

CYTOGENETICAL STUDIES ON THE IMPACT OF GAMMA RAYS TO INDUCE VARIATION *IN VITRO* IN PAULOWNIA (*PAULOWNIA TOMENTOSA*)

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Paulownia (*Paulownia tomentosa* L.) is the one useful trees in China. All parts of paulownia tree (leaves, flowers, wood, bark, roots and seeds) have been used for many medicinal and industrial purposes. Buds were cultured on $\frac{3}{4}$ Murashige and Skoog (MS) solid medium supplemented with 0.2 mg/l benzyl adenine and 0.1 mg/l kinetin. The survival percentage and mean shoot length of irradiated plantlets with gamma radiation doses of 10, 20, 30, 40 and 50 Gy were calculated after eight weeks. The results showed a decrease in the survival percentage and mean shoot length by increasing gamma radiation doses. The lethal dose was 50 Gy and the optimal dose for explant survival was 10 Gy. Anatomical and ultrastructure of un-irradiated and irradiated paulownia were examined based on photonic and electron microscopy for plantlets leaves after 16 days from culturing. Anatomical studied of leaf surface showed variations in epidermal cells, trichomes, stomata and mesophyll cells. The ultrastructure sections showed formation of plastoglobule and starch granules which indicates a reduced carbon metabolism at sublethal dose of 40 Gy.

Keywords: paulownia, gamma radiation, *in vitro*, morphological characters, anatomical features, ultrastructure alterations

INTRODUCTION

Paulownia (*Paulownia tomentosa* L.) is a tree which is very well recognized for its wood quality and medicinal properties. The genus *Paulownia*, belonging to Paulowniaceae family, *Paulownia* species are widely distributed in China and in many countries around the world (Joshee, 2012 and He et al., 2016). Furthermore, trees have large, heart-shaped leaves, organized in reverse pairs on the stem. The presence of flowers are in early spring with a tubular purple corolla. The fruit is a dry capsule, containing thousands of minute seeds. Paulownia is very adaptable, widely distributed and extremely fast growing. Under optimum conditions, it is as a fast-growing

tree (Basuet al., 2015 and Vaughn et al., 2015). It is propagated through seeds and micropropagation techniques. The propagation through seeds is unreliable because of disease and pest problem, poor germination, and also slow growth (Ipekci and Gozukirmizi, 2003 and Atiqur Rahman et al., 2013). Therefore, micropropagation techniques are essential for the propagation of paulownia and have many advantages over seed germination. Additionally, it is a rapid way for producing high quality genetically uniform plants (Chunchukov and Yancheva, 2015 and Magar et al., 2016).

Exposure to ionizing radiation as gamma rays causes direct or indirect damage in plants. Direct damage occurs when the radiation energy is transferred to cells and DNA directly, leading to cell damage or cell death and inducing abnormalities (Desouky et al., 2015 and Choi et al., 2021). The production of free radicals is indirect damage of gamma radiation in the plant cells (Gill and Tuteja, 2010 and El-Khateeb et al., 2017). This reacts with genomic DNA and causes DNA damage. Moreover, it may cause harmful effects on the morphology, physiology, biochemistry, and anatomy in plants. Furthermore, these effects induce changes in plant cellular structure (Kovacs and Keresztes, 2002 and El-Fiki et al., 2018). The leaf is the most adaptable organ in its response to abiotic stress (Marchi et al., 2008). Furthermore, leaf structures reflect the effects of environmental conditions more clearly than those of stems and/or roots (Ennajeh et al., 2010). Modifications of anatomical structures of leaves are an adaptive mechanism for the plant species (Grigore and Toma, 2007). Additionally, the ultrastructure cellular structures are very useful in localizing any damages in cells and cellular alterations of organelles under stresses (Zahra et al., 2014).

The hereby work was an attempt to illustrate the change in morphology, anatomy, and plant cellular structures of paulownia plantlets *in vitro* to provide survival strategies active to overcome gamma radiation stress.

MATERIALS AND METHODS

1. Preparation of Plant Material

Paulownia (*Paulownia tomentosa*) actively growing shoots were obtained from the Faculty of Agricultural Ain Shams University. Surface sterilization of buds was carried out by washing in soap for 2 min, then washed under tap water. Subsequently, dipping in Clorox (30%; containing 5% sodium hypochlorite) for 15 min, followed by three rinses in sterile distilled water.

2. *In Vitro* Culture

The buds were grown on cultured medium (3/4 strength) of Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), supplemented with 3% sucrose, 0.2 mg/l benzyl amino purine (BAP) and 0.1 mg/l kinetin (KIN) and solidified by 6 g/l agar at pH 5.8 before autoclaving. The buds were cultured and incubated in a growth chamber at 25°C±2 under a 16 h

photoperiod. Furthermore, the effect of full-strength MS medium supplemented with 0.1 mg/l KIN, α -naphthalene acetic acid (NAA), indole-3-butyric acid (IBA), indole-3-acetic acid (IAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) was examined. The bud survival percentage (%) and mean shoot length (cm), rooting and callus formation were recorded after 8 weeks.

3. Gamma Irradiation Treatments

^{137}Cs (Candian gamma cell- 40) was used as a source of gamma rays with a dose rate of 10 Gy/23 min 34 s. Paulownia plantlets were exposed to gamma radiation doses of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 Gy at National Centre for Radiation Research and Technology, Cairo, Egypt. Then buds were cultivated on culture medium (3/4 MS medium + 0.2 mg/l BAP + 0.1 mg/l KIN + 6 g/l agar + 3% sucrose at pH 5.8 before autoclaving) to study some morphological, anatomical and ultrastructure aberrations. Also, bud survival percentage (%) and mean shoot length (cm) were recorded after 8 weeks.

