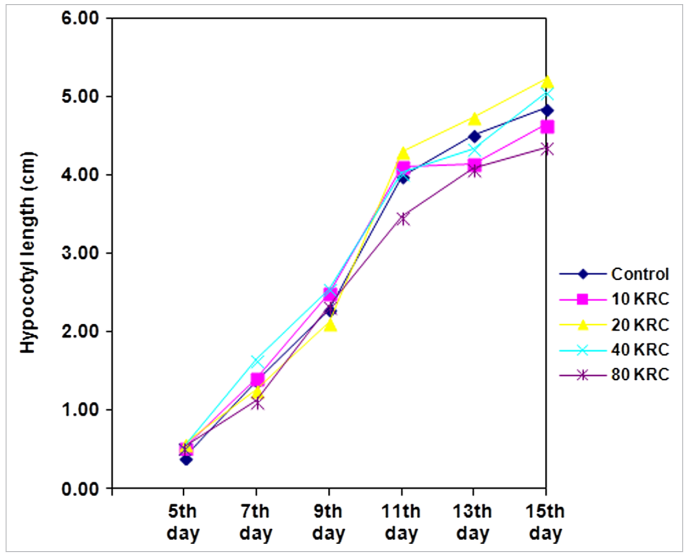

Figure 4.
Influence of Continuous Doses of Gamma Radiation on Hypocotyl Growth (cm) of A. chinensis. The data shown are mean ± SE of four replicates (error bars = ±SE).

dry weight of radicle does not show any significant difference between the treatments and control group. The fresh weight values of hypocotyl and cotyledon of all the treated seedlings had decreased in comparison to untreated seeds. Dry weights of hypocotyl were also found lower under all the continuous doses over control set (see Table 1). The dry weight of the cotyledons was not significantly different across all the treatments than the control, except 80 KRF (Fractionated kilorad) dose which recorded a significant stimulation in dry weight of cotyledon. A twofold improvement in the fresh weight of the shoot was obtained by 10 KR continuous dose (30.00 ± 0.82 mg) than the control (12.75 ± 1.08 mg), respectively; however, except 20 KR, the shoot dry weight does not differ significantly across all treatments and control group.

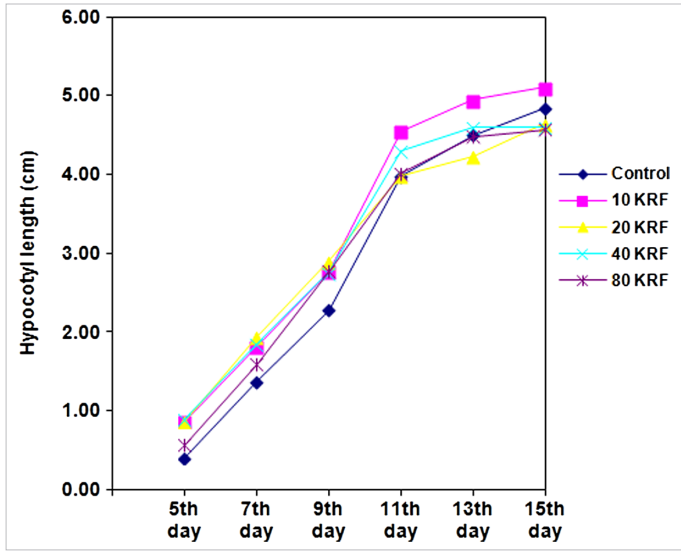

Figure 5.
Influence of Fractionated Doses of Gamma Irradiation on Hypocotyl Growth (cm) of A. chinensis. The data shown are mean ± SE of four replicates (error bars = ±SE).

