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THERMAL NEUTRON SENSITIVITY OF GERMINATION AND EMERGENCE ASSOCIATED TRAITS IN TOMATO (*Solanum lycopersicum* L.)

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Abstract
Non targeted mutation was used to test the sensitivity of germination and emergence traits in three genotypes of tomato using thermal neutrons. Radiation exposure time for the seeds were 6hrs 14mins, 12hrs 28mins, 18hrs 42mins and 24hrs 56mins respectively. Analysis of Variance indicated significant variation (P<0.01) in percentage germination, percentage emergence and opening of the primary leaves for Genotype and Thermal Neutron Doses. There were also statistically significant interactions (P<0.01) between Genotype and Thermal Neutron Doses for percentage germination, percentage emergence, opening of the primary leaves and length of primary leaves. The doses were observed to be purely stimulatory on characters such as germination and did not induce any variation in the plumule and radicle lengths. The absorbed dose was not sufficient to produce exceptional mutations in the traits studied and the equivalent doses which are the quantities representing the levels of genetic damage are thus deemed low. The tomato genotypes used can thus be considered to be tolerant for exposure to the doses of thermal neutrons used in this study and breeding efforts should utilize stronger doses to effect chromosomal damage that will increase the tendency for better genetic outcomes and the much sought-after useful mutants.

Key Words: Thermal Neutrons, Absorbed dose, Dosimetry, Emergence, Sensitivity, Tomato

Introduction
Mutagenesis utilizes ionizing radiation as tool used to genetically advance the usefulness of crop plants and this have served man for decades. It's most desirable radiobiological effect being genetic mutation due to chromosomal damage (Aggrawal, 2014). This type of mutagenesis cannot directly target genes of interest but rather works by random or chance mutation which is estimated to be 0.001 for useful mutants in each mutation event depending on the type of mutagen, the genotype been mutated, time of exposure and the concentration of buffer used in the case of chemical mutagens. The penetrating ability of ionizing radiation into living matter can cause varying degrees of damage depending on the type and degree of exposure resulting in part to the generation of highly reactive species such as hydrogen peroxide, hydroxyl and super oxide radicals that can cause oxidative stress and result in peroxidation, nucleic acids and severe overall cellular damage (FAO and IAEA 2018). Radiation can therefore directly harm plant physiological systems and DNA leading even to the permanent loss of proper repair function (Flowers *et al.,* 2019). This kind of damage may lead to the inhibition of cell division as well as death (Aggrawal, 2014).

It is necessary therefore to monitor the adverse responses of specific crop plants

to the effects of radiation energy so as to advance dosimetry that will be advantageous to and serve the principal purpose of mutation genetics which is to generate useful mutants. This is known as radiosensitivity. It measures the vulnerability of whole plants or their tissues and organs to the deleterious effects of ionizing radiation. Radiosensitivity which varies with plant species is assessed predominantly by measurement of growth inhibition, estimation of meristematic injury and damage to nuclear material which estimation will essentially be carried out by the physiological responses of the experimental subjects (FAO and IAEA 2018). And even though its assessment is not limited to the above mentioned criteria, this study will focus on the germination and emergence potential of embryo that have been exposed to thermal neutrons. They are known to penetrate deep into living matter having greater by far penetrating power than alpha rays. A single thermal neutron has equivalent energy of about 0.025 electron volts (Ahmed, 2015). Given that plant cells and tissue are mainly constituted by light elements such as carbon, hydrogen, nitrogen and oxygen, this gives the thermal neutron fairly high penetration power (Ahmed, 2015) which is sufficient energy to effect ionization in living cells mainly by the excitation of biologically active atoms and molecules especially when a high flux is used. The results presented in this study provide insights into the response of basic radiosensitivity parameters of tomato to various dose intensities of thermal neutrons thereby contributing to the optimization of the process for breeding programs that utilize this mutagen as a source of mutation.

