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RESPONSES OF DIFFERENT TOMATO VARIETIES TO DISEASE STRESS UNDER DIFFERENT CONDITIONS OF GRAVITY

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ABSTRACT

A study was carried out to evaluate the responses of different tomato varieties to fungal disease stress under different conditions of gravity, in Lafia, Nasarawa State Nigeria. Seeds of three local tomato varieties namely; UTC, Teema and Dan Syria were plated on plant agar inoculated with culture filtrates of Sclerotinia sclerotiorum and Pythium ultimum, and rotated on a clinostat at a speed of 20 revolutions per minute (rpm) for 96 hours in a dark growth chamber. Seedlings of Dan Syria subjected to the condition of normal gravity had the highest Frequency of germination (79.61%), seedling height (4.01mm) and stem girth (0.19mm). Teema had the highest width of vascular bundles (1.18mm) under normal gravity condition. Under conditions of microgravity, the highest number of trichomes (15.38), plant height (3.25mm), width of epidermis (0.19mm), and frequency of germination (66.63%), were observed in the UTC variety. The least frequency of germination (27.75%) and seedling height (1.06mm) was observed in Dan Syria under the condition of microgravity. Trichomes were absent in seedlings of Dan Syria under normal gravity. Seedlings of Dan Syria, UTC, and Teema subjected to disease stress revealed absence of stomata on exposure to microgravity conditions. Differences in effect of gravity conditions on morphological and anatomical features of the seedlings were significant ($P \le 0.05$). The UTC tomato variety showed higher disease tolerance under microgravity stress, and could be considered for genetic studies aimed at selection of stress tolerance genes in the tomato variety, for enhanced yield and productivity.

Key Words: Tomato Varieties, Fungal Pathogens, Microgravity, Normal Gravity, Disease Tolerance

INTRODUCTION

Tomato belongs to the family Solanaceae, which includes more than 3000 species, occupying a wide variety of habitats (Knapp, 2002). It is a flagship species in the Solanaceae, and has been extensively used in studies on the evolution and development of fruit characters in particular. The high demand for tomato fruits by consumers all over the world stems from its rich vitamin, carbohydrate, mineral and dietary fibre content (Cervoni, 2017), as well as other medicinal qualities such as its famed anticancer properties. Global production of tomatoes is about 89.8 million metric tonnes from an area of about 3,170.000 ha (Samuel et al., 2011). Nigeria is undeniably the 14th largest producer of tomatoes, second only to Egypt in Africa at 1.51 million metric tonnes, valued at N87 billion with a cultivated area of 254,430 in Sub-Sahara Africa (Adegbola et al., 2012).

Land degradation, particularly as a result of depletion in soil fertility and erosion, is a serious and irreversible constraint to agricultural productivity in Nigeria and beyond (Henao, 2014). The search for alternative growth environments for enhanced growth and productivity of critical crop plants such as tomato has become imperative.

Microgravity is a characteristic of a space environment, a condition of altered gravity which poses an abiotic stress on organisms' metabolism, growth and development. Various platforms have been used to simulate microgravity conditions, such as the dropping tower at the Centre for microgravity research in Bremen, Germany, the suborbital flight usually funded by National space agencies across the world, and the clinostat (Akomolafe et al., 2017). The International Space Station (ISS) has been an experimental platform to conduct research in true microgravity conditions. Several authors have simulated plants response to the condition of altered gravity (Moore and Cogoli, 1996; Jing et al., 2015). According to NASA (2009), the major importance of experiments carried out in microgravity is to explain the basic mechanisms by which plants perceive, transduce, and respond to gravitational force; understand the role of gravity and microgravity in the development and reproductive processes in plants; to learn how metabolic and transport processes are affected by gravity and microgravity and lastly to study the role of plants within recycling life support systems for space exploration.

Reports by several researchers have indicated that plants grown under conditions of microgravity show improved conditions in modifying the pattern of gene expression involved in secondary cell formation, shorter complete life cycle, delayed leaf senescence, morphogenesis and high yield as compared to ground controls (Cowles *et al.*, 1984). There is need to exploit microgravity for the possibility of enhanced disease tolerance, yield improvement and increased productivity of vegetables such as tomato in the Nigeria and beyond.