Table 1.
Average Fresh Weight and Dry Weight of Different Parts of A. chinensis Seedling as Influenced by Continuous Dose of Gamma Rays After 23 Days (unit: mg)

	Radicle (mg)		Hypocotyl (mg)		Cotyledons (mg)		Shoot (mg)	
Dose	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight
Control	3.50 ± 0.14^{c}	0.25 ± 0.018^{b}	49.75 ± 2.20^{a}	1.75 ± 0.04^{a}	22.25 ± 0.93^{a}	0.75 ± 0.09^{NS}	12.75 ± 1.08^{d}	0.75 ± 0.017^{b}
10 KRC	5.50 ± 0.36^{b}	0.25 ± 0.019^{b}	45.25 ± 2.02^{b}	1.00 ± 0.03^{d}	22.75 ± 0.73^{a}	0.75 ± 0.04^{NS}	30.00 ± 0.82^{a}	0.75 ± 0.015^{b}
20 KRC	7.75 ± 0.49^{a}	0.50 ± 0.022^{a}	31.00 ± 1.14^{d}	1.25 ± 0.02^{c}	15.00 ± 0.69^{bc}	0.75 ± 0.07^{NS}	15.25 ± 0.37^{c}	1.00 ± 0.019^{a}
40 KRC	$7.00 \pm 0.64^{a\backslash}$	0.25 ± 0.031^{b}	37.00 ± 1.14^{c}	1.00 ± 0.01^{d}	17.00 ± 0.90^{b}	0.75 ± 0.03^{NS}	17.25 ± 0.35^{bc}	0.50 ± 0.022^{c}
80 KRC	7.25 ± 0.18^{a}	0.25 ± 0.017^{b}	42.00 ± 1.37^{b}	1.50 ± 0.07^{b}	12.25 ± 0.71^{c}	0.75 ± 0.04^{NS}	19.50 ± 1.40^{b}	1.00 ± 0.048^{a}
One-way analysis of variance	*	*	**	**	*	NS	**	**

The data shown are mean ± SE of four replicates. Means within a column followed by the same letter are not significantly different ($p \leq .05$). Different letters a, b, c, d, and e denote significant differences ($p \leq .05$) between different treatments.
*$p \leq .05$.
**$p \leq .001$.
Note: NS = not significant. 10,20,40,80 KRC (KiloRad Continous doses); KRC = KiloRad Continuous.

Table 2.
Average Fresh Weight and Dry Weight of Different Parts of A. chinensis Seedlings as Influenced by Fractionated Dose of Gamma Rays After 23 Days (unit: mg)

	Radicle (mg)		Hypocotyl (mg)		Cotyledons (mg)		Shoot (mg)	
Dose	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight
Control	3.50 ± 0.14^{c}	0.25 ± 0.01^{NS}	49.75 ± 2.20^{a}	1.75 ± 0.04^{ab}	22.25 ± 0.93^{b}	0.75 ± 0.01^{b}	12.75 ± 1.08^{c}	0.75 ± 0.01^{b}
10 KRF	7.25 ± 0.18^{b}	0.25 ± 0.02^{NS}	47.75 ± 1.15^{ab}	2.00 ± 0.09^{a}	22.25 ± 0.79^{b}	0.75 ± 0.06^{b}	22.25 ± 0.93^{a}	1.00 ± 0.05^{a}
20 KRF	9.75 ± 0.30^{a}	0.25 ± 0.01^{NS}	43.25 ± 0.99^{b}	1.50 ± 0.02^{b}	24.00 ± 0.96^{b}	0.75 ± 0.03^{b}	18.50 ± 0.80^{b}	1.00 ± 0.05^{a}
40 KRF	10.00 ± 0.31^{a}	0.25 ± 0.04^{NS}	38.50 ± 0.38^{c}	1.50 ± 0.04^{b}	25.50 ± 0.47^{b}	0.75 ± 0.04^{b}	24.75 ± 0.93^{a}	0.75 ± 0.04^{b}
80 KRF	9.50 ± 0.26^{a}	0.25 ± 0.01^{NS}	42.25 ± 0.80^{b}	1.00 ± 0.09^{c}	40.25 ± 1.17^{a}	1.50 ± 0.10^{a}	17.00 ± 0.92^{b}	0.50 ± 0.02^{c}
One-way analysis of variance	*	NS	**	*	*	*	**	**

The data shown are mean ± SE of four replicates. Means within a column followed by the same letter are not significantly different ($p \leq .05$), Different letters a, b, c, d, and e denote significant difference ($p \leq .05$) between different treatments.
*$p \leq .05$.
**$p \leq .001$.
Note: NS = not significant. 10,20,40,80 KRF doses are (KiloRad Fractionated dose); KRF = KiloRad Fractionated dose.