4. Acclimatization of Paulownia Plantlets

The un-irradiated and irradiated plantlets were adapted to transfer to soil. Rooted shoots were washed from media by running water. The plantlets; 5 cm high were transferred to pots containing peat moss and sand (1:1). The pots were capped with plastic sheets. After three days, the plastic sheets were pored, then were removed after one week. The plantlets stayed in the growth chamber until transferred to greenhouse after two weeks.

5. Microscopic Examination of Leaves

The leaves of un-irradiated (control) and irradiated plantlets with doses of 10 and 40 Gy were examined using juvenile plantlets after 16 days from culturing to distinguish any changes in the anatomical features appeared. Specimens were prepared as described by John and Lonnie (1998). One mm² of leaves were fixed in 1% potassium permanganate for 5 min at room temperature, then washed three times in distilled water for 15 min for each. Subsequently, specimens were dehydrated in graded ethanol (30% to 90%), then in absolute ethanol, followed by passage through a graded propylene oxide ethanol series. Finally, maintained in pure propylene oxide. Dehydrated specimens were embedded in an epoxy resin composed of 20 ml dodecyl succinic anhydride (DDSA) (hardener), 16 ml nadic methyl anhydride (NMA) (softener) and 8 ml 2,4,6-dimethylamin-ethylphenol (DMP) (accelerator). Samples were polymerized in an oven at 60°C for 48 h. Sections (1 μm) were cut and examined with Leica in Electron Microscope Unit, Center for Mycology and the Regional Biotechnology, Al Azhar University.

6. Scanning Electron Microscopy (SEM)

The leaf samples were gold sputtered for 12 min by using the ion sputtering device model JEOL (JFC 1100 E). The samples surface were

investigated by using scanning electron microscope JEOL-5400 at National Centre for Radiation Research and technology.

7. Transmission Electron Microscopy (TEM)

Specimens of irradiated and un-irradiated leaves were prepared for TEM analysis according to John and Lonnie (1998). Sections (1 μm) were cut with Leica Ultra-microtome, mounted on copper grids, and stained with 0.5% uranyl acetate and lead citrate for 1 5min (for each) in line with Reynolds (1963) in Electron Microscope Unit, Center for Mycology and the Regional Biotechnology, Al Azhar University. Observations were examined by using JEOL TEM 100 CX, transmission electron microscope at 80 kV in National Centre for Radiation Research and Technology.

8. Statistical Analysis

The data were statistically analyzed using ANOVA analysis to determine the level of significant differences between means as compared to the control at $P \leq 0.05$ level of significance. The statistical software Costat (<http://www.cohort.com/costat.html>) was used for all analyses.

RESULTS AND DISCUSSION

1. In Vitro Culture

Tissue culture technique is used in a large scale for economically programs to produce a healthy plant of paulownia trees (Zayova et al., 2013). Paulownia plantlets were propagated *in vitro* by buds cultured on 3/4 strength MS medium supplemented with 0.2 mg/l BAP and 0.1 mg/l KIN. Additionally, the effect of 0.1 mg/l of KIN, NAA, IBA, IAA and 2,4-D was recorded. Also, a comparison between full strength MS medium and 3/4 strength MS medium was studied. Data in Table (1) and Fig. (1) show that the medium no. 1 (3/4 MS medium + 0.2 mg/l BAP + 0.1 mg/l KIN) positively affected the growth of buds, with bud survival of 96% and the mean shoot length of 5.7 cm. The lowest growth was recorded on full strength MS medium (No. 5) supplemented with 0.1 mg/l IAA, it recorded the lowest bud survival percentage and mean shoot length of 66.66% and 2.31 cm, respectively. Moreover, IAA promoted roots formation more than the other treatments. Furthermore, the full strength MS medium with KIN, NAA and IBA negatively affected bud survival percentage and mean shoot length. Additionally, full strength medium supplemented with 2,4-D (No. 6) induced callus formation. So, data in Table (1) and Fig. (1) indicate that medium no.1 (3/4 MS medium supplemented with 0.2 mg/l BAP and 0.1 mg/l KIN) was the best medium for micropropagation of paulownia. Propagation of paulownia is possible by seed, stem and root explants. The slow rate of growth and poor germination of seeds encourage the *in vitro* propagation of paulownia (Ipekci and Gozukirmizi, 2003 and Hamza, 2019). Consequently, the results showed that the tissue culture technique offers a rapid mean of propagation of

paulownia by maintaining the genetic gain (Bergmann and Moon, 1997 and Salki et al., 2018). Accordingly, in this investigation, nodal explants considered an excellent explant source for direct micropropagation protocol of *Paulownia tomentosa* in Egypt. This agree with Hamza (2019).

Table (1). Responses of *Paulownia tomentosa* explants to different culture media.

| No. | Treatment | Complete plantlet formation | Root formation | Callus formation | Bud survival percentage (%) | Mean shoot length (cm) |
|-----|---|-----------------------------|----------------|------------------|-----------------------------|------------------------|
| 1 | 3/4 MS medium + 0.2 mg/l BAP + 0.1 mg/l KIN | + | + | - | 96.00a | 5.70a±0.1 |
| 2 | MS medium + 0.1 mg/l KIN | + | + | - | 83.30b | 3.16b±0.34 |
| 3 | MS medium + 0.1 mg/l NAA | + | + | - | 74.00c | 2.70c±0.28 |
| 4 | MS medium + 0.1 mg/l IBA | + | + | - | 72.00d | 2.58d±1.2 |
| 5 | MS medium + 0.1 mg/l IAA | + | ++ | - | 66.66e | 2.31e±0.45 |
| 6 | MS medium + 0.1 mg/l 2,4-D | - | + | + | 0.00 | 0.00 |

Values followed by different letters are significantly difference at = 0.05 level.