MATERIALS AND METHODS

Pure cultures of *Pythium ultimum* and *Sclerotinia sclerotiorum* were obtained from the culture bank of the Plant Pathology Unit of Federal University Lafia and maintained on Potato Dextrose Agar (PDA) slants at ambient temperature $(28^{\circ}C \pm 2^{\circ}C)$.

Fruits of three local tomato varieties namely; Dan Syria, UTC, and *Teema* used for this study were purchased from the Lafia Tomato Market and conveyed in sterile polyethylene bags to the Department of Botany laboratory, Federal University Lafia, for further processing. Identification of sampled tomatoes was carried out as described by Harris and Harris (2001).

Sampled tomato fruits were cut longitudinally and seeds extracted by detaching seeds from fruit tissues with the aid of a clean knife. Extracted seeds were washed, air dried and package in paper bags prior to plating.

Ten 6cm diameter mycelia plugs were obtained from actively growing regions of 7 days old cultures of *Pythium ultimum* and *Sclerotinia sclerotiorum* on Potato Dextrose Agar (PDA) and transferred into 900-ml flasks containing 500 ml of Potato Sucrose Broth (PSB) (Potato infusion – 200g; Sucrose – 20g; Water – 1L), and incubated at ambient temperature ($28^{\circ}C\pm 2^{\circ}C$) for 21 days. Cell-free culture filtrates were obtained by passing the liquid through four layers of cheesecloth and Whatman no. 1 filter paper. The experiment was laid out in Completely Randomised Design (CRD).

The pour plate technique was used as described by Van Soestbergen and Lee (1969). Five milliliters (5 ml) of *Pythium ultimum* and *Sclerotinia sclerotiorum*culture filtrates were separately dispensed into sterile petri dishes, and mixed with 15ml of growth medium (3% w/v of Duchefa Biochemie Plant Agar-Agar; 1.5 %w/v agar-agar in 100 mL of tap water) and allowed to solidify for about 30 minutes.

Plating of Seeds for Growth under Normal Gravity and Microgravity Conditions

To plate seeds for growth under normal gravity condition, three petri dishes each containing

solidified agar were mixed with culture filtrates of a particular test organism were separately plated with 9 seeds of each tomato variety and incubated in the dark at ambient temperature under normal gravity conditions. A total of 6 petri dishes and 54 tomato seeds (9 seeds per variety and 27 per fungal pathogen) were laid out in a Completely Randomized Design (CRD comprising of 2 fungal pathogens, 3 tomato varieties and 9 replicates (2 x3 x9 in the laboratory.

The experimental set up for plant growth under microgravity was same as described for normal gravity, but instead of incubating under conditions of normal gravity, 6 petri dishes containing a total of 54 tomato seeds and 2 fungal filtrates were attached to a clinostat and rotated at a speed of 20 revolutions per minute (rpm) for 96 hours inside a dark growth chamber.

Seedling Growth Bioassay and Anatomical Studies

The food poisoning technique reported by Sangayomi (2004) was adopted for the determination of effect of pathogen and gravity stress on different tomato cultivars as follows:

Following the plating of tomato seeds on agar impregnated with fungal filtrates under normal and microgravity conditions, seeds were monitored daily for the onset of germination. Digital images of germinated seedlings were taken 5 days after germination and used for the measurement of seedling height with the aid of ToupView Camera Software Version 3.7.

Percentage germination inhibition was determined using the formula:

% GI=% GI =
$$\frac{\text{No. GSC} - \text{No. GSTP}}{\text{No. GSC}} \times 100$$

Where; % GI = Percentage Germination Inhibition No. GSC = No. of Germinated Seeds in Control No. GSTP = No. of Germinated Seeds in Treated Plates

The methods of Oloyede *et al.* (2011) were adopted in the anatomical studies on effect of pathogen and gravity stress on assayed tomato varieties as follow:

Thin transverse sections of stems of infected tomato plants were made using a sharp scalpel blade, placed on clean glass slides, stained with Safranin O purple stain and coated with few drops of 25% glycerol. Stained tissues were further covered with cover slips and viewed on the microscope. Anatomical features of examined stem tissues were evaluated with the aid of Toup View Camera Software Version 3.7. The following features were evaluated: Thickness of Epidermis (mm), thickness of cortex (mm), diameter of vascular bundle (mm), number of subsidiary and epidermal cells, and number of crystal sand.