The effect of various doses of gamma radiations on the plant biomass accumulated in different parts has been documented, which showed that fresh weight of radicle and hypocotyls was increased by all doses of gamma irradiations. However, the dry weight of radicle and hypocotyl was not influenced by the different treatments of gamma radiations. Increase in the overall plant biomass was dose dependent. The fractionated doses were found effective in increasing the plant biomass (see Table 2) over the control. However, the lower doses of continuous pattern showed the same results. The higher continuous doses were found to be lethal. A similar pattern was observed in *A. julibrissin* in which the irradiated plants accumulated more biomass than control plants (Singh & Vandana, 2008). Higher gamma irradiation doses significantly reduce mitotic activity, which in turn reduces seedling growth and biomass (Charumathi et al., 1992; Khan et al., 2000; Wi et al., 2007), low doses of gamma irradiation, on the other hand, could be used as a safer and more stimulatory tool to improve these plant growth characteristics (Kumari & Singh, 1996).

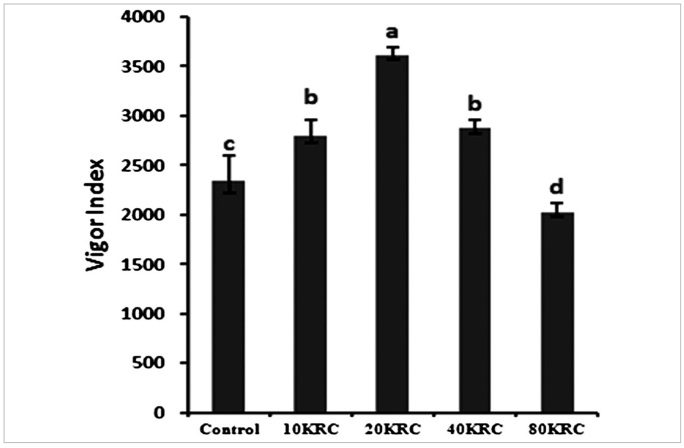

Figure 6.
Effect of Continuous Doses of Gamma Irradiation on the Vigor in Seedlings of A. chinensis. Bars indicate standard error; n = *4; different lowercase letters above the bars indicate significant differences* ($p \leq .05$).

The vigor index is a result of the total length of the seedlings and the percentage of germination when exposed to various doses of gamma radiation (see Figures 6 and 7). The highest vigor index (3916) was observed