Materials and Methods

Thermal Neutron Treatment of Experimental Subjects

Clean healthy looking seeds of three distinct genotypes namely Roma VF, UC 82 and Tropimech were tested for viability by planting fifty seeds in germination trays for each genotype. This was replicated three times. The procedure yielded a germination percentage of above 98% for each of the genotypes. Four sets of one thousand seeds were then packaged, sealed, properly labelled and taken to the Centre for Energy Research and Training (CERT), Ahmadu Bello University, Zaria, Nigeria for exposure to thermal neutrons. An irradiation equipment with an Americium Berylium source was used to expose the seeds at the rate of 1.32×10^4 cm^2 per second for 6 hours 14 minutes, 12 hours 28 minutes, 18 hours 42 minutes and 24 hours 56 minutes. The fifteen treatment combinations of three genotypes and five thermal Neutron radiation treatments were the experimental subjects.

The radiation dose is equal to the flux or strength of radiation field multiplied by the total length of time that exposure lasted. Flux rate of the Americium Beryllium source = 1.32×10^4 m^2 per second. Therefore, dose for each treatment (D) = Flux X Time

Germination Experiment

Fourty-five petri-dishes were thoroughly washed, rinsed with distilled water and labelled. Fresh cotton wool was spread over the inner surface of the dish and moistened with distilled water. Five seeds from each of the fifteen treatment combinations including the controls were placed and equally spaced on the wet cotton wool. This set up was replicated three times and laid out in a Randomized Block Design (RBD). The experiment was set up in a well-lit section of the lab at ambient temperature of 27°c.

Emergence Experiment

The selected experimental site was a plain and even field with sandy loam soil which was thoroughly cleared and prepared for planting. The experiment was laid out in a Randomized Complete Block Design (RCBD) consisting of three blocks and fifteen plots per block, each measuring one metre by one metre. Each plot was separated by 0.5m as well as the blocks.

This gave the experimental field the dimensions of 22m x 4m. Four furrows were made with a hand trowel 2.5cm deep. And the seeds were placed in one after the other, five seeds per furrow spaced twenty centimetres apart. They were then covered up with soil and gently watered. The plots were watered lightly every day which continued even after emergence.

Data Collection

Germination was measured by the emergence of the rudimentary root (radicle) from the split seed coat. Number of germinated seeds were recorded daily till Day 12 of the experiment and percentage germination estimated.

Plumule and **Radicle** lengths were taken for three randomly selected seedlings in each treatment replication using a digital calliper.

Data for the **Opening of the primary leaves** was collected daily by counting the number of seedlings with opened primary leaves until the experiment ended.

Seedling emergence of each treatment was obtained by hand counting daily after sowing and the data recorded. Percentage emergence was computed afterwards.

Seedling height was taken on the eleventh day after sowing on the field with the aid of a graduated rule. Three randomly selected seedlings were measured and recorded.

Length of the primary leaves of three randomly selected plants were taken on the eleventh day after sowing by measurement with a digital calliper. The data was recorded.

The **number of secondary leaves** were counted off three randomly selected seedlings on the field and the results recorded on the eleventh day after sowing.

Exposure Time(s) was the length of time taken to subject the seeds to direct thermal neutron radiation from the Americium Berylium Source. The exposure time were 6hrs 14mins, 12hrs 28mins, 18hrs 42mins and 24hrs 56mins.

Thermal neutron Dose (cm^2) was computed by the formula: Neutron flux X Time = Dose

Mass of the seeds exposed to thermal neutrons were obtained in grams using an electronic balance and then converted to kilograms.

Absorbed dose (Jkg^{-1}) was calculated based on the definition of Liamngee *et al.,* 2020.

Equivalent dose (Jkg^{-1}) was estimated based on the definition of Zaid *et al.,* 2019.

Radiation weighting factor (W_R) was calculated based on the formular and conditions specified by the 2007 recommendations of the International Commission on Radiological Protection (ICRP).

Germination percentage (G), Mean germination time (MGT), Coefficient of velocity of germination (CVG), Germination rate index (GRI) were estimated using the formulars suggested by Al-Mudaris (1998). Mean germination rate (MGR) was estimated by the formular suggested by Ranal *et al.,* (2009).