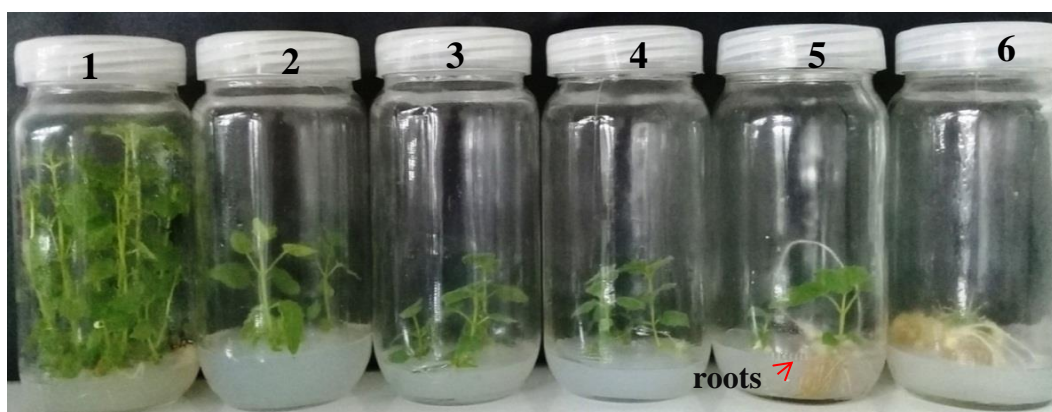


Fig. (1). Response of *Paulownia tomentosa* explants to different culture media.

The micropropagation is affected by physical and chemical conditions as the type of explant, strength of basal medium, carbohydrate source, light, temperature and different concentrations of growth regulators, according to Hesami and Daneshvar (2018). MS medium is widely used with valuable success, also BAP among various tested growth regulators, was the most effective in paulownia proliferation (Nguyen et al., 2005; Ozaslan et al., 2005 and Clapa et al., 2014). The concentration of 0.2 mg/l BAP induced shoots proliferation according to Awad et al. (2018). Also, KIN improved growth in paulownia (Abd El-Kader, 2004 and Ghatas, 2016). In this study,

the combination of BAP and KIN were used to induce shoots and roots. Shoot and root formations were found to be better using both BAP and KIN than BAP or KIN alone, This agrees with Uddin (2006). Results appeared in harmony with Chunchukov and Yancheva (2015), who illustrated that auxin promoted root induction. Moreover, 2,4-D is classified as an auxin and used for tissue culture at low concentrations and induced callus formation (Conger, 2018 and Costa et al., 2020).

2. Effect of Gamma Irradiation on *in Vitro* Growth

Ionizing radiation successfully used for crop and ornamental plants improvement through induced mutagenesis. Determination of optimum dose, radio sensitivity and treatment conditions are most vital for genetic manipulation through the induction of mutation (Khah and Verma. 2015). In this investigation, paulownia plantlets were irradiated with gamma irradiation doses of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 Gy (Table 2 and Fig. 2).

Table (2). Effect of gamma radiation doses on bud survival percentage and mean shoot length in *Paulownia tomentosa* plantlets.

| Radiation dose (Gy) | Bud survival percentage (%) | Mean shoot length (cm) |
|---------------------|-----------------------------|------------------------|
| Control | 96.66a | 5.70a±0.2 |
| 10 | 85.00b | 4.90b±0.11 |
| 20 | 81.66bc | 5.37c±0.31 |
| 30 | 72.71d | 3.50d±0.14 |
| 40 | 41.72 e | 3.04e±0.3 |
| 50 | 23.33 f | 1.41f±0.05 |

Values followed by different letters are significantly difference at = 0.05 level.



Fig. (2). Effect of gamma radiation doses on bud survival and shoot length of *Paulownia tomentosa* plantlets.

The results showed that low doses of gamma radiation caused significantly decreasing in both survival percentage and mean shoot length. Furthermore, complete mortality of explants was caused by high doses. The dose of 10 Gy caused decreasing in bud survival percentage to about 11.66%. The same dose decreased mean shoot length to 0.8 cm. The usage of dose 20 Gy was induced mortality in bud culture of about 15%. The mean shoot length was affected negatively with the same dose to 0.33 cm. Moreover, the dose of 30 Gy decreased survival percentage to 23.95%, and mean shoot length to 2.2 cm. Furthermore, the mortality was increased with the dose of 40 Gy to 54.94%. Also, mean shoot length decreased to 2.66 cm. The severe depression in survival percentage to 73.33%, and the shoot length was decreased to 4.29 cm with the dose of 50 Gy. Consequently, the sub lethal dose was 40 Gy and lethal dose was 50 Gy. The dose of 10 Gy was the optimal dose for explant survival as shown in Table (2) and Fig. (2). However, the doses of 60, 70, 80, 90 and 100 Gy caused 100% lethality of explants. These results are in harmony with radiation sensitivity test done by El-Fiki et al. (2015 and 2016) for tobacco. Taheri et al. (2014) reported that the mortality rate of three varieties of *Curcuma alismatifolia* increased with increasing gamma radiation dosage and mortality reached the highest rate at 100 Gy of radiation. Hence, results showed the presence of differences in morphological and growth parameters between un-irradiated and irradiated treatments. This agree with Wi et al. (2007), who found that the plants exposed to relatively low dose of gamma rays developed normally, while the growth of plants irradiated with a high dose of gamma rays (50 Gy) was significantly inhibited. So, these effects agree with Gaafar et al. (2017), who clarified the decrease in shoot and root lengths at higher gamma radiation doses on the basis of reduction in the mitotic activity of plant tissues.

