Journal of
NUCLEAR
Technology in Applied Science
ISSN 2314-8209 e-ISSN 2314-8217

J. Nucl. Tech. Appl. Sci., Vol. 11, PP. 31 : 45 (2023)

Morphological and Cellular Alterations by Gamma Radiation and Grew Under Drought Stress in Wheat *(Triticum aestivum L.)*

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Received: 11/05/2023
Accepted: 05/08/2023
DOI: 10.21608/jntas.2024.250094.1058

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ABSTRACT

Gamma radiation and polyethylene glycol (PEG) are widely used in various fields of research to induce stress and study their effects on plants. In this investigation the morphological characterization, cellular, and extracellular structure were studied of wheat cv. Sids 13. Gamma radiation doses 50 and 100 Gy were affected on shoot and root length negatively. Where caused decreasing in both shoot and root length with 3 cm, 8 cm; and 2 cm, 5 cm; respectively. While polyethylene glycol concentrations 10, 15, and 20% induced decreasing in shoot and root length with (8, 11 and 13cm) and (5, 7 and 8.5 cm) respectively. The combined effect of gamma ray doses and PEG also caused severe decreasing in both shoot and root length with (10, 12 and 15cm) and (5, 7 and 8.5 cm) respectively. Gamma radiation and PEG also affect the cellular and ultracellular structure of wheat. These effects investigated using various microscopy techniques, such as light microscopy, transmission electron microscopy, and scanning electron microscopy. These techniques reveal changes in cell size, cell wall thickness, organelle morphology, and other cellular structures. This study aims to investigate the effect of some radiation doses, different PEG concentrations and the combined effect on morphological and cellular changes in wheat variety Sids 13.

KEYWORDS

Wheat, Gamma Radiation, Polyethylene Glycol (PEG), Morphological Characters, Anatomical Features, Ultrastructure Alterations, Programmed Cell Death (PCD).

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is a major staple crop worldwide, and its productivity is crucial for global food security. Various techniques have been employed to improve wheat's yield and quality, including the use of radiation and polyethylene glycol (PEG) (**FAOSTAT Data, 2007; Jankowicz-Cieslak *et al*., 2017**). Gamma radiation is a type of ionizing radiation with high energy that can induce mutations in DNA, potentially leading to beneficial traits. These mutations can lead to changes in plant morphology, physiology, and biochemistry. Gamma radiation has been used in wheat breeding for several decades to create new varieties with improved traits like disease resistance, yield potential, and grain quality (**El-Demerdash *et al*., 2015; Adly and El-Fiki, 2016**). Polyethylene glycol (PEG) is a water-soluble polymer with a wide range of applications in various industries. In agriculture, PEG has been used to induce stress in plants, which can trigger various physiological and metabolic responses. PEG can also be used as a carrier for delivering other molecules, such as nanoparticles, into plant cells (**Shafeeq *et al*., 2014**). Understanding the combined effects of these two factors gamma radiation and Polyethylene glycol (PEG) on wheat's morphological, cellular, and ultracellular structure is crucial for optimizing their use in agricultural practices. These effects can be studied using various microscopy techniques, such as light microscopy, transmission electron microscopy, and scanning electron microscopy. These techniques can reveal changes in cell size, cell wall thickness, organelle morphology, and other cellular structures (**El-Mogy and Saleh, 2017**).

MATERIALS AND METHODS

Plant materials and culture conditions

The seeds of wheat cv. Sids 13 were obtained from the Egyptian Gene Bank, located at the Agricultural Research Centre in Giza, Egypt. Irradiation was conducted using a ^{60}Co source at the National Centre for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt. The seeds were exposed to gamma rays in doses of 50 and 100Gy, with a dose rate of 0.708 rad/sec. prior to irradiation, the seeds were soaked in water for approximately 24 hours. To facilitate germination, each treatment consisted of 40 seeds that were placed in water inside foam nets, as depicted in Figure 1. In order to induce water deficit, Polyethylene glycol 6000 (PEG) from AppliChem GmbH in Germany was used. Three different PEG concentrations (10%, 15%, and 20%) were employed to achieve specific osmotic potentials (-1.0, -1.5 and -2.0MPa), as described by **Michel and Kaufman (1983)**. The irradiated seeds were then germinated using different PEG concentrations.

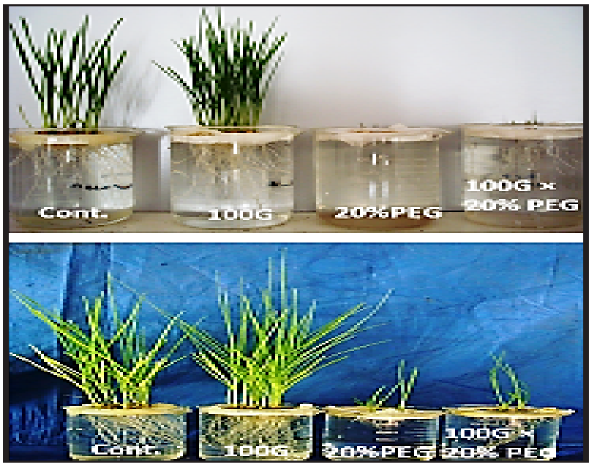

Fig. (1): Germination and seedling phenotypes after treatment with both gamma radiation and PEG.