Results and Discussion

Table 1: Thermal Neutron Quantitative Parameters for the Exposure of Tomato Embryos

Exposure Time	Thermal Neutron Dose (Jcm^{-2})	Absorbed dose (Jkg^{-1})	Equivalent dose (Jkg^{-1})
6hrs 14mins	2.96×10^{8}	5.20×10^{-7}	2.05×10^{-10}
12hrs 28mins	5.92×10^{8}	2.05×10^{-6}	4.42×10^{-10}
18hrs 42mins	8.88×10^{8}	4.62×10^{-6}	6.65×10^{-10}
24hrs 56mins	1.18×10^{8}	8.18×10^{-6}	8.85×10^{-10}

***Flux=1.32 x 10 cm^2 s^{-1}**

Table 2 : Mean Squares of Traits and Significance for Sources of Variation

Source of Variation	Percentage Germination	Percentage Emergence	Length of Plumule	Length of Radicle	Opening of Primary Leaves	Seedling Height	Length of Primary Leaves	Number of Secondary Leaves
Genotype	104.330**	3982.040**	23.069*	8.505^{NS}	12.775**	5.023*	14.710**	1.158^{NS}
Thermal Neutrons	413.750**	188.880**	2.829^{NS}	1.156^{NS}	5.195**	1.664^{NS}	0.760^{NS}	0.279^{NS}
Interaction(AXB)	619.380**	151.960**	4.501^{NS}	4.934^{NS}	8.452**	2.640*	3.710**	0.679^{NS}
Error	96.190	9.680	6.177	3.907	0.746	1.087	0.020	0.429
Total (SS)	18.797	10225.800	278.79	178.303	192.330	114.135	1.843	53.992

NS-Not Significantly different *significant at 95% confidence level **significant at 99% confidence level

Table 3 : Effects of Thermal Neutrons on Sensitivity Traits of Tomato Mutant Seedlings

Thermal Neutron Doses (Jcm^{-2})	Percentage Germination	Percentage Emergence	Length of Plumule	Seedling Height	Opening of Primary Leaves	Length of Primary Leaves
Control	92.083^{a}	36.611^{b}	2.633^{a}	4.541^{NS}	2.541^{a}	1.255^{NS}
2.96 x10^{12}	83.333^{c}	35.388^{b}	3.555^{a}	4.108^{NS}	2.333^{a}	1.222^{NS}
5.92 x 10^{12}	89.583^{ab}	46.777^{a}	2.311^{a}	4.837^{NS}	2.333^{a}	1.288^{NS}
8.88 x 10^{12}	84.166^{bc}	37.222^{b}	3.577^{a}	4.400^{NS}	1.458^{b}	1.233^{NS}
1.18 x 10^{13}	91.666^{a}	37.611^{b}	2.922^{a}	4.429^{ab}	1.708^{b}	1.177^{NS}
F-LSD	7.67	2.48	1.98	0.81	0.67	0.11
SE	1.790	0.920	0.741	0.190	0.158	0.042
p-value	0.003	0.000	0.766	0.199	0.000	0.559
Roma VF	86.000^{b}	38.066^{b}	2.393^{b}	4.062^{b}	2.225^{a}	1.373^{a}
UC 82	84.500^{b}	55.333^{a}	4.426^{a}	4.735^{a}	1.450^{b}	1.240^{b}
Tropimech	94.000^{a}	22.766^{c}	2.180^{b}	4.592^{a}	2.550^{a}	1.093^{c}
F-LSD	7.67	2.48	1.98	0.81	0.67	0.11
SE	1.270	0.656	0.524	0.135	0.112	0.029
p-value	0.000	0.000	0.036	0.012	0.000	0.000

Means followed by the same letters are not significantly different from each other

Table 4: Interaction Effect of Thermal Neutrons Doses and Genotype on Sensitivity Traits in Tomato Mutants

