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Anatomical studies of root, stem and leaves of three pepper cultivars in response to gamma irradiation exposure

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Vessel elements play an essential role in the transportation of water and minerals from roots to leaves of a plant. This present study was aimed at evaluating the response of some anatomical traits in pepper to gamma rays. The seeds of Shombo, Tatase and Nsukka yellow pepper varieties were exposed to varied dosages of gamma irradiation (50, 100, 150 and 200 Gy from ^{60}Co source) and planted using a 3 x 5 factorial experimental design laid out in a Completely Randomized Design (CRD) with 15 replicates. After harvest, transverse sections of the stem, root and leaves were made using a Reichert sledge microtome in the Anatomy Laboratory in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The data collected were analyzed via multivariate analysis and the analysis of variance for most of the anatomical features revealed high significant differences across dosages, cultivars and interactions. Leaves from 150 Gy treatment dose had the highest number of xylem vessel per row (4.00 ± 0.44), epidermal layer diameter ($3.91 \pm 0.76\mu\text{m}$) and palisade mesophyll diameter ($6.26 \pm 0.43\mu\text{m}$). The stem xylem vessel diameter varied across treatments from 1.32 to $4.84\mu\text{m}$ while the vessels diameter in the roots ranged from 3.24 to $6.20\mu\text{m}$. In conclusion, it is evident that the anatomical features peppers were sensitive to gamma irradiation which could influence the physiological processes of the plant. These changes could be exploited for the improvement of these plants.

Keywords: Climate change; Gamma rays; Pepper; Plant Anatomy; Vessel element; Transverse section; Xylem.

INTRODUCTION

The genus *Capsicum* shows both intra- and inter-specific diversity in fruit type, color, shape, taste, and biochemical content (Dagnoko et al., 2013). The species encompass a wide variety of shapes and sizes of peppers, both mild and hot, ranging from bell to chili peppers and other aromatic pepper types in Nigeria. The significance of peppers cannot be overemphasized as they are third in relevance among cultivated vegetables and are being consumed by every household (Abu et al., 2011). The remarkable cherished flavour and the desirability of carotenoid pigments in food coloring continually increase consumers

demand on pepper in local and urban markets (Abu et al., 2011).

Vessel element plays an essential role in water transportation from roots to leaves of a plant. It is also known as tracheary elements or conduits, which shows a wide anatomical variation with respect to their size, shape, arrangement, and grouping (Carlquist, 2001). Tracheary elements have been extensively studied by plant anatomists for many years and provide valuable information to a wide range of study fields, ranging from wood identification and palaeobotany to plant ecology and physiology (Tyree and Zimmermann, 2002; Fonti et al., 2010;

Pittermann, 2010; Gasson, 2011; Choat et al., 2012).

The essential elements of vascular systems are the xylem, concerned with the transport of water and dissolved salts, and the phloem, which translocates synthesized but soluble materials around the plant to places of active growth or regions of use or storage (Cutler et al., 2007). Xylem and phloem are normally associated and together form the vascular bundles which are often enclosed in a sheath of fibres. In addition, contains an outer sheath of parenchyma cells (the bundle sheaths) in some instances. Vascular bundles make up the 'plumbing system' of primary tissues, and organs without secondary growth in thickness (Cutler et al., 2007).

Vulnerability of plants to induced cavitation is directly associated with vessel diameter, whereby drought events are experimentally demonstrated to affect plants (Davis et al., 1999). There are anatomical evidence that species with wide treachery elements are more vulnerable to drought-induced cavitation than those with narrow conduits (Pockman and Sperry, 2000; Carlquist, 2001; Christman et al., 2012). The lengths of vessel elements, which reflect the length of fusiform cambium initial cells, have been used as a major criterion in establishing evolutionary traits in anatomy (Scholz et al., 2013). Vessel element has been suggested to be a sensitive character for xeromorphy or mesomorphy (Carlquist, 2007). Therefore, this present study seeks to evaluate the response of some anatomical traits in pepper to gamma rays.