3. Acclimatization of Plantlets

Micropropagated plantlets suffer high mortality when transferred from *in vitro* to *ex vitro* conditions (Chandra et al., 2010). Acclimatization is the physiological adaptation of plantlets to changes in climate or environment, such as light, temperature, or altitude. It also involves the changes in leaf structure, water relations and photosynthesis during acclimatization of plantlets to *ex vitro* conditions (Pospisilova et al., 1999). The un-irradiated and irradiated plantlets adapted to transfer to soil. The results showed that the acclimatization succeeded in control and irradiated treatments by doses 10, 20, 30 and 40 Gy as shown in Fig. (3). The most species developed *in vitro* need an adaptation process before moving them to the greenhouse and the field (Pospisilova et al., 1999 and Hazarika, 2003). In the same line, Yasmin et al. (2003) used sand and soil (1:1) for hardening of plants. These plants were irrigated with fine spray of water and covered with transparent polythene bags to prevent desiccation. Within 5-7 days, they were established, and polythene bags were removed.



Fig. (3). Acclimatization of *Paulownia tomentosa* plantlets.

3. Cytogenetical Studies

3.1. Antomical studies of leaf

The capability of plants to acclimate to gamma radiation doses involves alterations of morphological and anatomical characteristics. Transverse sections illustrate anatomical features of upper and lower epidermis, mesophyll cells, vascular bundles, stomata and trichomes in un-irradiated and irradiated paulownia leaf. The leaf of un-irradiated plantlets has a symmetric, heterogeneous structure (Fig. 4 a, b, c and d). Furthermore, the un-irradiated epidermis consisted of a single layer of closely packed cells, covered with a layer of cuticle. It had upper and lower epidermis (dorsiventral). The upper epidermis differed from the lower epidermis, which related to the exposure to sunlight. Epidermal cells were mostly thick walled, did not contain chloroplasts and formed a continuous cover. The epidermis serves to protect the inner tissues against dehydration, intense sunlight (especially ultraviolet light) and mechanical stress. The epidermis also contributes to limit prey by herbivores (insects, cattle) and damage by parasites. The main function of the epidermis is to give protection to the inner tissue called mesophyll. The most curious protection mechanisms were provided from trichomes (Fig. 4 a). The trichome features (e.g. shape, size, density) are the most useful taxonomic characteristics (Dinç and Öztürk, 2008 and Kahraman et al., 2010).

The minute openings were found on the lower epidermis are called stomata. Each stomata internally opened into an air chamber, which the exchange of gases takes place. Stomata were surrounded by a pair of bean shaped cells called guard cells. These guard cells are the only epidermal cells contain chloroplasts and are regulating opening and closing of stomata. Stomata are used for transpiration and gas exchange (Fig. 4 c and d). Stomata play an important role in the regulation of water and carbon cycle between the plants and the atmosphere (Weng et al., 2011). Stomata control the flux of CO₂ and water vapor by varying their aperture and they restrict the diffusion of gases. Various environmental factors (e.g. water availability, light intensity,

photoperiod) can affect stomata opening-closing behavior (Al-Ani and Bierhuizen, 1971; Pallardy and Kozlowski, 1979 and Bondada et al., 1994).