Source of variation		Sensitivity Traits				
Genotype	**Thermal Neutron Doses (Jcm^{-2})**	**Percentage Germination**	**Percentage Emergence**	**Opening of the Primary Leaves**	**Seedling Height**	**Length of Primary Leaves**
	Control	93.750^{ab}	41.500^{c}	2.125^{cd}	3.550^{d}	1.633^{a}
	2.96 x10^{12}	81.250^{cd}	44.166^{c}	2.125^{cd}	3.812^{cd}	1.400^{ab}
Roma VF	5.92 x 10^{12}	80.000^{cd}	35.500^{d}	3.125^{ab}	4.262^{bcd}	1.200^{bcdef}
	8.88 x 10^{12}	78.75^{cd}	34.000^{d}	0.750^{f}	4.275^{bcd}	1.366^{bc}
	1.18 x 10^{13}	96.250^{a}	35.166^{d}	3.000^{ab}	4.412^{bcd}	1.266^{bcde}
	Control	97.500^{a}	53.833^{b}	3.375^{a}	5.250^{ab}	1.133^{cdef}
	2.96 x10^{12}	75.000^{d}	44.500^{c}	1.625^{de}	3.700^{d}	1.266^{bcde}
UC 82	5.92 x 10^{12}	93.750^{ab}	69.333^{a}	0.625^{f}	5.837^{a}	1.333^{bcd}
	8.88 x 10^{12}	76.250^{cd}	54.000^{b}	1.125^{ef}	4.350^{bcd}	1.300^{bcd}
	1.18 x 10^{13}	80.000^{cd}	55.000^{b}	0.500^{f}	4.537^{bcd}	1.166^{bcdef}
	Control	85.000^{bc}	14.500^{g}	2.125^{cd}	4.825^{abc}	1.000^{f}
	2.96 x10^{12}	93.750^{ab}	17.500^{fg}	3.250^{ab}	4.812^{abc}	1.000^{f}
Tropimech	5.92 x 10^{12}	95.000^{a}	35.500^{d}	3.250^{ab}	4.412^{bcd}	1.333^{bcd}
	8.88 x 10^{12}	97.500^{a}	23.666^{e}	2.500^{bc}	4.575^{bcd}	1.033^{ef}
	1.18 x 10^{13}	98.750^{a}	22.666^{ef}	1.625^{de}	4.337^{bcd}	1.100^{def}
SE		**2.530**	**1.310**	**0.223**	**0.269**	**0.059**
P- value		**0.000**	**0.000**	**0.000**	**0.019**	**0.004**

Means followed by the same letters are not significantly different from each other

Table 5: Germination Statistics of Three Genotypes of Thermal Neutron Induced Tomato Mutants

Genotype	Thermal Neutron Doses (Jcm^{-2})	Percentage Germination	Mean Germination Time (days)	Mean Germination Rate	Germination Rate Index	Coefficient of Velocity of Germination
	Control	82.00	8	0.22	54.16	22.96
	2.96 x10^{12}	70.00	10	0.16	45.00	16.10
Roma VF	5.92 x 10^{12}	69.00	3	0.13	35.00	13.11
	8.88 x 10^{12}	69.00	9	0.20	36.44	20.70
	1.18 x 10^{13}	82.00	4	0.22	39.16	22.96
	Control	89.00	5	0.20	47.00	20.01
	2.96 x10^{12}	64.00	5	0.10	33.33	10.24
UC 82	5.92 x 10^{12}	81.00	5	0.23	39.83	23.49
	8.88 x 10^{12}	64.00	9	0.22	29.27	22.40
	1.18 x 10^{13}	64.00	10	0.20	21.33	20.44
	Control	73.00	5	0.18	35.33	18.98
	2.96 x10^{12}	83.00	6	0.22	43.66	22.41
Tropimech	5.92 x 10^{12}	82.00	5	0.23	40.33	23.52
	8.88 x 10^{12}	84.00	4	0.28	41.66	28.56
	1.18 x 10^{13}	88.00	4	0.19	25.00	19.36