MATERIALS AND METHODS

The pepper seeds of the three varieties (Tatase, Shombo and Nsukka yellow pepper) were divided into five sets and exposed to varied gamma irradiation concentrations of 0, 50, 100, 150 and 200 Gy (Grey) in the Gamma Irradiation Facility (GIF), Nuclear Technology Centre (NTC), Sheda Science and Technology Complex, Abuja.

Nurseries of the exposed seeds were raised according to the separate treatments in nursery baskets filled with well-filtered topsoil mix according to the specification of (Uguru, 1999) as adopted by (Ojua and Abu, 2018; Ojua et al., 2019). Seedlings were then transplanted at 6 weeks when the seedlings were 5 to 10 cm tall to individual polybags filled with well-filtered topsoil in the Botanical Garden of the University of Nigeria, Nsukka. The experiment was carried out using a 3 x 5 factorial experimental design laid out

in a completely randomized design (CRD) with 15 replicates.

The sectioning was carried out at the Anatomy Laboratory in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. Three mature plants from each treatment were randomly uprooted at harvest. Sections were made at 1 cm from the root tip, 1 cm away from the first branch on the stems and the fifth leave on a branch. Transverse sections of the stem, root and the leaves were made using a Reichert sledge microtome. The sections were placed into labeled specimen bottles containing 70% absolute alcohol to preserve the sections until they were needed for microscope studies. The samples for study were mounted on 2 drops of iodine. Two drops of phloroglucinol and 2 drops of concentrated hydrochloric acid were added while glycerin was used as the mountant (Purvis et al., 1964). The prepared sections were then viewed under Motic light microscope at X40 and X100 magnifications and microphotographs of the specimen were taken using a microscopic moticam.

Data collected were subjected to analysis of variance (ANOVA) using IBM Statistical Package for Social Science (SPSS) software version 20. Significant means were separated with least significant difference (LSD) test at $P \leq 0.05$ from GenStat Discovery Edition 4 software.

RESULTS

Xylem vessels (vessel elements) are formed as a result of the activities of the pro-vascular tissues (procambium), which are meristematic in nature. Cells at this differentiation stage could be very sensitive to the slightest change in both external and internal environments. The TS of the leaf/petiole, stem and root depicts the epidermis, parenchyma cells, palisade mesophyll, leaf blade, cortex, phloem, xylem, xylem vessels and pith were evaluated (Plate A, B, and C).

The analysis of variance (ANOVA) for all the anatomical features revealed high significant differences across dosages, cultivars and interaction, except between cultivars for parenchyma cell diameter of the stem and palisade mesophyll diameter of the petiole/leaf (Table 1).

Table 2 divulges the mean gamma irradiation dosage effect on the anatomy traits. The number of xylem vessel per row, epidermal layer diameter and palisade mesophyll diameter were significantly higher with 150 Gy dose (4.00 ± 0.44 , 3.91 ± 0.76 and $6.26 \pm 0.43 \mu\text{m}$ respectively).