Semi thin sections of roots

The untreated roots and roots treated with a gamma radiation dose of 100 Gy, PEG 6000 with a concentration of 20%, and the combined effect of a dose of 100 Gy and 20% PEG were analyzed. The untreated roots and roots treated with a gamma radiation dose of 100 Gy, PEG 6000 with a concentration of 20%, and the combined effect of a dose 100 Gy x 20% PEG were investigated. The roots were prepared following the methodology outlined by

John and Lonnie (1998). Segments of the root tips were fixed in a solution of 1% potassium permanganate for 5 minutes at room temperature. Afterward, they were rinsed three times with distilled water for 15 minutes each. Subsequently, the specimens were dehydrated using a series of ethanol solutions ranging from 30% to 90%, followed by absolute ethanol, and finally passed through a series of propylene oxide ethanol solutions, ultimately being preserved in pure propylene oxide. The dehydrated specimens were then embedded in an epoxy resin consisting of 20ml of dodecyl succinic anhydride (DDSA) as the hardener, 16ml of nadic methyl anhydride (NMA) as the softener, and 8ml of 2,4,6-dimethylaminethylphenol (DMP) as the accelerator. Finally, the samples were polymerized in an oven at 60°C for 48 hours. Thin sections measuring 1µm were cut and examined using a Leica electron microscope at the Electron Microscope Unit of the Center for Mycology and The Regional Biotechnology, Al Azhar University in Cairo, Egypt.

Scanning electron microscopy (SEM)

Roots samples were gold sputtered for 12min 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, Egyptian Atomic Energy Authority, Cairo, Egypt.

Transmission electron microscopy (TEM)

Specimens of untreated and treated roots were prepared for TEM analysis according to **John and Lonnie (1998)**. Sections (1µm) were cut with Leica Ultra- microtome, mounted on copper grids and stained with 0.5% uranyl acetate and lead citrate for 15min (for each) in line with **Reynolds (1963)**, in Electron Microscope Unit, Center for Mycology and The Regional Biotechnology, Al Azhar University Cairo, Egypt. Examinations were done by using JEOL TEM 100 CX, transmission electron microscope at80kV in National Centre for Radiation Research and Technology, Egyptian Atomic Energy Authority, Cairo, Egypt.

Statistical analysis

The data were statistically analyzed using ANOVA analysis to determine the level of significant differences between treatments means as compared to the control at $P \leq 0.05$ level of significance. The statistical software Costat was used for all analyses.

RESULTS AND DISCUSSION

The wheat seeds of Sids 13 were exposed to gamma radiation at doses of 50 and 100Gy. The application of radiation had a detrimental effect on the percentage of seed germination, as well as the length of shoot and root in the seedlings. The germination percentage of seeds was decreased by approximately 13 to 22% with an increase in the gamma radiation dose. Similarly, the length of shoots exhibited a decline from 3cm with a 50 Gy dose to 8 cm with a 100 Gy dose. The root lengths also experienced a negative impact, ranging from 2cm with a 50 Gy dose to 5cm with a 100 Gy dose, as depicted in Table (1). These findings clearly demonstrate that the gamma radiation doses significantly reduced the survival of buds and altered the morphological characteristics of the wheat cultivar at a level of significance of $P \geq 0.05$, as illustrated in Table (1). Notably, the number of tillers was only observed in the control group. Interestingly, a dose of 50 Gy positively influenced the early emergence of spikes, as depicted in Figure (2). Morphological characterization revealed significant changes in wheat plants exposed to gamma radiation treatment compared to control plants. Gamma radiation is a type of ionizing radiation with high energy, capable of penetrating deep into biological tissues. It can induce various effects in living organisms, including DNA damage, cell death, and mutations (**Ali *et al*., 2015**). In wheat, gamma radiation has been shown to reduce seed germination and plant growth. Whereas high doses of gamma radiation can damage the embryo and inhibit germination (**Arif *et al*., 2015**). This leads to reduced plant growth and yield. Gamma radiation can alter the genetic makeup of cells, leading to mutations. These mutations can be beneficial or harmful, depending on their nature. Also, gamma radiation can cause changes in the size, shape, and structure of plants. This can include stunted growth, abnormal leaf development, and reduced fruit production (**Hameed *et al*., 2019**). Polyethylene glycol (PEG) is a safe and non-toxic polymer that is neutral and non-ionic. It has excellent solubility in water. When the molecular weight of PEG is high, specifically 6000 or above, it is unable to pass through the pores in the cell wall. As a result, water is lost from both the protoplast and the cell wall. The use of PEG solutions is beneficial as it prevents metabolic disruptions that are associated with the use of ionic or low molecular weight osmotic substances, which can enter the cells and cause plasmolysis. However, one drawback of PEG solutions is their high viscosity, which hinders the diffusion of oxygen to the roots. This was highlighted in a study conducted by **Verslues *et al*. (2006)**. The study aimed to examine the impact of water deficit induced by osmotic potential of PEG 6000 on various aspects of plant growth, including seed germination percentage, shoot length, and root length. The results of the study revealed that as the concentration of PEG increased, the seed germination percentage decreased, reaching a minimum of 29% with a 20% PEG concentration. Similarly, the mean lengths of the shoots and roots decreased with increasing osmotic potential of PEG 6000. The reduction rates were observed to range between 8cm and 13cm for shoot length and 3cm and 7cm for root length, as indicated in Table (1) and Figure (3). Polyethylene glycol (PEG) is a synthetic polymer with various applications in medicine, cosmetics, and agriculture. In plants, PEG can act as an abiotic stressor, inducing changes in cellular processes and metabolism (**Shu, 2008**). Studies have shown that PEG can decrease water uptake by plants, leading to drought stress. Plants exposed to PEG often show increased levels of antioxidants, which help to protect cells from damage. PEG can alter the expression of genes involved in stress response, growth, and development (**Shafeeq *et al*., 2014**).