Germination Percentage

The effect of thermal neutron doses on the germination of tomato genotypes used in this study indicated highly significant ($P<0.01$) variation. The means did not show a dose related reduction in germination. The control had the highest germination percentage followed by the 1.18 x 10^{13} Jcm^{-2} dose even though there was no significant difference between them (Table 3). In the Roma VF treatments, the control took four extra days to complete germination as the 1.18 x 10^{13} Jcm^{-2} dose completed four days earlier (Table 5). The quantity of absorbed radiation energy by the seeds clearly did not suffice to drastically affect embryo survivability but the inhibitory effects of thermal neutrons on the germination of tomato seeds in this study were however obvious as mean germination percentages were reduced for almost all the doses. While there were no stimulatory effects on mean germination itself contrary to reports in other studies using other radiation types such as gamma rays (Beyaz *et al.,* 2016) there were changes in the mean germination time which says how long the germination process took per treatment. This happened only in Roma VF and Tropimech treatments. It is thus clear that the impact of thermal neutrons on the germination percentage and germination time are unrelated and may not have followed the same path of action. Stimulatory effects of this nature have been reported in rice by Bora *et al.,*(1959). Inhibition of seed germination could be attributed to the damage in vital seed tissue, damage to chromosomes and subsequent mitotic retardation. Also the production of free radicals that are first of all directly and thoroughly destructive to embryo tissue and can cause other physiological disturbances which could very well prevent germination (FAO & IAEA, 2018). Critical injury to chromosomes by ionization radiation which involves breaks and shifts in chromosome segments effecting chromosomal aberrations such as deletions, inversions, and translocation (Griffiths, 1999) or mistakes in the cell induced chromosome repair (Sachs *et al.,* 1997) could impair germination related genes such as those for the biosynthesis of gibberellic acid which is known to induce germination (Ravan *et al.,* 1992). Genetically controlled processes associated with the activity of enzymes which mediate biochemical transformations such as the conversion of stored proteins, carbohydrates or oils to simple substances crucial to germination are some processes which could also be impaired. It is to be considered that since thermal neutrons rearrange whole segments of chromosomes, its effect on the genetic scale may be all or none since ionization energy is not commonly associated with point mutations. The strongest percentage germination among the genotypes was shown by Tropimech with a 94% percent germination (Table 3). This was significantly different ($P<0.01$) from Roma VF and UC 82 which were not significantly different from each other with lower germination percentages of 86.0 and 84.5 percent respectively (Table 3).

Interaction effect of tomato genotypes and thermal neutron doses on percentage germination

Percentage germination indicated statistically significant ($P<0.01$) differences in the interactions between the doses of thermal neutrons and genotype of tomato. The means ranged from 75.000 to 98.750 percentage germination. The combined effect of the thermal neutron dose 1.18 x 10^{13} Jcm^{-2} and Tropimech genotype produced the strongest germination of 98.750 closely followed by Tropimech at 8.88 x 10^{12} Jcm^{-2} and UC 82 control both with 97.500 percent even though there was no significant difference ($P<0.01$) between them (Table 5) thus stimulatory effects of thermal neutrons on the germination of Tropimech gave the best results. The best of which was found to be at the two highest doses. It became obvious that the germination process did not show any sensitivity to the three lower doses which

leads us to doubt that themal neutrons are potent enough to alter the process positively. 3.5 x 10^{-11} Jcm^{-2} and 4.64 x 10^{-11} Jm^2 which corresponds to the radiation energy absorbed by the seeds in the two highest doses (8.88 x 10^{12} and 1.18 x 10^{13} m^2) delivered may be the threshold behind which no positively significant impact might be felt with respect to germination. The interaction of UC 82 and 2.96 x10^{12} showed the least germination percentage at 75.000 ($P<0.01$). It may be observed that the Tropimech dose interactions produced two of the five overall strongest percentage germinations outperforming the other genotype dose interactions (Table 3) Meanwhile, the UC 82 dose interactions had two of the least performing interactions (Table 3). This was not surprising giving the drop in energy absorbed by the seeds with a decrease in dose (Table 1). The Tropimech dose interactions might be considered for strong and competitive germinations in research works involving thermal neutrons to ensure survivability and improve the chances for the identification of useful mutations.