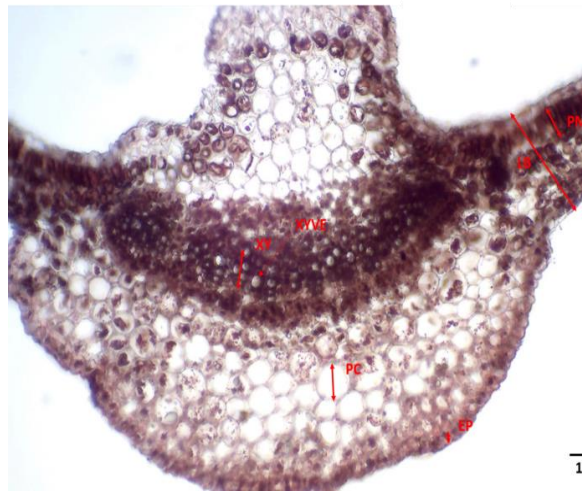
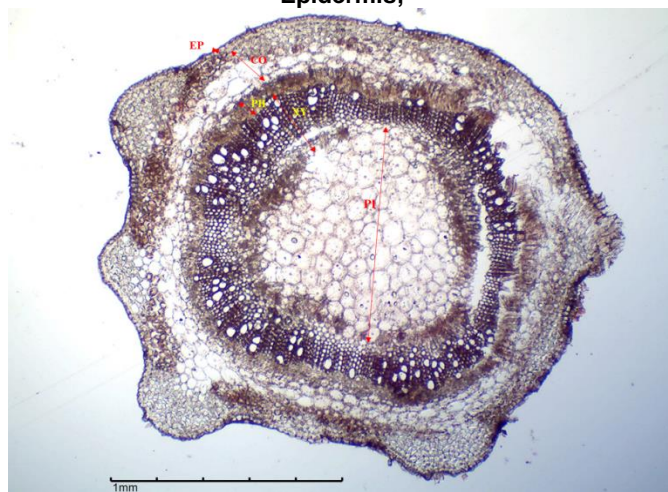
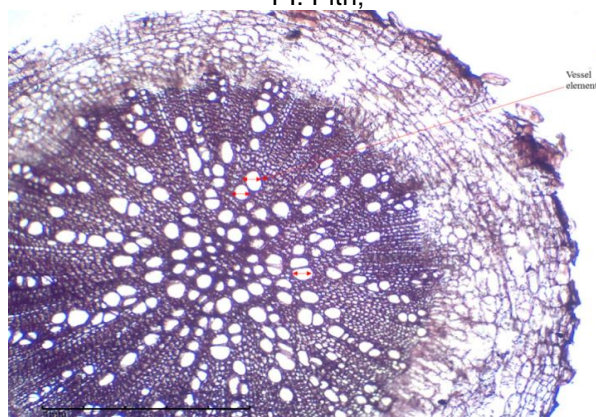


PLATE A: TS of the leaf/petiole: PC: parenchyma cells; PM: palisade mesophyll; LB: leaf blade; EP: Epidermis;



B. TS of the stem: EP: Epidermis; CO: Cortex; PH: Phloem; XY: Xylem, XYVE: xylem vessel element; PI: Pith;



C. TS of the root

Table 1: Summary of ANOVA table for the Anatomical features of *C. annuum*.

Source of Variation	Mean Squares								
	Number of xylem vessels per row in the petiole/leaf	Palisade mesophyll diameter of the petiole/leaf	Leaf blade	Epidermal diameter of the petiole/leaf	Epidermal cell Diameter of the stem	Parenchyma cell diameter of the stem	Cortex diameter of the stem	Xylem vessel diameter of the stem	Xylem vessel diameter of the root
Dosage	2.31**	17.60***	89.22***	12.27***	0.50***	5.03***	34.06***	1.43***	5.05***
Cultivar	17.76***	0.11 ^{NS}	81.84***	9.62***	21.94***	2.17 ^{NS}	72.00***	16.58***	9.50***
Dosage x Cultivar	1.74**	9.55***	165.10***	14.14***	1.38***	14.75***	54.14***	5.21***	2.48***
Residual	0.56	0.51	2.26	0.19	0.04	0.85	2.14	0.24	0.29

** - Significant at P < 0.01; ***- Significant at P < 0.001; NS- not significant

Table 2: Mean Effect of gamma irradiation dosages on the anatomy characteristics (μm) of *C. annuum*