Table (1) : *The effect of gamma radiation doses, PEG 6000 and combined effect of gamma radiation dose 100Gy and PEG 6000 with concentration 20 % on seed germination percent and mean of shoot and root length .*

Parameters	Gamma radiation doses /Gy				Osmotic potential (PEG6000)				Gamma radiation dose x Osmotic potential (PEG6000)			
	Cont.	50	100	LSD 5%=	10%	15%	20%	LSD 5%=	100 Gy × 10% PEG	100 Gy × 15% PEG	100 Gy × 20% PEG	LSD 5%=
Germination%	$94^{a}\pm1.04$	$81^{b}\pm1.4$	$72^{c}\pm1.8$	0.982	$61^{b}\pm0.3$	$44^{c}\pm2.4$	$29^{d}\pm2.5$	4.106	$43^{b}\pm2.56$	$35^{c}\pm1.75$	$21^{d}\pm2.41$	4.654
Mean of shoot length (cm)	17±1.45	14±0.25	9±2.29		9±0.64	6±2.4	4±0.1		7±1.65	5± 1.7	2±0.8	
Mean of root length (cm)	10±1.66	8±1.22	5±0.56		7±1.25	5±0.35	3±1.12		5±0.35	3± 0.65	1.5±0.9	

Values (mean±sd) followed by different letters are significantly difference at = 0.05 level

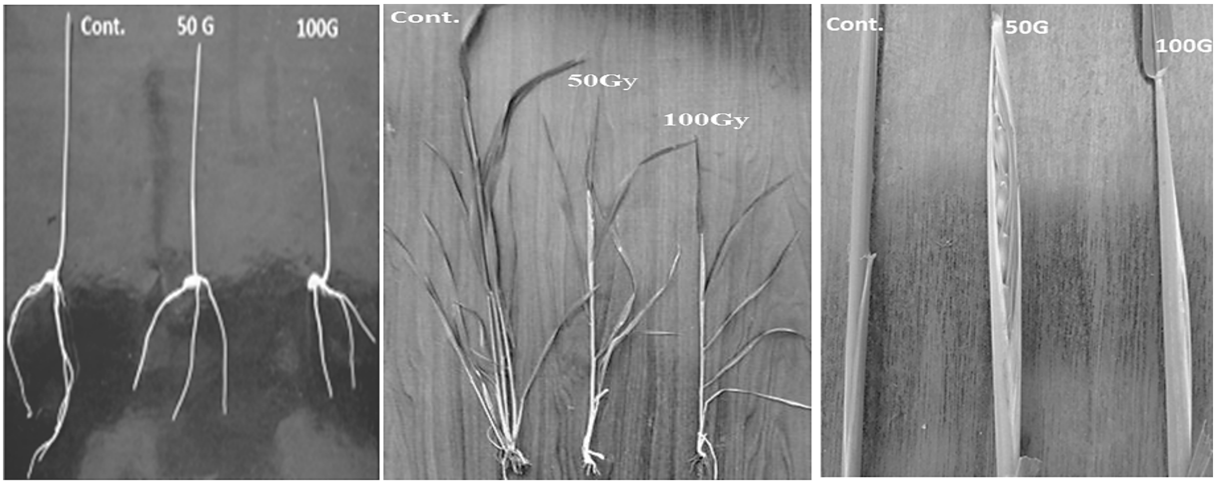

Fig. (2): The impact of gamma radiation doses on seed germination, shoot and root length, tillering and expulsion of the spikes.

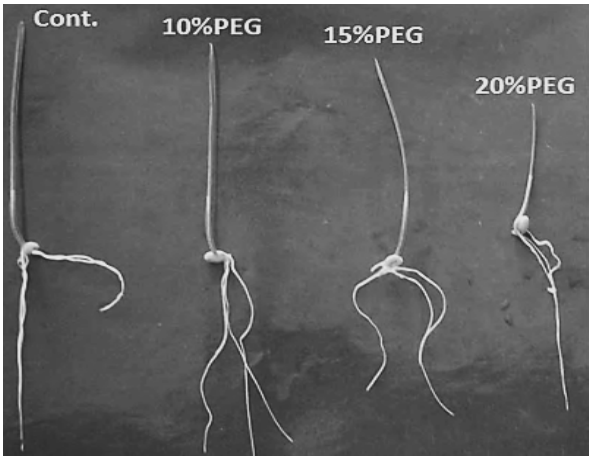

Fig. (3): The effect of different osmotic potential PEG 6000 concentrations 10, 15 and 20% PEG 6000 con¬centrations on seed germination.

In the same line, the combined impact had an adverse effect on plant growth, seed germination, plant height, and root length, as evidenced by the data presented in Table 1 and figure 3b. The mortality rate of seeds increased significantly, reaching 51% and 59% with the combined treatments of 100Gy x 10% PEG and 100Gy x 15% PEG, respectively. Notably, the treatment involving 100Gy x 20% PEG exhibited the highest seed lethality, reaching a staggering 73% compared to the control. The decrease rate was (10, 12 and 15cm, respectively) in mean of shoot length and (5, 7 and 8.5 cm. respectively) in mean of root length as depicted in Table (1) and Figure (4). The

combined effects of gamma radiation and PEG on wheat are not well understood. However, some studies suggest that these two factors can interact synergistically, leading to more pronounced effects than either factor alone. Gamma radiation combined with PEG stress significantly reduced the germination rate and root length of wheat seedlings compared to controls. Morphological characterization involves studying the external features of plants, including their size, shape, and structure (**Abdel-Moneam and El-Demerdash, 2017**).

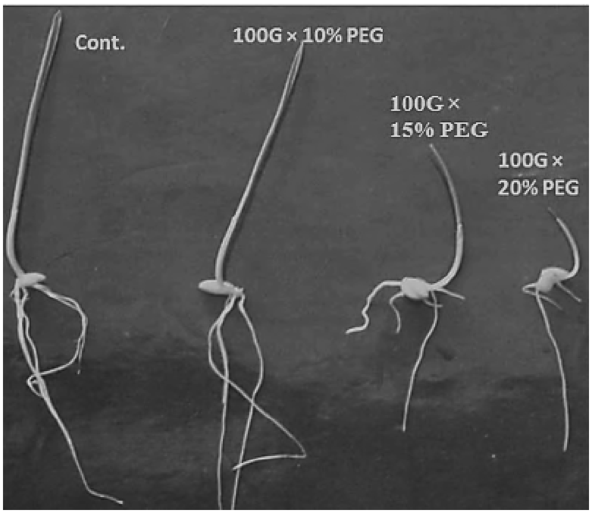

Fig. (4): The effect the combined effect of gamma radiation doses 100Gy with 10, 15 and 20% PEG 6000 con¬centrations on seed germination.