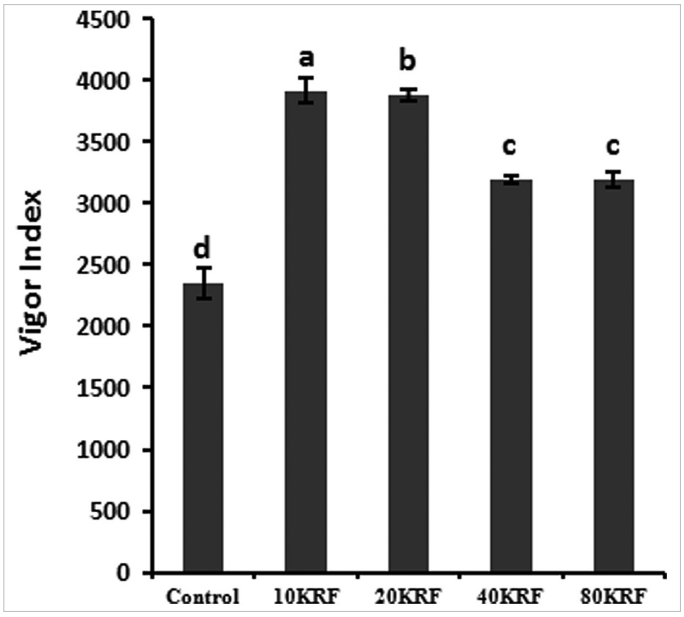

Figure 7.
Effect of Fractionated Doses of Gamma Irradiation on the Vigor in Seedlings of A. chinensis. Bars indicate standard error; n = *4; different lowercase letters above the bars indicate significant differences* ($p \leq .05$).

for the plants from seeds irradiated with 10 KRF, followed by the plants of seeds irradiated with 20 KRF (3876), 20 KRC (3612.4), 80 KRF (3194), 40 KRF (3192), 40 KRC (2873), 10 KRC (2803), and 80 KRC (2028.72), respectively. An increase in the vigor index was recorded under all the doses of fractionated doses than the control set. However, the lower continuous doses increase the vigor index than the higher doses. The increase in the vigor index may have happened as a result of their adapted resistance to radiation stress and the stimulatory effect of lower gamma irradiation doses (Luckey, 1998). It also affects enzyme activity since the higher seedling vigor is associated with germination efficiency. It is possible that the seedling vigor is stimulated by the higher amylase activity, which is enhanced by the lower continuous and fractionated gamma irradiation doses (Afzal et al., 2008; Kumagai et al., 2000). The findings of the present study agreed with those of reports on *Terminalia arjuna* and *Pterocarpus santalinus* (Akshatha et al., 2013; Akshatha & Chandrashekar, 2013), where it was seen that the lower doses of gamma irradiations stimulate the vigor index of the seedlings. The vigor of the seedlings in *Bambusa arundinacea* was improved by low doses of gamma radiation (Jan et al., 2011). These radiopotential doses can aid in the development of seedling stock with better establishment, which can be used to supplement the issues with this tree's natural regeneration in forests.

Witherspoon and Taylor Jr (1969) exposed the apical meristem of *Pinus uriginia*is acute closes of fast neutron radiation (100 and 300 rads) and were collected 2 weeks later. Apies from the plant receiving 100 rads showed necrosis of cells in central mother cell zone. There is considerably less work has been reported of irradiation on the process of initiation and matriration of vascular tissues (Haber & Foard, 1964). Transverse section of hypocotyl of the seedlings treated with 20 KRC + GA3 show five-layered sclerenchyma in contrast the control set which had only two-layered sclerenchyma (see Tables 3 and 4). The seedlings treated with 20 KRF + STIK showed one-layered sclerenchyma in the hypocotyl. The numbers of xylem layers were more in the seedlings treated with gamma rays and STIK combinations. Seedlings treated with 80 KRF+ STIK combination had five-layered xylem where the untreated set has only three-layered xylem. The gamma rays and GA3 combination has two-layered xylem and the size of xylem cells remains unchanged. As far as cell layers in the cortex are concerned, STIK combinations with gamma rays resulted in an increase in the cell layers of cortex. The 20 KRF + STIK combination has 12-layered cortex, 80 KRC + STIK has 14-layered cortex, 20 KRF + STIK dose showed only six layers, and 80 KRF + STIK combinations had 10 layers where the control set showed only six layers and 80 KRF + STIK combination had 10 layers where the control set showed only five-layered cortex cells. GA3 and gamma ray combinations were more or less similar to the control set. The size of the cortical cells was also found increased in STIK combinations. The 80 KRC + STIK combination had 60.50 µm where the control set showed 32.04 µm cortical cell sizes.