Data obtained from the study were subjected to Analysis of Variance (ANOVA) at 5% level of probability.

RESULTS AND DISCUSSION

Table 1. Morphological and Anatomical Responses of the UTC Tomato Variety to Disease Stress by Different Fungal Pathogens under Different Conditions of Gravity.

	Plant Response										
		FREGM (%)	HP (mm)	SG (mm)	WEP (mm)	WC (mm)	WVB (mm)	NT (mm)	LT (mm)	FREST (%)	
Growth condition	Fungal Pathogen										
Normal gravity	Pythium ultimum	44.40	1.14	0.10	0.93	3.86	1.14	0.11	0.17	0.11	
	Sclerotinia sclerotiorum	55.53	4.08	0.19	0.11	3.68	0.97	2.89	0.50	0.22	
Microgravity											
	Pythium ultimum	77.73	3.36	0.20	0.23	1.31	0.61	13.89	1.86	0.00	
	Sclerotinia sclerotiorum	55.53	3.13	0.15	0.15	1.28	0.65	16.89	2.93	0.00	
LSD		22.20	3.00	0.30	1.00	3.00	0.32	11.00	1.07	0.50	

HP= Height of plant, SG= Stem Girth, WVB= Width of vascular bundle, NT= Number of trichomes, LT= Length of trichomes WEP=Width of epidermis, FREG= Frequency of germination, FST= Frequency of stomata

Seeds of the UTC tomato variety challenged with culture filtrates of *Pythium ultimum* under normal gravity had the least frequency of germination (44.40%), seedling height (1.14mm), stem girth (0.10mm), and number of trichomes (0.11mm) (Table 1; Figure 1). The highest seedling height (4.08mm) and frequency of stomata (0.22%) were observed in seeds treated with culture filtrates of *Sclerotinia sclerotiorum* under normal gravity condition. However, under microgravity condition, seeds treated with culture filtrates of *Pythium ultimum* had the highest frequency of germination (77.73%), stem girth (0.20mm) and least width of vascular bundles (0.61mm). Stomata were absent in seedlings challenged with culture filtrates of *S. sclerotiorum* and *Pythium ultimum* under microgravity condition. Differences in effect of culture filtrates of fungal pathogens on morphological and anatomical features of UTC seedlings were significant ($P \le 0.05$).

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Figure 1: Germination of Diseased Seedlings of the UTC Tomato Variety under; (A) Normal Gravity, (B) Microgravity

Table 2. Morphological and Anatomical Responses of the *Dan Syria* Tomato Variety to Disease Stress by Different Fungal Pathogens under Different Conditions of Gravity

		Plant Response										
		FREGM (%)	HP (mm)	SG (mm)	WEP (mm)	WC (mm)	WVB (mm)	NT (mm)	LT (mm)	FREST (%)		
Growth condition	Fungal Pathogen											
Normal gravity	Pythium ultimum	81.44	4.57	0.22	0.10	3.27	1.18	0.00	0.00	0.22		
	Sclerotinia sclerotiorum	77.77	3.46	0.17	0.11	3.64	0.67	0.00	0.00	0.00		
Microgravity												
	Pythium ultimum	44.40	1.60	0.12	0.15	1.60	0.74	3.78	0.67	0.00		
	Sclerotinia sclerotiorum	11.10	0.51	0.02	0.12	4.94	0.73	0.56	0.21	0.00		
LSD		33.30	1.86	0.15	0.03	1.67	0.51	3.22	0.46	0.50		

HP= Height of plant, SG= Stem Girth, WVB= Width of vascular bundle, NT= Number of trichomes, LT= Length of trichomes WEP= Width of epidermis, FREG= Frequency of germination, FST= Frequency of stomata