Radiation induced mutations has been widely used in crop improvement as a source of phenotypic and genotypic diversity, as well as an important driver of evolutionary diversification (**Jankowicz *et al*., 2017; Fayed *et al*., 2020**). Mutation induction is valuable in plant breeding to increase food production needs in order to meet the demands of a continually growing population (**Ray *et al*., 2013**). In this investigation, irradiated seeds with gamma radiation doses 50 and 100 Gy had a negative influence on the seed germination percentage, shoot and root length, this in the same line with (**Borzouei *et al*., 2010; Ahumada-Flores *et al*., 2021**). **Preuss and Britt (2003)** stated that the high radiation dose causes growth inhibition in Arabidopsis due to many damages in the entire genome through somatic cell division in G2/M phase, which might block the development of RNA polymerase and inhibit the expression of genes necessary to control cell cycle development. Moreover, the usage of different concentrations of osmotic potential PEG 6000 in this study had a negative impact also on seed germination percentage, shoot and root length. PEG, a chemical that creates physiological drought is commonly used under laboratory conditions to screen out drought resistant varieties at the early stage of seedlings. PEG affects seed germination process through increases external osmotic potential that reduces water uptake during imbibition (**Munns and Tester, 2008**). Drought stress is considered as a serious threat to wheat production. Seed germination is a major problem of wheat production and crucial stage for seedling establishment under stress environment including salinity, high temperature, and drought (**Wahid, 2007**). Seed germination is an important stage in the life history of the plant and affecting seedling development and survival, and population dynamics. Additionally, Seed germination had a complex multi-stage developmental process and regulated by internal and external factors. Internal factors include proteins, plant hormones (gibberellins/ABA balance, ethylene and auxin) chromatin related factors such as methylation, acetylation, related genes (maturating genes and hormonal and epigenetics-regulating genes), non-enzymatic processes, seed age, seed size, and structural components of seed including (endosperm and seed coat). Also, external factors containing moisture, light, salinity, temperature, acidity, and nutrient (**An and Lin, 2011; Finch-Savage and Leubner- Metzger, 2006**). Seed germination begins with the water uptake of dry seed (imbibition) and ends with radicle protrusion. So, seedling development provides a practical and convenient assay to investigate the extent of seed sensitivity to abiotic stress (**Adly and El-Fiki, 2016**). In this study, Drought stress (PEG 6000) affected the water uptake and reduces turgor pressure was leading to reduction germination percentage , decreases shoot length and causes reduction of root length of wheat this agree with (**Khakwani *et al*., 2011; Raza *et al*., 2012; Almaghrabi, 2012**). The harmful effects of PEG may result decrease in photosynthesis and increase in respiration rate leading to a shortage of assimilates to the developing organs,thus slowing down growth or stopping it entirely. The cell division and differentiation are inhibited which adversely affects metabolic and physiological processes (**Khakwani *et al*., 2011**). In general, the size, morphology of the root will determine the ability of plants to acquire water and nutrients (**Passioura, 1988**) and influence the relative size and growth rate of the shoot (**Vamerali *et al*., 2003**). Stress combination is a term used to describe a situation in which a plant is simultaneously subjected to two or more abiotic stresses (**Mittler, 2006**). In general, the combination of two or more abiotic stresses has a negative impact on plants that is greater than that of each of the different stresses applied individually (**Choudhury *et al*., 2017**). In this investigation, the impact of each radiation and drought was very clear in Figure (4) by decrease the mean of shoot and root lengths. The ability of a plant to alter its phenotype in response to the environment is called phenotypic plasticity and affects morphology, anatomy, development, or changes in resource allocation (**Sultan, 2000**). Phenotyping has been a major challenge for plant breeders to improve abiotic stress tolerance in crop plants. It includes genetically complex traits that are extremely difficult to measure, and would be ideal to assist plant breeders for using in breeding program (**Sharma *et al*., 2016**). Studies indicate that roots play a crucial role in water stress as well as adaptation and tolerance to water-deficit stress (**Geng *et al*., 2018**). Root phenotyping is as important as shoot phenotyping, because plant's ability to uptake moisture and nutrients mainly depends on root phenotyping and function (**Zhu *et al*., 2011**). Therefore in this investigation, study the root phenotyping which is important for crop breeding, although under field conditions, screening roots by phenotyping is a very difficult task. However, selection for deep and fast growing roots may enhance soil water harvesting and help in yield stabilization under water stress conditions. Stress combination led to higher reduction in photosynthetic activity and enhanced production of ROS (**Sachdev *et al*., 2021**). The highly reactive ROS induced DNA (deoxyribonucleic acid) breaks may induce a spectrum of genomic and chromosomal abnormalities, which result in severe damage to cell structures and cell death (**Gudkov *et al*., 2019**). ROS damage the macromolecules including proteins, carbohydrates, nucleic acids, and lipids, or cellular structures like membranes, resulting with inhibition of seed germination (**Ibrahim, 2016**).

ANATOMICAL STUDIES OF ROOTS

Semi thin sections

Transverse sections of the roots illustrated the variations of anatomical features of the unstressed and stressed wheat cv.sids 13. Roots are the first to sense abiotic stresses and adjust their genetic program for post-embryonic development to survive the stress (**Lynch, 1995**). The external form and internal anatomy of the roots is also changed in plant response to drought. In this investigation, control samples of wheat showed that the outermost layer of the root is a single layer of epidermis. The epidermal cells are live, on the periphery, they are elongated Fig. (5a). The root cross sectional under different abiotic stress depends on the area of its cortex cells. However, the larger root cross sectional area in wheat under drought stress and combined effect between drought stress and gamma radiation has larger cortex area which was composed of a high proportion of aerenchyma than untreated wheat roots. On the other side, under radiation wheat root had a lowest cortex area Figs. (5a, b, c and d).