Transverse sections of radicals were viewed under the microscope and it was observed that the cortical was seven layered and xylem was three

Table 3.
Influence of Continuous Doses of Gamma Irradiations and Its Combinations on Number and Size of Cell Layers in Cortex, Xylem, and Sclerenchyma of Hypocotyl in A. chinensis

Dose	Number of Cell Layers in Cortex	Average Size of Cortical Cells (µm)	Number of Cell Layers in Xylem	Average Size of Xylem Cells (µm)	Number of Cell Layers in Sclerenchyma
Control	7.0 ± 0.31^{d}	32.04 ± 0.70^{cd}	3.0 ± 0.07^{ab}	13.75 ± 0.84^{NS}	2.0 ± 0.06^{ab}
20 KRC	7.0 ± 0.36^{d}	36.71 ± 1.21^{c}	3.0 ± 0.04^{ab}	13.75 ± 0.60^{NS}	4.0 ± 0.07^{a}
20 KRC + GA3	8.0 ± 0.21^{c}	27.50 ± 0.98^{d}	2.0 ± 0.04^{b}	13.75 ± 0.56^{NS}	5.0 ± 0.06^{a}
20 KRC + STIK	12.0 ± 0.55^{a}	50.46 ± 1.36^{b}	4.0 ± 0.06^{a}	13.75 ± 0.47^{NS}	3.0 ± 0.04^{b}
80 KRC	8.0 ± 0.35^{c}	32.04 ± 1.29^{cd}	2.0 ± 0.03^{b}	13.75 ± 0.50^{NS}	1.0 ± 0.02^{b}
80 KRC + GA3	8.0 ± 0.17^{c}	32.04 ± 0.64^{cd}	2.0 ± 0.04^{b}	13.75 ± 0.51^{NS}	2.0 ± 0.04^{ab}
80 KRC + STIK	9.0 ± 0.42^{b}	60.50 ± 1.17^{a}	3.0 ± 0.06^{ab}	13.75 ± 0.21^{NS}	3.0 ± 0.05^{a}
One-way analysis of variance	*	**	*	NS	*

The data shown are mean ± SE of four replicates. Means within a column followed by the same letter are not significantly different ($p \leq .05$), Different letters a, b, c, d, and e denote significant differences ($p \leq .05$) between different treatments.
*$p \leq .05$.
**$p \leq .001$.
Note: NS = not significant. 20,80KRC = KiloRad Continuous; GA3 = Gibberellic acid (100 ppm); STIK = Synthetic Plant Growth Regulators (100 ppm).

Table 4.
Influence of Fractionated Doses of Gamma Irradiations and Its Combinations on Number and Size of Cell Layers in Cortex and Xylem of Hypocotyl in A. chinensis

Dose	Number of Cell Layers in Cortex	Average Size of Cortical Cells (µm)	Number of Cell Layers in Xylem	Average Size of Xylem Cells (µm)	Number of Cell Layers in Sclerenchyma
Control	7.0 ± 0.31^{ab}	32.04 ± 0.70^{b}	3.0 ± 0.07^{c}	13.75 ± 0.84^{NS}	2.0 ± 0.06^{a}
20 KRF	8.0 ± 0.28^{ab}	32.04 ± 0.61^{b}	2.0 ± 0.04^{d}	13.75 ± 0.41^{NS}	2.0 ± 0.03^{a}
20 KRF + GA3	7.0 ± 0.37^{ab}	32.04 ± 0.80^{b}	4.0 ± 0.04^{a}	13.75 ± 0.21^{NS}	3.0 ± 0.05^{a}
20 KRF + STIK	6.0 ± 0.40^{b}	41.04 ± 0.50^{a}	2.0 ± 0.03^{d}	13.75 ± 0.35^{NS}	1.0 ± 0.02^{a}
80 KRF	7.0 ± 0.17^{ab}	13.75 ± 0.21^{c}	3.0 ± 0.02^{c}	13.75 ± 0.27^{NS}	2.0 ± 0.04^{a}
80 KRF + GA3	8.0 ± 0.35^{ab}	13.75 ± 0.17^{c}	3.0 ± 0.05^{c}	13.75 ± 0.19^{NS}	2.0 ± 0.04^{a}
80 KRF + STIK	10.0 ± 0.62^{a}	41.25 ± 0.35^{a}	5.0 ± 0.04^{a}	13.75 ± 0.14^{NS}	3.0 ± 0.06^{a}
One-way analysis of variance	*	**	*	NS	*