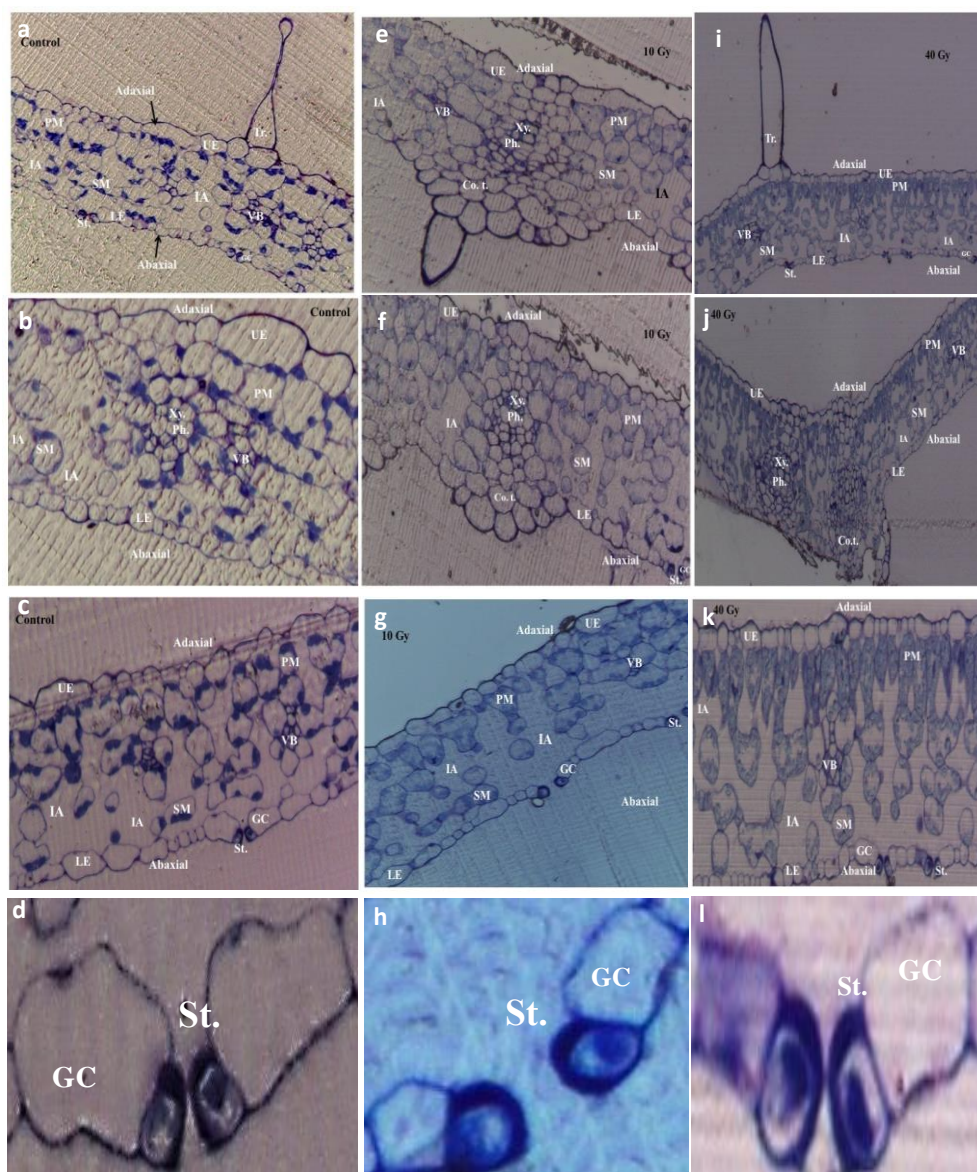


Fig. (4). Diversity in cross sections of *Paulownia tomentosa* with leaves of (a, b, c and d), un-irradiated and irradiated doses of (e, f, g and h). 10 Gy and (i, j, k and l). 40 Gy.

Collenchyma tissue (Co.t.), guard cell (GC), intercellular airspace (IA), lower epidermis (LE), phloem (Ph), palisade mesophylle (PM), spongy mesophylle (SM), stomata (ST), trichome (TR), upper epidermis (UE), vascular bundle (VB), xylem (X).

The mesophyll or chlorenchyma (chloroplast-rich tissue) is the tissue specialized in photosynthesis. The entire mesophyll (GK meso = in the middle, phyllome = leaf) was sandwiched between an upper and lower epidermis. The mesophyll had two layers, an upper palisade layer and a lower spongy layer. Palisade parenchyma cells were seen beneath the upper epidermis. It consisted of less rounded cells in one layer. These cells were compactly arranged and were generally without intercellular spaces. The function of palisade parenchyma is photosynthesis. Spongy parenchyma cells were rounded shaped. The spongy parenchyma had an open and net-like structure and very loosely arranged with numerous airspaces. The air space that was found next to the stomata is called respiratory cavity or sub stomatal cavity to aid gaseous exchange between the leaf and the outside atmosphere through the stomata. The spongy parenchyma connects the veins with the palisade parenchyma (Fig. 4 c).

Veins or vascular bundles of the leaf were irregularly distributed throughout the mesophyll. The vascular bundles contained xylem and phloem cells. Xylem transport water and minerals to the leaves. The phloem transports the photosynthetic products from the leaf to the other parts of the plant. The bundle sheath plays numerous roles, including water and nutrient transport *via* channels and pumps, phloem loading and water storage (Sack and Scoffoni, 2013). Vascular bundles were conjoint, collateral and closed. They were surrounded by a compact layer of parenchymatous cells called bundle sheath or border parenchyma. Collenchyma cells had an explicit thick wall and appear in variable forms and they are living cells. They were often found close to vascular bundles, in particular, at the base of the midrib vein (Fig. 4 b and c). While transverse sections samples subjected to 10 Gy appeared changes in the shape of trichomes (Fig. 4 e). Stomata were opened and the guard cell was smaller than in un-irradiated sample (Fig. 4 g and h). Furthermore, xylem elements in the vascular bundle were more than that in un-irradiated ones as seen in Fig. (4 f). On the other hand, many modifications were induced by dose 40 Gy as the cells of epidermis were more circular than un-irradiated sample (Fig. 4 k). Moreover, trichome had different shapes (Fig. 4 i). The guard cells of stomata were smaller than in un-irradiated sample as illustrated in Fig. (4 k and i). Ahuja et al. (2014) reported that the higher gamma radiation doses resulted in inhibition of stomatal conductance. Palisade parenchyma cells were cylindrical and elongated cells. Both of palisade parenchyma and spongy parenchyma contain more chloroplast compared with un-irradiated sample as shown in Fig. (4 k). Xylem elements in vascular bundles were more in dose 10 than in un-irradiated sample (Fig. 4 j and k). The leaf is one of the most important organs in plants. Its structure is generally bifacial flat and thin and consists of three tissues; epidermal, mesophyll, and vascular tissues (Ouk et al., 2020). The anatomy of leaves is mostly related to plant function (Rossatto and Kolb, 2009) and always have modifications with environmental changes (Bosabalidis and Kofidis, 2002 and

Poorter and Bongers, 2006). The internal physiology and gene expression are also affected (Zhang et al., 2015 and Chang et al., 2017). These factors cause morphological and structural changes to the leaves of trees. Furthermore, the free radicals, which produced from gamma radiation doses can damage or modify important components of plant cells and affected morphology, anatomy, biochemistry, and physiology of plants depending on the irradiation dose (Wi et al., 2005).