The result indicates that root tip swelling due to water stress is mainly caused by enlargement of cortex cells rather than by an increase in the number of cortex cells and larger of root aerenchyma cells. Deformed shape of the epidermis and outer cortical cells In contrast, the epidermal cells closer to the root cap remained small and compact like the cells in unstressed seedling root apex. One such feature in the root anatomical adaptation is the development of root cortical aerenchyma (RCA). Aerenchyma is a specialized root tissue and formed via PCD by water stress which may contribute to reduced radial conductivity (**Evans, 2004**). During the formation of aerenchyma in the root cortex, nuclei of cells undergoing programmed cell death contain fragmented DNA (**Mittler and Lam, 1995**). This indicates the activation of DNA degradation mechanism prior to the final disruption of the nucleolus that occurs during the autolysis in the cell group. The swollen cortex cells of root tips were significantly increased. It can be formed either by deferential growth and cell separation or in the case of lysogenic by the selective death of cells in the mid cortex. The autolysis and disappearance of cortex or pith parenchyma cells leads to the formation of aerenchyma. Aerenchyma is the general term for tissue with large intercellular spaces (**Esau,**

1977). The induction of RCA by nutrient stress in oxygenated roots prompted the hypothesis that RCA enhances adaptation to nutrient stress by converting living cortical tissue including cell wall to form air space (channels) for oxygen transport, thereby reducing the respiratory and nutrient cost of soil exploration (**Lynch and Brown, 1998**). RCA serves many functions for the plants, which include the continued exploration of nutrients in the soil when they are in limited supply. The important role played by aerenchyma in the root tissues which provides an internal passage for oxygen transfer, also brings down the number of cells that consume oxygen (**Singhal and Mehar, 2020**). During the development of aerenchyma there is enlargement of cortical cells. It has been shown that cells to reasoning for the enlargement of cells where the cellulose enlarged radially, and some of them disintegrated, leading to the development of intercellular spaces (**Kawase, 1974**). The expansion of cells was reasoned to be due to the change in cell walls, and the radial cell enlargement was an induction of cellulase acting on the microfibrills which are normally transversely oriented (**Horton and Osborne, 1967**).

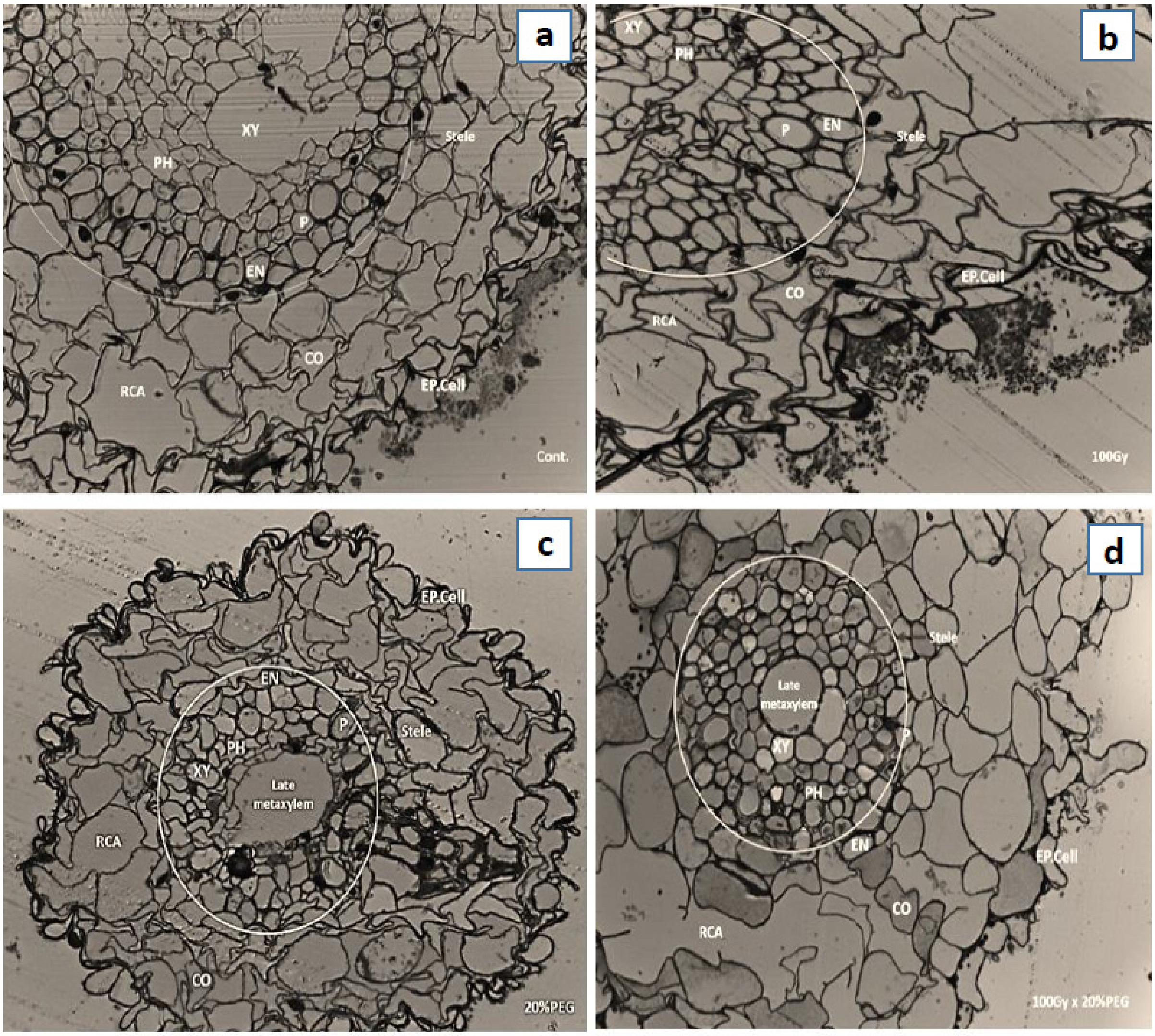

Fig. (5): Cross-sections of anatomical and environment variations in wheat root tip
(a) Control (b) Radiation (c) Drought (d) combined effect
CO: cortex; EN: endodermis; P: pericycle; PH: phloem; RCA: root cortical aerenchyma; XY: xylem.