The data shown are mean ± SE of four replicates. Means within a column followed by the same letter are not significantly different ($p \leq .05$). Different letters a, b, c, d, and e denote significant differences ($p \leq .05$) between different treatments.
*$p \leq .05$.**$p \leq .001$
Note: NS = not significant. KRF = KiloRad Fractionated dose; GA3 = Gibberellic acid; STIK = Synthetic Plant Growth Regulators.

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Table 5.
Influence of Continuous Doses of Gamma Irradiations and Its Combinations on Number and Size of Cell Layers in Cortex and Xylem of Radicle in A. chinensis

Dose	Number of Cell Layers in Cortex	Average Size of Cortical Cells (µm)	Number of Cell Layers in Xylem	Average Size of Xylem Cells (µm)
Control	6.0 ± 0.21^{a}	55.00 ± 1.58^{ab}	3.0 ± 0.08^{b}	27.50 ± 1.13^{a}
20 KRC	7.0 ± 0.16^{a}	50.46 ± 2.20^{b}	5.0 ± 0.04^{ab}	13.75 ± 0.44^{b}
20 KRC + GA3	8.0 ± 0.32^{a}	48.13 ± 1.14^{b}	7.0 ± 0.10^{a}	27.50 ± 0.65^{a}
20 KRC + STIK	6.0 ± 0.14^{a}	59.00 ± 0.14^{a}	4.0 ± 0.08^{ab}	27.50 ± 1.02^{a}
80 KRC	6.0 ± 0.18^{a}	55.00 ± 0.98^{ab}	4.0 ± 0.14^{b}	13.75 ± 0.98^{b}
80 KRC + GA3	8.0 ± 0.33^{a}	37.81 ± 0.64^{c}	2.0 ± 0.06^{b}	13.75 ± 0.85^{b}
80 KRC + STIK	6.0 ± 0.46^{a}	34.38 ± 1.13^{c}	5.0 ± 0.13^{ab}	13.75 ± 0.72^{b}
One-way analysis of variance	NS	**	*	*

The data shown are mean ± SE of four replicates. Means within a column followed by the same letter are not significantly different ($p \leq .05$). Different letters a, b, c, d, and e denote significant differences ($p \leq .05$) between different treatments.
*$p \leq .05$.**$p \leq .001$.
Note: NS = not significant. 20,80 KRC = KiloRad Continuous; GA3 = Gibberellic acid (100ppm); STIK = Synthetic Plant Growth Regulators (100 ppm).

Table 6.
Influence of Fractionated Doses of Gamma Irradiations and Its Combinations on Number and Size of Cell Layers in Cortex and Xylem of Radicle in A. chinensis

Dose	Number of Cell Layers in Cortex	Average Size of Cortical Cells (µm)	Number of Cell Layers in Xylem	Average Size of Xylem Cells (µm)
Control	6.0 ± 0.21^{a}	55.00 ± 01.58^{a}	3.0 ± 0.08^{ab}	27.50 ± 1.13^{a}
20 KRF	8.0 ± 0.30^{a}	44.69 ± 01.14^{b}	3.0 ± 0.06^{ab}	13.75 ± 0.47^{b}
20 KRF + GA3	7.0 ± 0.14^{a}	55.00 ± 01.31^{a}	4.0 ± 0.04^{a}	13.75 ± 0.69^{b}
20 KRF + STIK	8.0 ± 0.21^{a}	79.06 ± 01.69^{a}	3.0 ± 0.09^{ab}	27.50 ± 0.94^{a}
80 KRF	8.0 ± 0.47^{a}	51.56 ± 01.28^{b}	2.0 ± 0.06^{b}	13.75 ± 0.35^{b}
80 KRF + GA3	6.0 ± 0.17^{a}	55.00 ± 0.90^{a}	4.0 ± 0.10^{a}	13.75 ± 0.21^{b}
80 KRF + STIK	7.0 ± 0.24^{a}	79.06 ± 01.84^{a}	4.0 ± 0.09^{a}	27.50 ± 0.78^{a}
One-way analysis of variance	NS	**	*	**