3.2. Scanning electron microscopy sections of trichomes

Trichome has vital microscopic features that can be used for comparative systematics. Difference in trichome types can afford vision into the evolutionary relationships within and among species (Payne, 1978 and Theobald et al., 1979). Taxonomic characters of particular types of trichomes have been studied in several plant species such as *Arabidopsis* (Valverde et al., 2004), tomato (*Solanum lycopersicum*) (Levin, 1973), and eggplant (*Solanum elongata*) (Frery et al., 2003). The special structure of trichomes can play a protecting role in the interaction between plants and biotic or abiotic stresses (Xiao et al., 2017). Morphology of the trichomes was observed by using a scanning electron microscope. Plants produce glandular trichomes and their particular metabolites for their interaction with the environment. Glandular trichomes are epidermal outgrowths. Moreover, they are sites of biosynthesis and storage of great amounts of metabolites. Additionally, it considered signify a main first line of plant protection, making a physical and chemical barrier against several environmental sours such as excessive light radiation, extreme temperature and herbivory (Werker, 2000, Martínez-Natarén et al., 2018 and Schuurink and Tissier, 2020).

The structure of trichomes of paulownia plantlets is a key for thoughtful how these plantlets adjusted to radiation. Responses of trichomes structure to different treatments have been recognized in Fig. (5). Trichome in un-irradiated sample was a hair- or scale-like extension of the epidermis of a plant. The structure of trichomes in leaves of paulownia plantlets was capitate, straight, un-branched glandular with a one celled head. The basal cells were arisen from epidermis cells. Trichomes consisted of long-stalked three long cells. The capitate glandular trichomes commonly had rounded to pear shaped heads (Fig. 5 a and b). This is in harmony with Turner et al. (2000), who showed that the glandular trichomes are usually multicellular, consisting of differentiated basal, stalk and head cells. Gamma radiation caused deformation of trichome shape.

The dose of 10 Gy caused increase in the trichome length, compared to un- irradiated sample. The first cell in stalk no.1 was longer and thicker than un-irradiated sample, but the second and third cells of stalk were shorter than un-irradiated sample as shown in Fig. (5 c and d). However, at the dose of 40 Gy, trichome length was shorter than in un-irradiated sample. The three stalk cells were thicker and shorter than un-irradiated as seen in Fig. (5 e and f). Trichomes play a key role in development and evolution of plant which are

epidermal appendages covering the surface of plants. Trichome are known as general adaptive responses of plants to survive under biotic and abiotic stresses. Trichomes that create a thin layer to reduce the rate of transpiration by blocking air flow across the leaf surface (Zhang et al., 2020). So, trichomes have an extensive role in plant–environment interactions (Xiao et al., 2017). Also, they are a model structure for cell differentiation, cell cycle regulation, cell polarity and cell expansion according to its different distributions on leaves (Xiao et al., 2017). Furthermore, they have a major importance in plant taxonomy, plant ecology, plant protection, useful in medical industry and they will inspire non-conventional human applications (Liu et al., 2017).

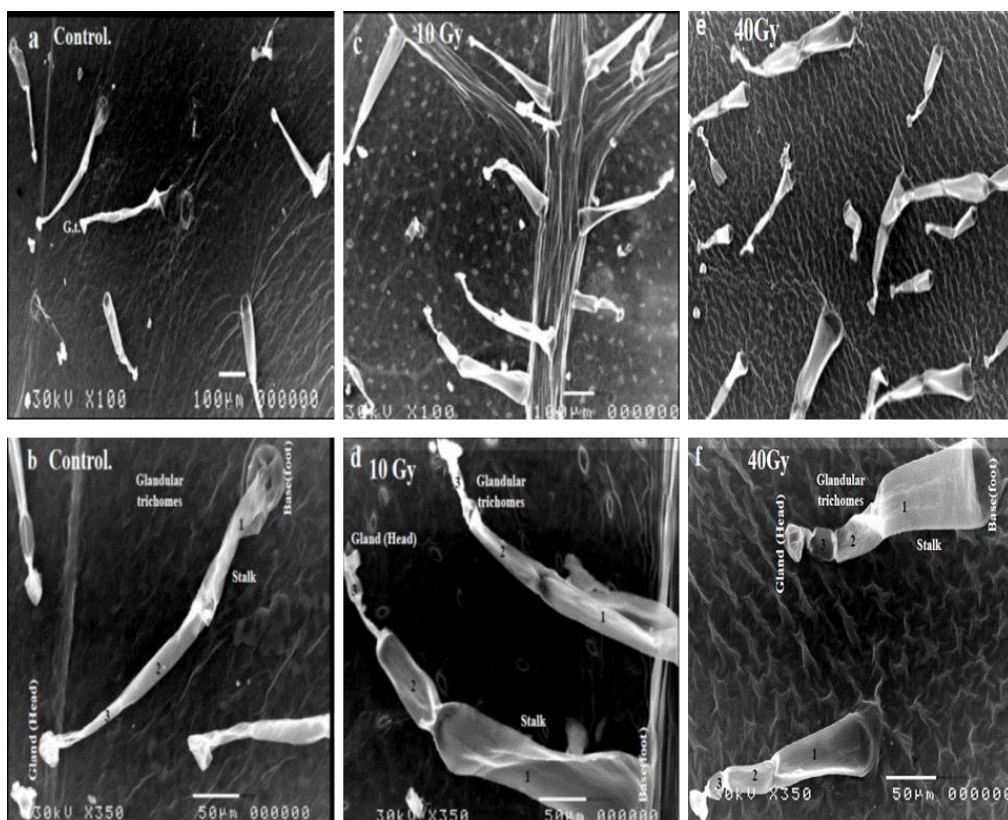


Fig. (5). Diversity in scan electron micrographs of the leaf of *Paulownia tomentosa* plantlets having tall glandular trichomes with three stalk cells and unicellular head in response to different irradiation treatments *in vitro*. (**a and b**), glandular trichome of control, (**c and d**), after dose of 10 Gy, and (**e and f**), dose of 40 Gy.