Dosage	0 Gy (Control)	50 Gy	100 Gy	150 Gy	200 Gy
Number of xylem vessels per row	3.00 \pm 0.2 ^b	3.13 \pm 0.22 ^b	3.20 \pm 0.26 ^b	4.00 \pm 0.44 ^a	3.27 \pm 0.23 ^b
Leaf Epidermal layer diameter	2.37 \pm 0.24 ^b	1.95 \pm 0.13 ^c	1.80 \pm 0.17 ^c	3.91 \pm 0.76 ^a	1.73 \pm 0.07 ^c
Leaf Parenchyma cell diameter	4.81 \pm 0.32 ^a	5.07 \pm 0.34 ^a	4.01 \pm 0.26 ^b	4.89 \pm 0.63 ^a	3.77 \pm 0.38 ^b
Palisade mesophyll diameter	5.24 \pm 0.34 ^b	5.43 \pm 0.29 ^b	3.63 \pm 0.31 ^c	6.26 \pm 0.43 ^a	3.98 \pm 0.19 ^{bc}
Leaf blade diameter	19.66 \pm 1.77 ^a	16.49 \pm 0.7 ^b	14.34 \pm 1.14 ^c	20.18 \pm 2.76 ^a	16.49 \pm 0.88 ^b
Stem Epidermis diameter	1.55 \pm 0.12 ^b	1.85 \pm 0.29 ^a	1.48 \pm 0.20 ^b	1.75 \pm 0.28 ^a	1.42 \pm 0.24 ^b
Stem Cortex diameter	9.64 \pm 0.32 ^c	12.42 \pm 1.58 ^{ab}	11.13 \pm 0.45 ^b	13.03 \pm 0.71 ^a	9.85 \pm 0.23 ^c
Stem xylem vessel diameter	3.37 \pm 0.19 ^a	3.60 \pm 0.20 ^a	3.57 \pm 0.18 ^a	3.36 \pm 0.43 ^a	2.83 \pm 0.37 ^b
Root xylem vessel diameter	5.17 \pm 0.17 ^b	4.06 \pm 0.20 ^c	5.65 \pm 0.16 ^a	4.85 \pm 0.33 ^b	4.80 \pm 0.24 ^b

Data are presented in mean \pm standard error and significant means are separated with different alphabets each horizontal array using least significant difference test (F-LSD) at P \leq 0.

The varied irradiation dosages appears not to have affect the stem vessel diameter, except where plant raised from seeds exposed to 200 Gy had significantly the smallest diameter (Table 2). Significant variations also exist in xylem vessel diameter of the root with 100 Gy recording significantly the highest xylem vessels ($5.65 \pm 0.16 \mu\text{m}$), while 50 Gy induced a significant reduction ($4.06 \pm 0.20 \mu\text{m}$) as compared to the control (Table 2).

Variation in the anatomy traits was not solely dependent on the irradiation dosage but also as a result of varietal differences. Cultivar effect ranged approximately from 2 – 4 μm , 2 – 3 μm , 4 – 5 μm , 4.5 – 5.0 μm and 16 – 19 μm in number of xylem vessel per row, epidermal layer diameter, parenchyma cell diameter, palisade mesophyll diameter and leaf blade diameter correspondingly (Table 3). Furthermore, the cultivar effect showed

variants ranging from 1 – 3 μm , 9.5 – 13 μm and 2.5 – 4.5 μm in the stem epidermis, cortex and xylem vessel respectively. Consequently, the stem epidermis, cortex and xylem vessel were all significantly higher in Shombo as compared to other cultivars (Table 3).

The Nsukka yellow pepper cultivar exposed to 150 Gy showed a significant increase in the xylem vessels frequency per row and palisade mesophyll in the leaf with an average of 5.80 ± 0.66 vessels per row (Table 4). Generally, diameter of the leaf blade ranged from 9.87 μm to 28.27 μm in Shombo and Tatase plants exposed to 150 Gy respectively (Table 4). The stem xylem vessel diameter varied across interactions from 1.32 to 4.84 μm . The vessels increased in Shombo and Tatase, but reduced in Nsukka yellow pepper across dosages as compared to their controls (Table 5).

Table 3: Mean Effect of cultivars on the anatomy characteristics (μm)of *C. annuum*