The data shown are mean ± SE of four replicates. Means within a column followed by the same letter are not significantly different ($p \leq .05$). Different letters a, b, c, d, and e denote significant differences ($p \leq .05$) between different treatments.
*$p \leq .05$. **$p \leq .001$. *Note:* NS = not significant. 20,80 doses KRC = KiloRad Continuous; GA3 = Gibberellic acid (100 ppm); STIK = Synthetic Plant Growth Regulators (100 ppm).

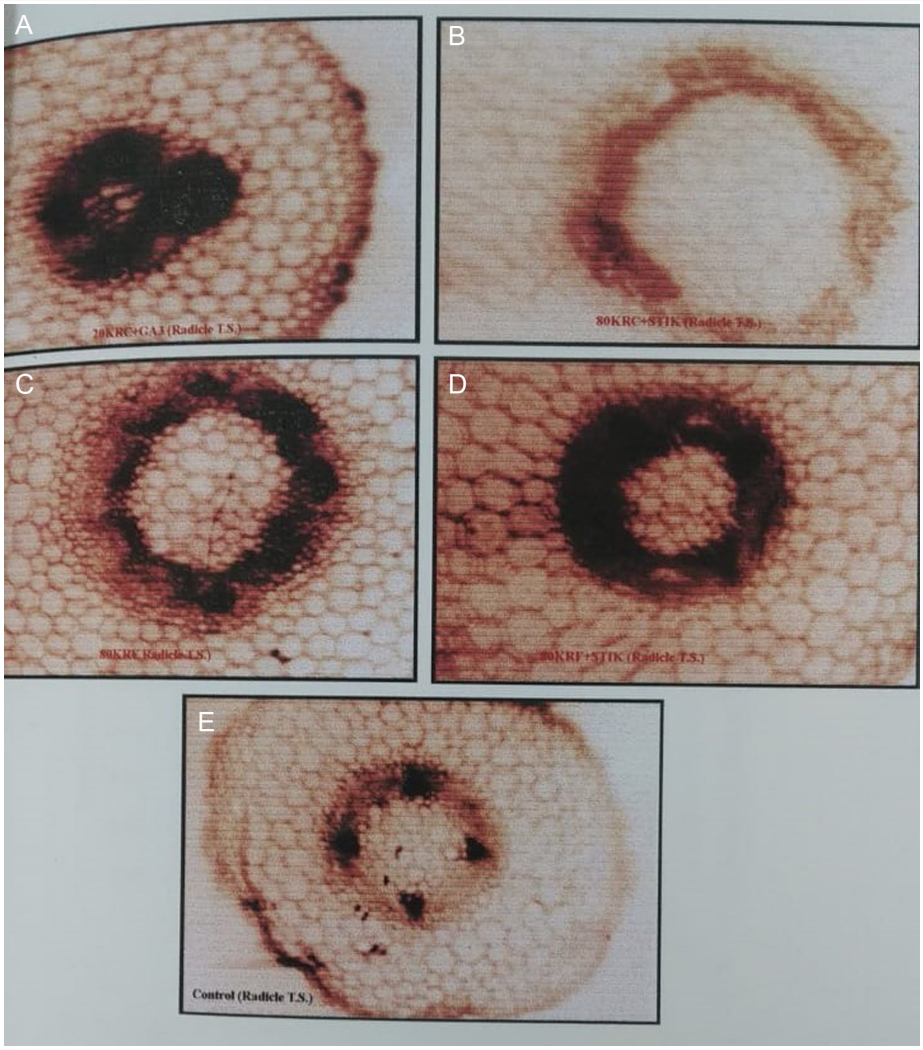

Figure 8.
(A–E) Transverse Sections of Radicle Showing Anatomical Variations During Seedling Growth After Treatment with Gamma Rays and Combined Doses with GA3 and STIK. All the treated seedlings show higher number of xylem layers and its bigger size cortical size has also increased after hormonal combination treatment.