3.3. The ultrastructure of leaf

Ultrafine sections of leaves of paulownia confirmed variations in irradiated leaves which appeared in photonic microscopy sections. Electron

micrograph (Fig. 6 a, b, c and d) illustrates the ultrastructure of mesophyll cells and chloroplasts in un-irradiated paulownia plantlet. The mesophyll cells were intact. Moreover, chloroplasts were organized along plasma membrane. There was large intercellular space between mesophyll cells. The micrographs of irradiated treatments by doses 10 and 40 Gy showed the alterations in structure of chloroplast. At dose 10 Gy, chloroplast disorganized along plasma membrane. By dose 40 Gy, chloroplasts increased in number. Chloroplast characterized by the presence of large and numerous starch granules and appearance of plastoglobule (Fig. 6 j, k, l, m and n). On the other side, the dose of 10 Gy did not induce any starch granules in chloroplasts and plastoglobule as seen in Fig. (6 e, f, g and h). Ultrastructural configurations of chloroplasts represented the ideal system to maximize photosynthesis (Bondada and Oosterhuis, 2003). Chloroplast ultrastructure in *in vitro* culture was diverged in their function, chemical compounds, and inner structure (Solymosi and Aronsson, 2013). The present results agree with Wi et al. (2007), who stated that the higher doses inhibit chloroplast development and cause changes in the synthesis of nucleic acid in plastids.

In the present investigation, the dose of 40 Gy caused the appearance of starch granules in the chloroplasts, this agrees with Bondada and Oosterhuis (2003). The accumulation of starch within the chloroplasts accompanied by damage and disorientation of grana and thylakoids would indicate the inhibition of carbohydrate transport (Bondada and Oosterhuis, 2003). This indicates that those structures are remarkably sensitive to ionizing radiation. Therefore, the structural changes are affected by an increase in free radical formation under gamma radiation (Wi et al., 2007).

The changes in chloroplasts are typical in plants stressed by extreme temperatures, low light, saline stress and water stress (Munné-Bosch et al., 2001; Navarro et al., 2007; Fu et al., 2011 and 2013). Plastids serve as reliable abiotic and biotic stress marker. Plastids perform many essential functions in plant metabolism including photosynthesis, synthesis of metabolites, and stress signaling (Zechmann, 2019). By the increase of the radiation doses, then number of chloroplasts, plastoglobuli and starch granule were increased in this study at dose 40 Gy (Fig. 6 j, k, l, m and n). The plastoglobuli may play a role in the synthesis and recycling of lipophilic products and oxidative stress defense. Plastoglobuli have a function in the storage of thylakoid components, such as lipids, plastohydroquinone, and tocopherol (Munné-Bosch et al., 2001). Plastoglobuli number can increase in response to abiotic conditions that promote stress in photosynthetic apparatus (Austin et al., 2006 and Polesi et al., 2019). Changes in ultrastructure of chloroplasts can be attributed to the accumulation of reactive oxygen species (ROS), especially hydrogen peroxide, in this organelle (Simon et al., 2013 and Rossi et al., 2017). ROS leads to chloroplast degradation, reduction of photosynthetic efficiency, and a decrease of chlorophyll (Luschin-Ebengreuth and Zechmann, 2016 and

Wojciechowska et al., 2018). Also, activates apoptotic like programmed cell death (PCD) (Ambastha et al., 2015 and Veloso and van Kan, 2018).

