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Original Research Article

Bioprospecting Nigeria tomato cultivars against *Fusarium oxysporum lycopersici* under polyethylene glycol-induced drought stress

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Abstract

Among the vegetables, tomato is one of the economically important vegetables even at the global level. The nutritional and health benefits of tomato are so numerous to the extent that it has gained so much value in Africa due to its low-cost cultivation and contribution to livelihoods. However, *Fusarium oxysporum lycopersici* (FOL) and drought have been identified as potential threats to tomato production in Nigeria. To manage these challenges, one of the approaches is to identify Nigerian tomato cultivars that can exhibit resistance to FOL and drought. This study therefore investigates four Nigeria tomato cultivars; NGB00714, NGB00715, NGB00725 and NGB00740 to ascertain their resistance attributes to FOL and drought. Prior to their evaluation, Petri - dish seed germination bioassay was used *in-vitro* to assess the germination potentials of the tomato seeds. NGB00725 had the best germination, followed by NGB00714 and NGB00740 while the least was NGB00715. Using the Petri - dish *in-vitro* blotter technique, each tomato seed cultivar was separately exposed to FOL spores and FOL metabolites under drought stress induced by polyethyleneglycol (PEG-6000). Cultivar NGB00725, followed by NGB00740 exhibited resistance to FOL under PEG-induced drought stress based on their seed germination potentials. Bioprospecting resistant Nigeria tomato cultivars in this study is an indication of sustainable production of tomatoes in Nigeria against FOL under drought stress.

Biological: Agriculture.

Key words: *Plant diseases, climate change, fungicides, environmental stress, crop tolerance*

1. INTRODUCTION

Tomatoes are utilized in many cuisine preparations by different tribes in Nigeria, where they are considered both fruits and vegetables in certain regions. This led to a rise in the demand for fresh tomatoes. As a result of this, Nigeria was placed 14th globally in terms of tomato output, contributing 10.8% of Africa's 1.2% global tomato production (Emenike, 2020). Nigeria had the largest production in 2010 ($687,610,000), primarily due to an expansion of the cultivated area that had grown by 5% a year during the previous ten years. With an annual production of 2.3

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million tonnes, Nigeria emerged as the second-largest producer of fresh tomatoes in Africa in 2016 (PwC, 2018). On this note, tomato production and cultivation have been regarded as one of the agricultural products that might help diversify Nigeria's economy, based on its economic worth and gross domestic input (Athanasius, 2019). But in contrast to the global average yield of 38.1 tonnes/ha, tomato yields have been extremely low from the end of 2016 and up to this point. On average, they are only 5.47 tonnes/ha. Tomato seeds are subjected to a variety of biotic and abiotic stresses throughout cultivation, which may account for the low tomato production (Rivelli *et al*., 2012).

Fusarium oxysporum lycopersici (FOL), one of the biotic fungi that used to cause *Fusarium* wilt, appears when extreme environmental conditions, like drought, are present (PwC, 2018; Ogunsola and Ogunsola, 2021, Pierre *et al*., 2023). (Fig. 1). Drought stress can influence a plant at any stage of its life cycle, affecting its water relations throughout the entire plant, as well as its organs, cells, and molecules (Begna, 2023; González, 2023). Low yield changed flower and grain-filling production, and general disturbance of a plant's growth and development are the usual outcomes of this (Farooqi *et al*. 2020). Additionally, soon after stomatal closure, there is a steady decline in net photosynthetic activity and water-use efficiency, which significantly reduces plant productivity (Ozeki *et al*., 2022). In theory, drought stress causes the cell membrane to rupture, which increases the membrane's porosity when it dehydrates. Dryness also causes membrane proteins to shift, which compromises membrane integrity, selectivity, cellular disruption, compartmentalization, and enzyme performance. According to Abiala *et al*. (2023), dehydration can also lead to a rise in electrolyte concentration, which can disrupt cellular metabolism and induce osmotic disturbances. Furthermore, this type of stress results in slower cell division, less leaf expansion, and reduced vegetative development. These reductions in transpiration have an impact on agricultural productivity (Li *et al*., 2024).

During the dry season, 80% of the majority of tomato varieties in Nigeria were lost, according to Adetula (2017). This is due to the fact that a significant water scarcity brought on by a drought might seriously harm tomato plants in addition to causing wilting and leaf loss, dehydration, and plant death. Furthermore, enhanced cell permeability under dryness typically results in nutrient linking into the apoplast, which can promote the growth of some diseases like FOL, which is known to be harmful to tomatoes. FOL can spread quickly, and by the time a tomato plant exhibits symptoms, it's too late and it will have withered and died. The results of Cantore *et al*. (2016) highlighted that the combined impacts of FOL and drought have a major economic impact on tomato production anywhere in the world, with yield reductions of up to 50–80% possible.