Percentage Emergence

The 5.92 x 10^{12} dose of thermal neutrons deposited 2.05 x 10^{-6} Joules of radiation energy in the seeds (Table 1) which produced the highest percentage emergence of 46.777, performing statistically better ($P<0.01$) than the control which produced just 36.611 (Table 3). All other doses were not significantly different ($P<0.01$) from each other including the control. It is worthy of note that 2.05 x 10^{-6} joules was one of the lowest amounts of energy absorbed by any of the treatments but it also led to a reduction in germination time which was the best amongst the Roma VF treatments. This exposure time and its corresponding dose might be noted for reducing germination time of tomato seeds. The UC 82 genotype out performed Roma VF and Tropimech with the best percentage emergence of 55.333 (Table 4) and was also significantly different ($P<0.01$) from them. Roma VF and Tropimech were also significantly different from each other with Roma VF showing an emergence of 38.066 percent (Table 4). The most important summative cytological activity the cell embarks on after germination is mitosis, which is central to the emergence process after germination. Explanations for the positive values indicated by the percentage emergence of tomato at 5.92 x 10^{12} dose only further proves the stimulatory action of thermal neutrons as Bora *et al.,* (1959) confirmed that exposure of dry rice seeds to thermal neutrons improves on growth and early flowering which had a three week head start over the control as well as shorten its life cycle even though concerns have arisen about some purely stimulatory effects without any genetic consequences. Reductions in emergence percentages would involve more impairments in the genetic structures which control the complex processes involved in post germination mitosis than it will concern direct damage to tissue resulting in low germination which is normally the case with chemical mutagens. A chance exist though that direct physical damage inflicted on the embryo tissue, a good proportion which are stem cells, by free radicals just before germination could still affect the emerging seedling to varying degrees depending on the intensity of the dose and which genes were actually affected as is typical of untargeted mutagenesis. During mitosis, the initial aspects of the S phase and the M phase are under tight control, to verify that chromosomes are coupled appropriately and that the correct order is followed (Rao *et al.,* 1994), to eliminate mistakes and ensure the daughter cells do not carry defaultive or impaired genes. This regulation is crucial because any cell which contain mistakes in its Deoxyribo Nucleic Acid (DNA) replication and installation process is destroyed if repair fails. Apoptosis, which is a programmed procedure for this purpose is then initiated to carry this out basically through autophagic destruction of the cytoplasm and

chromosomal disassembly (Filonova *et al.,* 2008). Given that the purpose of this study was to induce genetic mutation in the DNA of tomato embryos using a potent mutagen such as thermal neutrons, it is reasonable to expect that there are a lot of cells with mutated and mutilated DNA which will be subjected to the programmed apoptitic process of certain death which could negatively affect emergence. It is not surprising then that emergence percentages were much lower than germination percentages (Table 4). This gives a fair understanding of what took place in the seed while in the soil during the emergence experiment. Germination was good, but emergence was poor and apoptosis could very well be a good explanation.

Interaction Effect of tomato genotypes and thermal neutron doses on seedling emergence

The interaction effects ranged from a 69.333 percent emergence in the UC 82 at 5.92 x 10^{12} dose to 17.500 in the Tropimech at dose 2.96 x10^{12} (Table 5). The interaction of the factors produced their best results in the UC 82 treatments at dose 5.92 x 10^{12} producing the strongest emergence of 69.333 percent followed by UC 82 at dose 1.18 x 10^{12} with 55.000 percent and UC 82 control with a 53.833 performance (Table 5). The interaction of Tropimech genotype and thermal neutron doses were the worst performing producing emergence percentage values as low as 17.500 and 14.500 (Table 5). It is noteworthy that they (Tropimech dose interactions) had the highest percentage germination while suffering the worst emergence values. On the other hand, UC 82 dose interactions seemed to have dismal performances in the germination but out performed all the other interactions to post the best emergence values. One reason for this could be the kick start of a number of parallel auxin biosynthesis after germination (Napier, 2003) for which the amino acid tryptophan is the precursor and after which a tryptophan independent pathway ensues. If the conversion of stored assimilates to ready to use substances is altered in any way by the deposition of radiation energy such as enzymatic processes that make amino acids available from stored proteins, tryptophan is likely to be unavailable which can truncate a tryptophan dependent pathway for auxin synthesis which will in turn reduce or completely eliminate stem elongation which is a critical feature of emergence. If this was the case, then the variance in the responses of different genotypes to radiation energy could explain why the interactions in this study produced different emergence results.