The morphological and anatomical effects of the different pathogenic fungi on the *Dan Syria* tomato variety differed considerably (Table 2; Figure 2 and Figure 3). Seeds treated with culture filtrates of *Pythium ultimum* under normal gravity had the highest frequency of germination (81.44%), seedling height (4.54mm) stem girth (0.22mm), width of vascular bundles (1.18mm) and frequency of stomata (0.22%). The least seedling height (0.51mm), stem girth (0.02mm) and frequency of germination (11.10%) were observed in *Dan Syria* seeds pre-treated with culture filtrates of *Sclertotinia sclerotiorum* under condition of microgravity. The least width of cortex (1.60mm) was induced in seeds treated with culture filtrates of *Pythium ultimum* (1.60mm) under microgravity. Trichomes and stomata were absent under normal and microgravity conditions respectively. Absence of stomata was also observed in plants exposed to filtrates of *S.sclerotiorum* under normal gravity. Differences in effect of fungal filtrates on the anatomy and morphology of *Dan Syria* variety under conditions of normal and microgravity were significant ($P \le 0.05$).



Figure 2: (A) Presence of Stomata in Stem Section of Tomato Seedling Growing under Normal Gravity; (B)

Absence of Stomata in Stem Section of Tomato Seedling Growing under Microgravity



Figure 3: (A) Dense Cluster of Trichomes in Stem Section of Tomato Seedling Growing under Microgravity; (B) Sparse Trichomes in Stem Section of Tomato Seedling Growing under Normal Gravity

Table 3. Morphological and Anatomical Responses of the *Teema* Tomato Variety to Disease Stress by Different Fungal Pathogens under Different Conditions of Gravity

	Plant Response											
		FREGM (%)	HP (mm)	SG (mm)	WEP (mm)	WC (mm)	WVB (mm)	NT (mm)	LT (mm)	FREST (%)		
Growth condition	Fungal Pathogen											
Normal gravity	Pythium ultimum	22.20	1.78	0.11	0.15	3.23	1.51	5.00	1.75	0.22		
	Sclerotinia sclerotiorum	77.73	5.07	0.20	0.12	3.51	0.84	1.89	0.86	0.11		
Microgravity												
	Pythium ultimum	22.20	0.51	0.05	0.09	1.61	0.84	17.78	2.16	0.00		
	Sclerotinia sclerotiorum	55.50	1.79	0.13	0.19	3.43	0.93	2.11	1.26	0.00		
LSD		22.23	3.28	0.15	0.20	1.62	0.58	12.78	0.90	0.25		

HP= Height of plant, SG= Stem Girth, WVB= Width of vascular bundle, NT= Number of trichomes, LT= Length of trichomes WEP= Width of epidermis, FREG= Frequency of germination, FST= Frequency of stomata.

Seeds of the RomaVF tomato variety treated with culture filtrates of *Sclerotina sclerotiorium* under normal gravity had the highest frequency of germination (77.73%), seedling height (5.07mm), stem girth (0.20mm) and width of cortex (3.51mm) (Table 3). Seeds pre-treated with culture filtrates of *Pythium ultimum* had the highest number of trichomes (5.00), width of vascular bundles (1.51mm) and frequency of stomata (0.22%) under normal gravity condition. The least frequency of germination (22.00%), stem girth (0.05mm), seedling height (0.51mm), width of cortex (1.61mm) and width of vascular bundles (0.84mm) were observed under microgravity in *Teema* seedlings challenged with culture filtrates of *P. ultimum*. Absence of stomata was also observed in plants exposed to filtrates of *S. sclerotiorum* and *Pythium ultimum* under microgravity condition. Differences in effect of culture filtrates of fungal pathogens on morphological and anatomical features of *Teema* under conditions of normal and microgravity were significant (P≤0.05).