Cultivar	Shombo	Tatase	Nsukkayellow pepper
Number of xylem vessels per row	2.44 ± 0.1^c	3.40 ± 0.17^b	4.12 ± 0.23^a
Leaf Epidermal layer diameter	2.29 ± 0.13^b	1.76 ± 0.14^c	3.00 ± 0.5^a
Leaf Parenchyma cell diameter	4.64 ± 0.15^{ab}	4.18 ± 0.45^b	4.72 ± 0.3^a
Palisade mesophyll diameter	4.98 ± 0.15	4.88 ± 0.29	4.86 ± 0.44
Leaf blade diameter	16.90 ± 1.38^b	19.45 ± 1.53^a	15.95 ± 0.93^c
Stem Epidermis diameter	2.69 ± 0.10^a	1.11 ± 0.08^b	1.03 ± 0.10^b
Stem Cortex diameter	13.06 ± 0.85^a	10.86 ± 0.63^b	9.72 ± 0.18^c
Stem xylem vessel diameter	4.28 ± 0.13^a	2.94 ± 0.14^b	2.82 ± 0.26^b
Root xylem vessel diameter	4.22 ± 0.19^c	5.41 ± 0.16^a	5.08 ± 0.19^b

Data are presented in mean \pm standard error and significant means are separated with different alphabets on the same column using least significant difference test (F-LSD) at $P \leq 0.05$

Table 4: Effect of gamma irradiation on the leaf/ petiole characteristics of three *C. annuum* cultivars

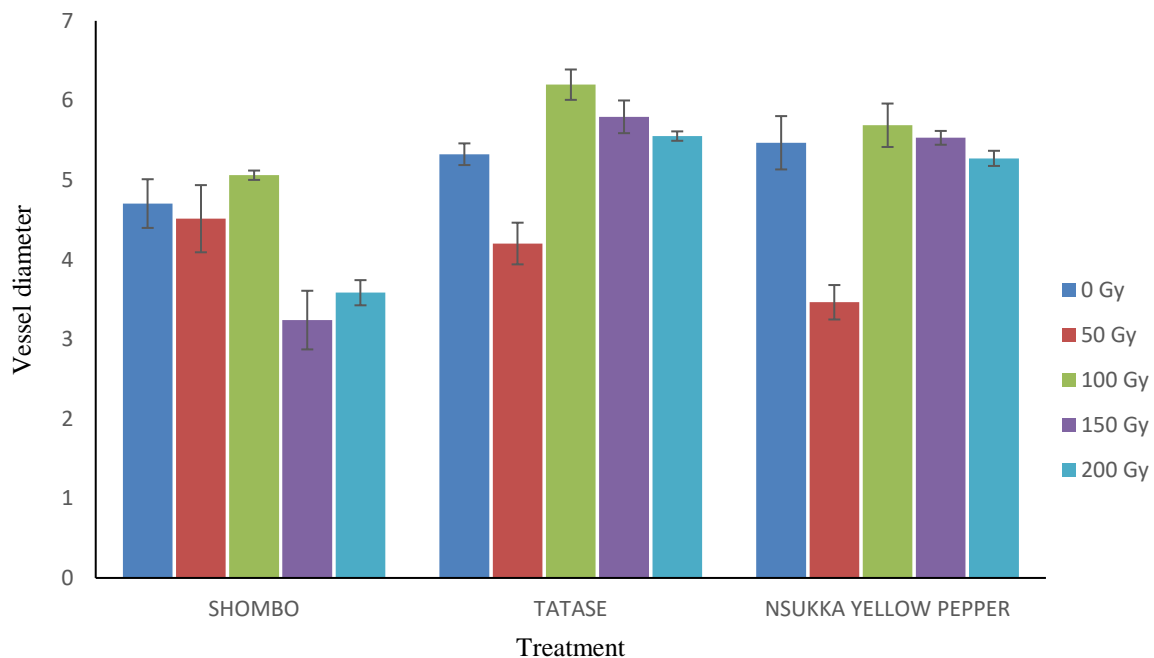
Cultivar	Dosage	Number of xylem vessels per row(μm)	Epidermal layer diameter (μm)	Parenchyma cell diameter (μm)	Palisade mesophyll diameter (μm)	Leaf blade diameter (μm)
Shombo	0 Gy	2.40 ± 0.24^d	3.22 ± 0.28^b	4.28 ± 0.33^{cde}	5.94 ± 0.27^{bc}	25.07 ± 2.51^b