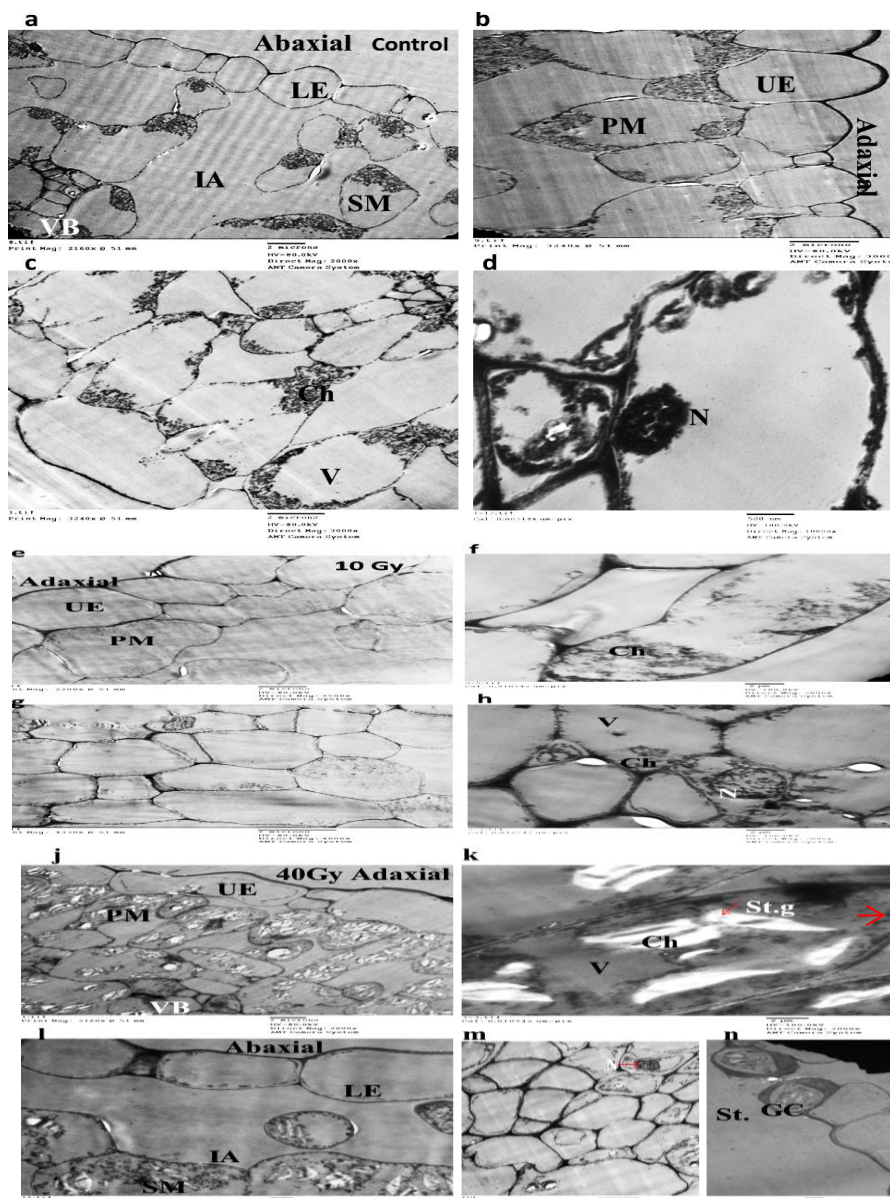


Fig. (6). Ultrastructural aspect of the *Paulownia tomentosa* leaf cells of (a, b, c and d). un-irradiated and irradiated by doses of (e, f, g and h). 10 and (j, k, l, m and n). 40 Gy.

chloroplast (ch), guard cell (GC), intercellular airspace (IA), lower epidermis (LE), nucleus (N), palisade mesophyll (PM), starch granule (St.g), spongy mesophyll (SM), stomata (ST), trichome (TR), upper epidermis (UE), vacuole (V), vascular bundle (VB)

Experimental evidence suggests that chloroplast is vulnerable to high-dose gamma irradiation, as it leads to the deformation of the thylakoid structure, thereby reducing photosynthetic efficiency (Wi et al., 2005 and 2007). Because the energy required for completing the plant life cycle is obtained through photosynthesis. The results showed the cellular organelle as chloroplast appears as major target of the stress. This is in harmony with Mitsuya et al. (2000). Ionizing radiation-induced decline in photosynthetic efficiency has harmful effects on plant growth, development, and reproduction (Caplin and Willey, 2018 and Choi et al., 2021). Furthermore, changes of the chloroplast structure presumably affect photosynthesis, resulting in increased starch in leaves, suppression of nitrate reductase activity and reduced growth (Ghosh et al., 2001). Assessment and adaptation mechanisms of plants which provide resistance to adversarial conditions is considered a maximum significance for biological science and future of humanity (Butnariu, 2015).

CONCLUSION

MS medium of 3/4 strength is an effective factor for the micropropagation of *Paulownia tomentosa* in combination with BAP and KIN. Furthermore, the usage of gamma radiation as a tool for inducing genetic variations in the plant characterizes an efficient method. Gamma irradiation had a negative impact on the growth rate. The ultrastructure changes are well suited as a stress marker for plants for radiation stress.

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دراسات وراثية خلوية لتأثير أشعة جاما على إحداث تباين في نبات الباولونيا معملياً

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الباولونيا هي أحد أهم الأشجار في الصين. حيث تستخدم جميع أجزاء الشجرة (الأوراق والزهور والخشب واللحاء والجذور والبذور) للعديد من الأغراض الطبية والصناعية. تمت زراعة براعم نباتات الباولونيا في المعمل على بيئة مورايشج وسكوج بقوة ٤/٣ مضافاً إليها ٠.٢ مجم/لتر بنزول أدنين و ٠.١ مجم/لتر كينيتين. تم تشجيع النبيتات الناتجة بجرعات أشعة جاما ١٠ و ٢٠ و ٣٠ و ٤٠ و ٥٠ جراي. تم حساب نسبة حيوية البراعم المشععة ومتوسط طول النبيتات بعد ثمانية أسابيع. أظهرت النتائج أنه مع زيادة جرعات الإشعاع كان هناك انخفاض في نسبة حيوية البراعم ومتوسط طول النبيتات وكانت الجرعة ٥٠ جراي هي الجرعة المميّنة بينما كانت الجرعة ٤٠ جراي هي الجرعة المثلى لنمو البراعم. علاوة على ذلك، تم الفحص المجهرى الضوئي والإلكتروني لأوراق النبيتات المشععة والكنترول بعد ١٦ يوماً من الزراعة. وقد أوضحت الدراسة التشريحية وجود تباين في خلايا البشرة، والشعيرات، والثغور، وخلايا الميزوفيل كما أوضح الفحص المجهرى الإلكتروني الماسح التغيرات في شكل الشعيرات بينما أوضح الفحص المجهرى الإلكتروني النافذ أن الجرعة ٤٠ جراي من أشعة جاما تسببت في ظهور حبيبات النشا في البلاستيدات الخضراء مما يشير إلى انخفاض التمثيل الغذائي للكربون. تقدم الدراسة الحالية نظرة ثاقبة لتكيف نباتات الباولونيا مع أشعة جاما من خلال التغير في الشكل المورفولوجي والتشريحي والتركيبي الدقيق لخلايا الورقة.