Scanning electron microscopy

The Scanning electron microscopy micrographs illustrated the changes dramatically of root tips structure after exposure to gamma radiation dose and water stress. Moreover, it has been shown that water stress triggers a swelling of root. The plants under drought stress (20% PEG) have the maximum of swollen in root tip Fig. (6c). The combination between gamma radiation dose 100Gyx20% PEG showed less swelling compared with drought treatment Fig. (6 d). Whereas, the root tip of irradiated plants with dose 100Gy was the lowest swelling root tip compared with control. Root tip swelling was a common response to mild and moderate water stress. Ji et al. (2014) stated that the root swelling was a general morphological response in both monocot and dicot plants to PEG mediated mild and moderate water stress. The root tip swelling is a mechanism for plant roots to absorb more water and protecting root meristem cells from water stress.

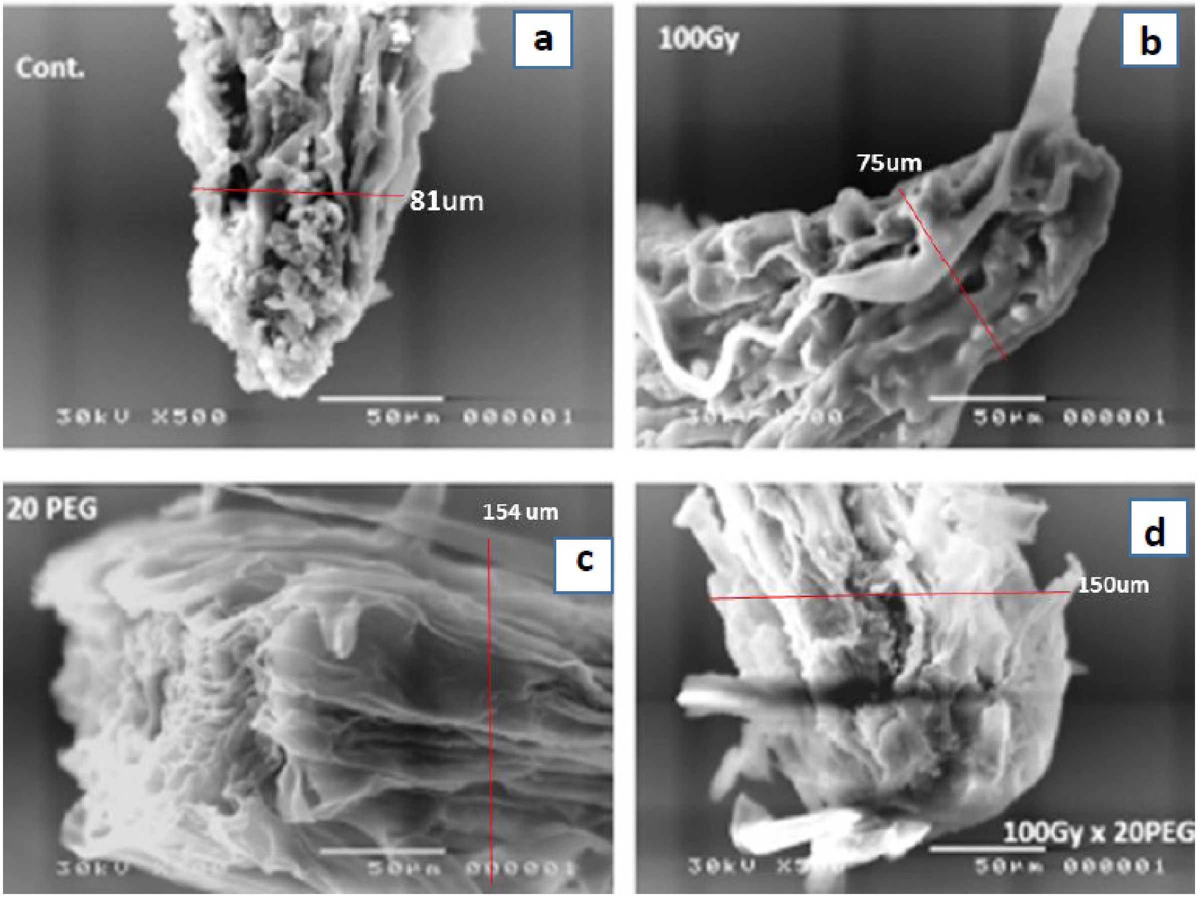

Fig. (6): Scanning electron microscopy micrograph of the phenotype root tip swelling in wheat under.
(a) Control (b) Irradiated root tip (c) Swollen of root tips under drought stress (d) Combined effect stress.