	50 Gy	2.60 ± 0.24^d	2.28 ± 0.05^c	4.32 ± 0.34^{cde}	4.72 ± 0.22^{ef}	15.60 ± 0.55^f
	100 Gy	2.20 ± 0.20^d	2.34 ± 0.07^c	4.80 ± 0.09^{cd}	5.04 ± 0.05^{cde}	18.07 ± 0.20^{de}
	150 Gy	2.60 ± 0.24^d	1.86 ± 0.26^{cde}	5.26 ± 0.47^{bcd}	4.82 ± 0.19^{def}	9.87 ± 0.33^g
	200 Gy	2.40 ± 0.24^d	1.76 ± 0.08^{cde}	4.52 ± 0.15^{cde}	4.40 ± 0.41^{efg}	15.90 ± 0.35^{ef}
Tatase	0 Gy	3.00 ± 0.32^{cd}	2.24 ± 0.45^c	6.28 ± 0.31^{ab}	6.22 ± 0.24^b	19.93 ± 0.24^d
	50 Gy	2.80 ± 0.20^d	2.08 ± 0.28^{cd}	6.48 ± 0.59^{ab}	5.80 ± 0.18^{bc}	19.03 ± 0.66^d
	100 Gy	3.80 ± 0.49^{bc}	0.92 ± 0.09^f	2.78 ± 0.29^{fg}	2.64 ± 0.24^j	10.27 ± 0.22^g
	150 Gy	3.60 ± 0.40^{bc}	1.98 ± 0.10^{cde}	1.98 ± 0.10^g	5.64 ± 0.19^{bcd}	28.27 ± 0.47^a
	200 Gy	3.80 ± 0.37^{bc}	1.60 ± 0.12^{de}	3.36 ± 1.12^{ef}	4.08 ± 0.21^{gi}	19.73 ± 0.27^d
Nsukka yellow pepper	0 Gy	3.60 ± 0.24^{bc}	1.66 ± 0.07^{de}	3.88 ± 0.23^{def}	3.56 ± 0.13^{gij}	13.97 ± 0.50^f
	50 Gy	4.00 ± 0.32^b	1.50 ± 0.09^e	4.42 ± 0.13^{cde}	5.76 ± 0.77^{bc}	14.83 ± 0.29^f
	100 Gy	3.60 ± 0.24^{bc}	2.14 ± 0.12^d	4.46 ± 0.12^{cde}	3.22 ± 0.41^{ij}	14.70 ± 0.52^f
	150 Gy	5.80 ± 0.66^a	7.88 ± 0.20^a	7.42 ± 0.40^a	8.32 ± 0.40^a	22.40 ± 1.69^c
	200 Gy	3.60 ± 0.24^{bc}	1.82 ± 0.14^{cde}	3.44 ± 0.15^{ef}	3.46 ± 0.20^{ij}	13.83 ± 0.41^f