Length of Plumule and Radicle

Thermal neutron doses did not induce any statistically significant changes in the length of the rudimentary shoot (Plumule). The genotypes however indicated significant differences ($P<0.05$) in Plumule length with UC 82 performing significantly better than Roma VF and Tropimech with a Plumule length of 4.426cm (Table 3). The means for Plumule length in Roma VF and Tropimech did not show any significant difference between them. This means all the radiation energy absorbed from the doses used in this study could not genetically or otherwise induce variation in the character. Stem cells constitute the apices of both the rudimentary stem and root which are self-renewing and undifferentiated (Miwa *et al.,* 2009). On them is placed the burden of post embryonic growth, development and the formation of organs on which the plant will come to depend as they form part of the meristems that provide cells for continuous growth. The root apical meristem is protected by the root cap as it finds its way into the soil, signalling its delicate nature. Bombardment of tomato embryo with thermal neutrons can break chemical bonds and ionize atoms and molecules which can be devastating, causing irreparable damage to tissues and meristems which should have implications for post germination growth. The energy it carries is deposited in the embryonic tissue can disturb the delicately organised

intercellular communication which controls the organization and maintenance of the shoot apical meristem as well as cell differentiation (Miwa *et al.,* 2009). It is clear therefore that the maintenance of stem cells is the result of a well organised regulatory system which results in meristem homeostasis. It was curious then that the thermal neutron doses did not have a significant effect on this set up such as to effect a response on plumule or radical length. This could be explained by insufficient dosage of the ionization energy (thermal neutrons) or the simply reversal of the genetic mutations in the genes which regulate the nature and functions of these meristems. In a case where that is not possible, the cells with mutant DNA are degraded and killed (Filonova *et al.,* 2008). This is something the embryonic system by design is fully capable of.

The lark of effect of the treatment on the target meristems at all the doses used in this study may not be unrelated to the inability of thermal neutrons at the levels used to effect neither lethal nor advantageous changes in the growth patterns observed in their development especially as there is the well documented effect of other ionizing energy and chemical mutagens on embryonic meristems as evidenced in their subsequent growth. This phenomenon could however serve the geneticist or plant breeder who wants to study other concepts in germination and emergence using mutagenesis but will find damage or retardation in meristem activity a limiting factor. Furthermore, the mutation value of thermal neutrons in non targeted mutagenesis will rise if it is indeed confirmed that this potent mutagen has negligible effect on shoot and root development of tomato while creating significant genetic changes in other aspects of plant growth.

Opening of Primary Leaves

The opening of the primary leaves indicated a dose related decrease in the trait with the control out performing all the thermal neutron doses, even though this result was not statistically different ($P<0.01$) from dose 2.96×10^{12} and 5.92×10^{12}. The two highest doses, 8.88×10^{12} and 1.18×10^{13} were not only not significantly different ($P<0.01$) from each other but also were the least performing with 1.458 and 1.708 seedlings with opened primary leaves (Table 3). The trait was highly variable amongst the genotypes. Tropimech having the highest number of seedlings with opened primary leaves (2.550), this performance still did not differ significantly ($P<0.01$) from that of Roma VF (2.225). UC 82 had the least performance (1.450). Foliar initiation is an organogenic process that produces one of the important plant organs for plant survival. The leaf initiation is controlled by auxins from the shoot apical meristem and dictates minute details such as the determination of the location of serrations as well as the initiation of leaflets and lobes from the margin of leaf primordial (Swartz *et al.,* 2016). Also, genes proven to be required for the inhibition of lamina growth between leaflets are likely candidates of mutagen attack and can negatively affect leaf development. Consequently, it has been reported that the expression of these mutant genes can lead to a substantially reduced leaflet lamina in tomato (Hendelman *et al.,* 2012).

Appropriate and balanced foliar development will depend on the distribution of auxins at the proper and exact locations (Ben-Gera *et al.,* 2012) which are regulated by auxin response pathways involving transcriptional activators or repressors that regulate the expression of auxin-responsive genes (Ben-Gera *et al.,* 2012). The thermal neutron imposed injury to chromosome and other nuclear structures which control normal transcription and expression of auxin associated genes can slow down the expression of these genes resulting in reduced gene products could be responsible for the dose related decrease observed in the treatments.