Table 4: Mean Total Effect of the Different Conditions of Gravity on the Morphology and Anatomy of the Studied Tomato Varieties

	Plant Response									
		FREGM (%)	HP (mm)	SG (mm)	WEP (mm)	WC (mm)	WVB (mm)	NT (mm)	LT (mm)	FREST (%)
Growth condition	Fungal Pathogen									
Normal gravity	Dan Syria	79.61	4.01	0.19	0.11	3.46	0.92	0.00	0.00	0.11
	UTC	49.97	2.61	0.14	0.10	3.77	1.05	1.50	0.34	0.67
	Teema	49.97	3.42	0.16	0.13	3.78	1.18	3.44	1.31	0.67
Microgravity	Dan Syria	27.75	1.06	0.72	0.11	3.27	0.74	2.17	0.44	0.00
	UTC	66.63	3.25	0.17	0.19	1.30	0.63	15.38	2.40	0.00
	Теета	38.85	1.15	0.09	0.14	2.53	0.88	9.94	1.71	0.00
LSD		16.66	2.10	0.53	0.08	1.23	0.13	5.44	0.69	1.00

HP= Height of plant, SG= Stem Girth, WVB= Width of vascular bundle, NT= Number of trichomes, LT= Length of trichomes WEP= Width of epidermis, FREG= Frequency of germination, FST= Frequency of stomata.

Results of the mean total effect of the different conditions of gravity on the morphology and anatomy of different tomato varieties subjected to disease stress by various fungal pathogens are presented in table 4. Seedlings of Dan Syria subjected to the condition of normal gravity had the highest Frequency of germination (79.61%), seedling height (4.01mm) and stem girth (0.19mm). Teema was observed to have the highest width of vascular bundles (1.18mm) under normal gravity condition. Under conditions of microgravity, the highest number of trichomes (15.38) was observed in UTC. The least frequency of germination (27.75%), seedling height (1.06mm) was observed in Dan Syria under the condition of microgravity. Trichomes were absent in seedlings of Dan Syria under normal gravity. Seedlings of Dan Svria, UTC, and Teema revealed absence of stomata on exposure to microgravity conditions. Differences in effect of gravity conditions on morphological and anatomical features of the seedlings were significant (P<0.05).

Tomato seedlings challenged with disease stress under conditions of normal gravity showed higher vertical and horizontal growth indices than those grown under microgravity. Similarly, Zheng et al. (2015) and Akomolafe et al. (2017) reported higher growth rate in plants grown under conditions of normal gravity than in microgravity. Furthermore, an evaluation of quanti-qualitative traits (growth, yield and quality) of the dwarf tomato variety 'Micro-Tom' grown under simulated microgravity conditions by Colla et al. (2007) revealed that total fruit yield, small fruit yield, leaf area, leaf dry weight, fruit dry weight, total dry weight and shoot - root ratio were lower in the clinorotated tomato plants than those grown in the control treatment. Tripathy et al. (1996) also observed that photosynthetic functions such as the growth rate of wheat plants grown on space stations are affected by the microgravity environment. These differences could be as a result of abiotic stress encountered by growing shoots and roots as a result of sustained

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exposure to microgravity conditions which hinder normal physiological processes within the affected plants.

The extensive formation of trichomes in tomato plants grown under microgravity conditions as opposed to their relative sparse appearance in plants grown under normal gravity, is also indicative of the presence of substantial environmental stress posed by both microgravity and fungal metabolites. Similarly, Zheng *et al.* (2015) and Akomolafe *et al.* (2017) also reported that plants subjected to long periods of exposure to microgravity acclimatize to the stress by changing their metabolism, as well as internal cellular features such as reduction in the thickness of cells and rate of cell proliferation.

Stomata were absent in stem tissues of tomato plants subjected to fungal disease stress under microgravity. This further suggests that compared to normal gravity, plants grown under microgravity are more predisposed to effective disease initiation by pathogens that interfere with stomata formation. In support of this explanation is the report of Balcerowicz *et al.* (2014) that stomata development can be regulated by a number of environmental and hormonal factors.

The UTC tomato variety showed the highest disease tolerance under microgravity stress, by having the highest frequency of germination, width of epidermis, number of trichomes, length of trichomes and plant height. This could be as a result of the likely presence of genetic properties in the UTC tomato variety that conferred higher stress tolerance in the tomato variety, compared to others.

CONCLUSION

Tomatoplants grown under conditions of microgravity showed less disease tolerance compared to those grown under normal gravity. The UTC tomato variety showed higher disease tolerance under microgravity stress, and could be considered for genetic studies aimed at selection of stress tolerance genes in the tomato variety, for enhanced yield and productivity.

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