Data are presented in mean \pm standard error and significant means are separated with different alphabets on the same column using least significant difference test (F-LSD) at $P \leq 0.05$

Table 5: Effect of gamma irradiation on the stem features of three cultivars of *C. annuum*

Cultivar	Dosage	Epidermal diameter (μm)	Cortex diameter (μm)	Xylem vessel diameter (μm)
Shombo	0 Gy	2.05 \pm 0.11 ^d	9.44 \pm 0.45 ^{fg}	3.63 \pm 0.17 ^c
	50 Gy	3.29 \pm 0.18 ^a	20.43 \pm 1.21 ^a	4.41 \pm 0.10 ^{ab}
	100 Gy	2.49 \pm 0.06 ^c	11.82 \pm 0.85 ^{cd}	4.05 \pm 0.24 ^{bc}
	150 Gy	2.99 \pm 0.10 ^b	13.13 \pm 0.62 ^c	4.84 \pm 0.31 ^a
	200 Gy	2.64 \pm 0.07 ^c	10.49 \pm 0.37 ^{def}	4.49 \pm 0.30 ^{ab}
Tatase	0 Gy	0.99 \pm 0.05 ^{fg}	9.70 \pm 0.83 ^f	2.52 \pm 0.23 ^d
	50 Gy	0.83 \pm 0.02 ^g	7.60 \pm 0.28 ^g	2.69 \pm 0.06 ^d
	100 Gy	0.86 \pm 0.06 ^{fg}	11.62 \pm 0.85 ^{cde}	2.91 \pm 0.24 ^d
	150 Gy	1.79 \pm 0.11 ^e	15.72 \pm 1.00 ^b	3.90 \pm 0.40 ^{bc}
	200 Gy	1.10 \pm 0.06 ^g	9.68 \pm 0.44 ^f	2.67 \pm 0.21 ^d
Nsukka yellow pepper	0 Gy	1.61 \pm 0.04 ^{ef}	9.79 \pm 0.38 ^{ef}	3.97 \pm 0.16 ^{bc}
	50 Gy	1.43 \pm 0.13 ^f	9.24 \pm 0.56 ^{fg}	3.70 \pm 0.11 ^c
	100 Gy	1.10 \pm 0.08 ^g	9.96 \pm 0.46 ^{ef}	3.75 \pm 0.22 ^c
	150 Gy	0.47 \pm 0.03 ^h	10.23 \pm 0.29 ^{def}	1.34 \pm 0.09 ^e
	200 Gy	0.52 \pm 0.04 ^h	9.38 \pm 0.28 ^{fg}	1.32 \pm 0.13 ^e

Data are presented in mean \pm standard error and significant means are separated with different alphabets on the same column using least significant difference test (F-LSD) at $P \leq 0.05$

Figure 1: Effect of gamma irradiation on the xylem vessel diameter of three cultivars of *C. annuum*

Significant differences were observed in the root xylem vessel diameter across all dosages by cultivars interactions. The vessels ranged from 3.24 to 6.20 μm across the interactions; however Tatase plants exposed to 100 Gy had the widest vessels ($6.2 \pm 0.19 \mu\text{m}$). The variations induced by different dosages in specific cultivar depicted that 100 and 200 Gy had smaller vessels in Shombo plants than the control. In Tatase, 50 Gy and 100 Gy vessel diameter varied significantly from the control, while in Nsukka yellow pepper, only 50 Gy vessels were different from the control (Fig. 1).

DISCUSSION

The variations observed in the vessel diameter as compared to the control could be as a result of some changes in the original nature of the cambial precursor (procambium) because the procambium, which might be already established in the embryonic level in the seeds, could possibly have been affected by the exposure. It was also observed that the number of xylem vessels increased across treatments in the petiole while the diameter measured in the root reduced in most of the treatments. Similar differences had also been observed in the reports of Schuerger et al., (1997) on the anatomical features of pepper plants (*C. annuum* L.) grown under red light-emitting diodes supplemented with blue or far-red light. However, auxin concentration had been identified to affect size and density of vessels along the plant axis (Aloni and Zimmermann, 1983; Saeed et al., 2010). Therefore, the increase in vessel elements and decrease in vessel diameter as observed in this study could be a pointer that gamma irradiation affected a few genes involved in the auxin biosynthesis pathway.

Although it had been argued that wider vessels conduct water more efficiently than narrow vessels, Gutierrez et al., (2009) pointed out that wider vessel diameters having high relative hydraulic conductivity are vulnerable to cavitations, especially if they are growing in natural conditions with variations of environmental elements such as temperature and rainfall. Alternatively, if wider vessels are cavitated, narrow vessels are an alternative way for conducting water from roots to leaves by storing and moving water in a radial and tangential direction (Zimmermann, 2003). It should, therefore, be reasonable to indicate that selection geared towards the reduction of vessel diameter could be beneficial for developing lines that could withstand environmental variation mostly in this

climate change era.

CONCLUSION

Conclusively, it is evident that the anatomical features of pepper are sensitive to gamma irradiation which could influence the physiological processes of the plant. These changes could be exploited for the improvement of these plants and other plants.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

OEO performed the experiments, data analysis and also wrote the first draft of the manuscript. and. ENM, AGC and ANE designed the experiments and reviewed the manuscript. All authors read and approved the final version.

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