The ultrastructure of roots cells

Transmission electron microscopy micrograph showed dramatic ultrastructure changes in root cells occur during stress responses. In this study, the cortical cells of treated roots showed mitochondria that one of the main cellular components to be affected by gamma radiation, drought stress and combined effect. In this paper showed normal ultrastructure of untreated cells with intact cell walls and cytoplasm. Nuclei were large and spherical and containing fairly disperse chromatin and a prominent nucleolus in cortical cells of untreated roots. Nuclei were more or less centrally located in the cells. A relatively dense cytoplasm contained numerous mitochondria and variously shaped plastids. Under radiation stress treatment (100Gy) showed deformed in nucleus and mesophyll cell as shown as in figure (8). In severely drought stressed cells under PEG 20% illustrated the crescent-shaped mitochondria and mitochondria were fused together figure (9a) and crescent-shaped nuclei as figure (9b). Additionally, the plasma membrane had retracted from the cell wall with abundant vesicles present near the plasma membrane as seen as figure (9c). The mitochondria under combined stress showed a moderately dense stroma, which contained ribosomes, DNA and well developed cristae. The two membranes of the mitochondrial envelope were parallel and evenly spaced figure (10d). Figure (10c) revealed the increase number of mitochondria to increased respiration, as a less susceptible and more adaptive process than photosynthesis, can be regarded as a determinative factor for the survival of plants in extreme environment (**Vassileva *et al.*, 2011**). Mitochondria have their own genetic material; therefore, their replication is dependent on cellular division. The existing mitochondrion undergoes binary fission, producing two equivalent organelles. Conversely, one or more organelles can fuse together producing single mitochondrion. Additionally, mitochondria have the ability to change their architecture and distribution within the cytosol to be malleable with their function **Saad-Allah and Abdelsalam, (2020)**.

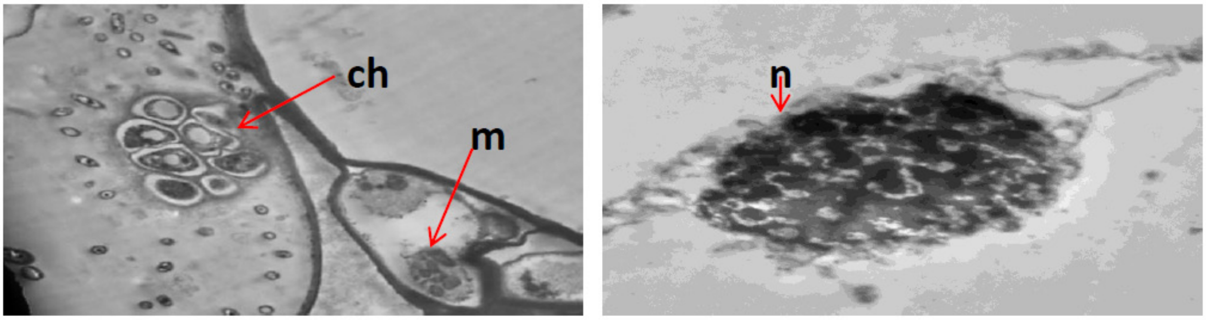

Fig. (7): Ultrastructure of mesophyll cells of untreated wheat seedlings
ch: chloroplast n: nucleus m: mitochondria. Scale bars = 2 μm.

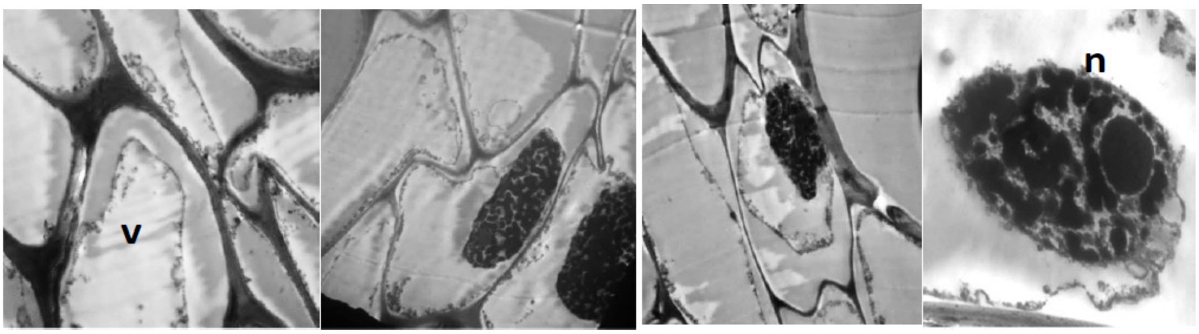

Fig. (8): Ultrastructure of deformed of nucleus and mesophyll cells of irradiated with dose 100 Gy wheat seedlings
n: nucleus v: vacuole Scale bars = 2 μm.

Mitochondrial dynamics is a delicate physiological balance between fission and fusion events of mitochondria, which is essential for their maintenance in the growing cells, regulation of cell death pathway, and removal of damaged mitochondria (**Hales, 2004; Youle, and Van der Bliek, 2012**). Mitochondrial morphology varies tremendously in the cells and tissues in response to external stimuli and availability of nutrients. Mitophagy is an autophagy-lysosome system that removes dysfunctional mitochondria through fusion with lysosomes (**Ding and Yin, 2012**). Mitochondria are the intracellular organelles which play a significant role in the cells by metabolizing nutrients, powerhouses of the cell via producing the “energy currency” adenosine triphosphate (ATP) and responsible for various processes such as energy metabolism, intracellular Ca^{2+} regulation, generation of free radical, reactive oxygen species production & scavenging, regulation of apoptotic cell death , activation of the caspase family of proteases, cell survival and death (**Kim *et al*., 2008; Halliwell and Gutteridge, 2007**).