Interaction effect of tomato genotypes and thermal neutron doses on opening of primary leaves

The combined effect of the factors was strongly significant ($P<0.01$) among the means as the mean number of seedlings with opened primary leaves was the UC 82 control at 3.375 seedlings. This mean was significantly higher than Tropimech at dose 2.96×10^{12} (3.250), Tropimech at 5.92×10^{12} (3.250) and Roma VF at 5.92×10^{12} (3.125) which were next in performance strength in that order (Table 4). UC 82 with dose 1.18×10^{13} had the least number of seedlings with opened primary leaves (0.500) at the end of the experiment. The interaction showed no pattern with respect to the delay or speeding up the process of opening of the primary leaves. The trait however indicated how fast the seedling was developing by its pace. Auxins are definitive requirements for plant cell division and given that they exercise such control over phyllotaxy (Napier, 2003), gives room for genotypic differences which when combined with an array of neutron doses in interaction gives the varied expression that characterizes this trait under study.

Interaction Effect of tomato genotypes and thermal neutron doses on seedling height

Mean values for seedling height indicated statistically significant ($P<0.05$) differences in the interactions between the doses of thermal neutrons and genotype of tomato. The means range from 5.837cm in the UC 82 at dose 5.92×10^{12} to 3.550cm in the Roma VF control (Table 5). Roma VF interactions showed a decrease in height with a decrease in neutron dose while the UC 82 interactions did not indicate an observable pattern of increase or decrease in the trait. Tropimech interactions on the other hand decreased with an increase in dose. Corso and Lecari (1997) reported that Ultraviolet radiation (UV) significantly lowered the height of tomato transplants even though this change was dose dependent, it corresponds to the observations made in the Roma VF interactions where a decrease in seedling height followed a decrease in dose. This obviously did not play out for UC 82 and Tropimech interactions which did not follow that pattern. Sidrak and Suess (1973) mentioned that height influenced sensitivity of traits in tomato to UV radiation.

Interaction Effect of tomato genotypes and thermal neutron doses on seedling length of primary leaf

The interactive effect indicated a highly significant variation ($P<0.01$) between the means for the trait. Interaction of the factors performed best in the Roma VF treatments with the Roma VF control having the longest mean primary leaves closely followed by and significantly different ($P<0.01$) from Roma VF at dose 2.96×10^{12}. Interactions recorded the shortest length of primary leaves with 1.000cm each at Tropimech control and Tropimech at dose 2.96×10^{12}. It is obvious that Roma VF dose interactions generally outperformed the other interactions followed by UC 82 dose treatments and the Tropimech dose interactions were the least performers. It is noteworthy however that UC 82 and Tropimech both interacted with the same dose (5.83×10^{12}) to produce the longest primary leaves for each of those genotypes. It is clear that leaf morphogenesis is dependent on the extent of the leaf initiation zone at the apical meristem circumference. Thus mutations that alter the process of leaf initiation can impact on the leaf form (Dengler and Tsukaya, 2001) such as the initiation of a leaf from the surface of another leaf which can make it longer than that of a non mutant. Even though evidence that such leaves can arise without shoot apical meristem mediation is not rife, the observation cannot denied (Dengler and Tsukaya, 2001).

4.0 Conclusion

The verifications from this study show that the doses of thermal neutrons used were insufficient to induce genetic mutations and

cause variation in most of the traits studied and were at best stimulatory in their effects which are not heritable therefore the genotypes were weakly sensitive to them. Increase in doses (cm^2) also reflect commensurate increments in absorbed dose (JKg^{-1}) and equivalent dose (JKg^{-1}) so the phenotypic response of the experimental subjects can be used to evaluate reasonably the genetic damage and the potency of the absorbed dose. We can also conclude that as a measure of biological damage, the lark of genetic mutation in many of the traits studied was reflective on the equivalent dose as weak. Thus plant breeders may not expect reasonable results from this range of equivalent doses in tomato.

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