layered in control set (see Tables 5 and 6). The number of cell layers in cortex did not show any significant variation among the various treatments. The cortical cells in 80 KRF + STIK were observed to be bigger than that of any other treatment, there average size was 79.06 µm where the size of cortical cells in control set was 55.0µm. Xylem was observed to be seven layered in 20 KRC + GA3. This was the highest number of layers formed in xylem among all treatments. In STIK combinations, five-layered xylem was found when the control set had three-layered xylem, but in the size of xylem, no significant variation was noticed among the treatments examined.

Iqbal (1973) reported that in *Caspium annuum*, the number of procambial cells sieve elements and vessel elements was influenced by irradiation of gamma rays (60 co) from 1 to 10 KR, and there was in general an increase in their number. In control seedlings, the first procambial strand when first observed in the axis comprised of —five to six cells, whereas in irradiated ones, the number of sieve elements and vessel elements was observed 7–15 days after the irradiation. The tissue differentiation could not be properly studied in seedlings 21 days following exposure because of increased cellular injury.

Data of the present investigation showed that the number of cell layers formed in the cortex, xylem, and sclerenchyma significantly altered in the treated seedlings in comparison to control set. The number of xylem layer and cortex layer was increased by the gamma irradiation + combination treatments. An increase in the form of wood might be due to some stimulus which enhances the cambium activity (Fischer et al., 2019), may be the stimuli induced by gamma irradiation. A specific IAA and gibberellic acid (GA) combination controls cambial activity in *Acer pseudoplatanus*, *Populus nigra*, and *Fraxinus excelsior* (Jian et al., 2022). The increase in the cell layers of xylem and cortex under the influence of gamma irradiation + combination treatments than the control may be due to the activity of cambium which might have been triggered. A significant increase in the xylem layers by different gamma irradiation + combination treatments will enhance the wood productivity of *A. chinnensis*.

Several studies on the stimulatory effects of gamma irradiation at low and high rates revealed a decrease in phytochemical content and plant anatomy (Jan et al., 2011; Widiastuti et al., 2010). According to Sakr et al. (2013), the epidermis, mesophyll, and diameter of the xylem and phloem vessels of leaves, stems, and root organs were changed by gamma ray irradiation rates of 5, 10, and 15 Gy. The findings of this study point to gamma irradiation adaptation and combination treatments as the most effective means of boosting wood quantity and quality.

Xylogensis is the process of xylem elements formation in vascular bundle during seed germination and later during secondary growth.Xylem elements develop from procambium or cambial initials in early vascular bundles and later during secondary growth, it get developed by lateral cambium. The molecular and biochemical studies have identified some of genes and protein involved in xylogenesis (xylem elements formation).

The early process involves the origination and development of procambial initials and these initials form the xylem elements, e.g., vessels, tracheids, fiber, and parenchyma. In the present study, the gamma ray doses influenced the periclinal division rate in vascular bundles and increase the number of cell layers in xylem. The combination effects of gamma irradiation with GA3 and STIK showed protagonist effect on cell layers of xylem and its size in hypocotyl and radicle in comparison to control set. GA3 favors the initial cell division and elongation of the cortical cell and STIK induces the cambium division and ultimately layers of the xylem (Figure 8).

Overall data of this study clarified the response of *A. chinnensis* toward various doses of gamma irradiations. The germination, growth, and biomass were enhanced by both continuous and fractionated doses in a dose reliant manner. In contrast, the gamma irradiation + combination treatments stimulated the xylogenesis mechanism. In general, the results could provide useful guidance for selecting an irradiation type and dose in accordance with the need to improve a particular parameter in *A. chinnensis*.

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