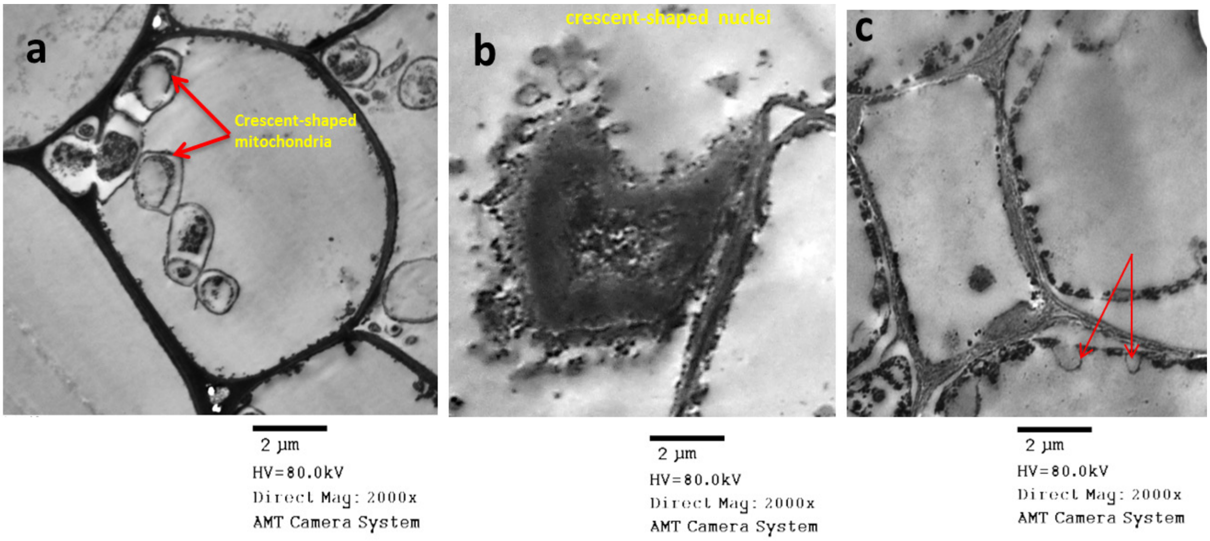

Fig. (9): Ultrastructure of (a) Crescent-shaped mitochondria, (b) Crescent-shaped nucleus and (c) Shrinkage of the plasma membrane from the cell wall and presence of vesicles between plasma membrane and cell wall. Arrows point to vesicles present near the plasma membrane of mesophyll cells of treated wheat root by 20% PEG.

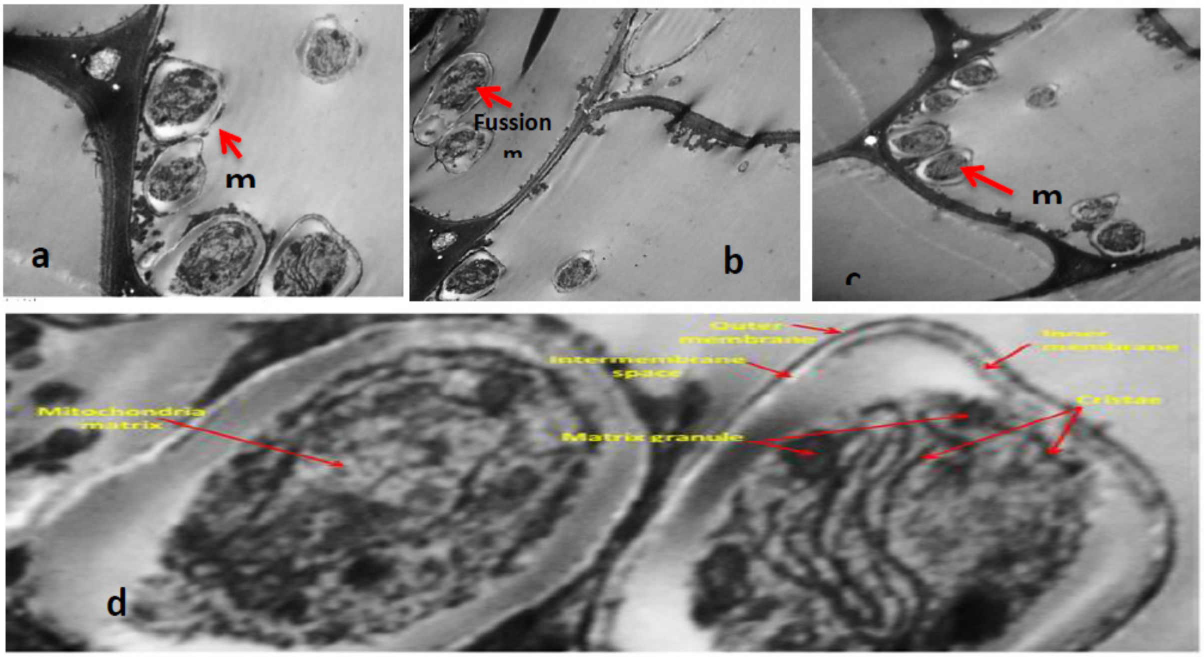

Fig. (10): Ultrastructure of (a) deformed shape of mitochondria,(b) Fussion mitochondria, (c) increase number of mitochondria and (d) structure of mitochondria in mesophyll cells of irradiated wheat root by 100 Gy and 20% PEG.

The higher mitochondrial number meets the increased demand for ATP under unfavorable conditions, when photosynthesis is greatly suppressed (**Stoyanova and Yordanov, 1999; Silva *et al*., 2010**), and also these organelles respond to stress by synthesis of numerous specific mitochondrial stress proteins (**Rizhsky *et al*., 2002 & 2004**). Mitochondria have a central role as mediators of programmed cell death (PCD) by inducing the generation large amounts of ROS signalling. PCD is an essential process controlling the elimination of cells during development, defence and stress responses to biotic and abiotic stresses (**Blackstone and Kirkwood, 2003; Sychta *et al*., 2021**). In plants, programmed cell death is essential machinery for growth, development and plays a role in response and adaptations adopted by plants to survive under stresses as biotic and abiotic (**Lam *et al*., 2001; Greenberg and Yao, 2004; Kratsch and Wise, 2000; Huh *et al*., 2002**). Plant PCD involves a complex regulatory network that is regulated at the gene and protein levels by multiple signal transduction pathways. Morphological changes in mitochondria are one of the early indicators of whether cells are affected by reactive oxygen species (ROS) stress. Stress promotes directly or indirectly. ROS are the intracellular signalling molecules regulating plant growth, development, responses to external biotic and abiotic stimuli, and PCD (**Ye *et al*., 2021**).

ACKNOWLEDGEMENT

The research work was supported by grants from National Centre for Radiation Research and Technology, Egyptian Atomic Energy Authority, Cairo, Egypt.

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