Technically, FOL has the ability to disrupt the genes responsible for resistance and exploit the drought to target the host plant's water conductivity vessels. Consequently, it has been determined that the combined effects of FOL and drought pose a risk to Nigeria's tomato crop. The resolution of the interaction complexes between FOL and drought on Nigerian tomato cultivars is exclusively achievable using molecular technology, as the response of plants to a mixture of stresses is a multifaceted characteristic that cannot be inferred directly from each individual stress that is applied. Furthermore, FOL pathotypes have shown resistance to chemical pesticides, and Nigerian farmers' continuous use of pesticides poses a risk to the public health. Furthermore, it was discovered that biological control agents and imported biofertilizers did not work on the native soils of Nigeria. As of as now, Nigeria is not seeing any success with any of the attempts to reduce FOL and/or drought (Adetula, 2017). At this point, farmers' search for more sustainable options

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has led to a variety of strategies, one of which is the search for tomato cultivars that are stress-tolerant, because these cultivars have a great deal of potential to guarantee high output. In this study, we screened and identified stress-tolerant tomatoes among Nigeria's commercially viable varieties using seed germination bioassay as a preliminary method. To create drought stress, seeds of various tomato cultivars were exposed to FOL (Schmey *et al*., 2023) and/or 15% polyethylene glycol (PEG) (Abiala *et al*., 2023). Furthermore, the evaluation of the crude metabolite of FOL was evaluated in relation to the germination of tomato seeds under drought stress caused by PEG.

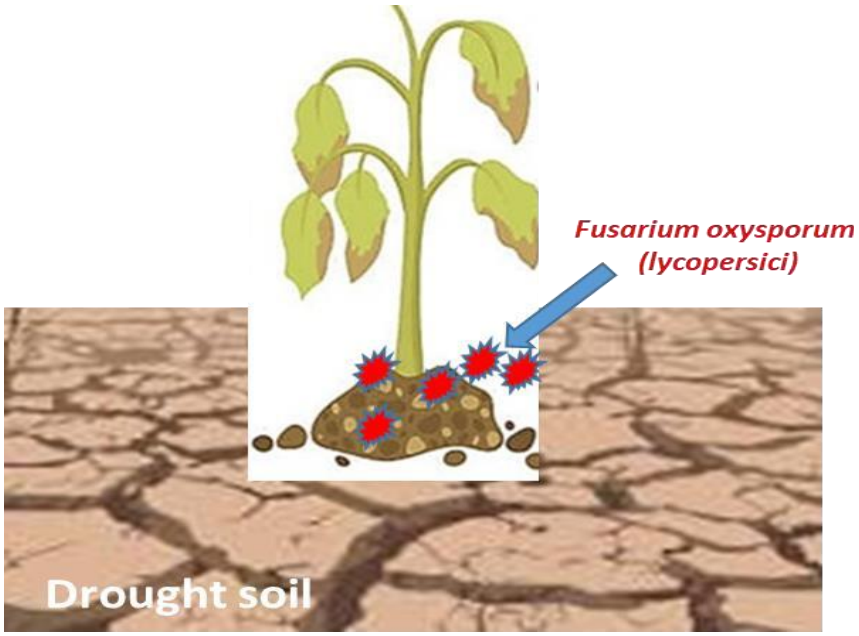

Figure 1 – Effect of *Fusarium oxysporum lycopersici* on tomato under drought stress

2. MATERIALS AND METHODS

2.1 Collection of tomato cultivars

The tomato cultivars: NGB00714, NGB00715, NGB00725 and NGB00740 were collected from the National Center for Genetic Resources and Biotechnology, Ibadan, while the FOL was collected from the National Horticultural Research Institute and Training, Ibadan, Nigeria.

2.2 Seed germination bioassay

Before applying stress, the tomato seed varieties were assessed to determine their capacity for germination using the seed germination bioassay. In a nutshell, 10 seeds of a tomato cultivar that had already been pre-sterilized were put in a petri dish covered with damp Whatman No. 1 filter paper. The seeds were kept under 420 $\mu molm^{-2}$ s^{-1} of photosynthetically active radiation in a growth chamber after being incubated for two days in the dark at a temperature of 25 ± 2°C and relative humidity (RH) of 75 ± 5%. They also spent five long days in the light at a temperature of 24 ± 2°C and RH of 65 ± 5%, during a photoperiod of 16 hours of light and 8 hours of darkness. On day 7, a radicle that protruded at least 2 mm from the testa was regarded a germination of the seed. According to Sharma et al. (2023), the germination rate of each cultivar was determined as the number of germinated seeds/total number of seeds x 100%. For every cultivar, there were ten replicates in the seed germination experiment.

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2.3 Preparation of FOL spores

After the germination status of each tomato cultivar had been established, we imposed FOL and/or drought stress using a 15% PEG concentration. Prior to the stress, the FOL inoculum size and crude metabolite of FOL were prepared accordingly. For the inoculum size, the mycelial growth of young cultures of each isolate on potato dextrose agar was harvested using a camel hairbrush and the resulting suspension was filtered through sterile double-folded cheesecloth to separate the conidia from the mycelia fragments. The sieved conidia solution was however counted and readjusted using haemocytometer to 3.5 x 10^6 conidia mL^{-1} (Schmey *et al*., 2023). Quantified inoculum was stored at - 60ºC. Before inoculation, spore germination percentage was determined and viable spores for FOL were re-diluted to concentration of 10^8 spores' mL^{-1}.

2.4 Preparation of FOL metabolites

A 5 mL mycelia disc containing the confluent growth of a 7-day-old culture of FOL was aseptically inoculated into individual 125 ml conical flasks containing 50 ml of sterile potato dextrose broth in order to obtain the crude metabolite of FOL. The flasks were then incubated for 7 days at a temperature of 28ºC and 150 rpm using a shaker incubator (Scigenics Biotech). After the incubation, the mycelia mat and the culture filtrate were filtered using sterile Whatman No. 1 filter paper. This was followed by vacuum filtration on a 0.22 um millipore membrane filter. In the sterile flask, the culture filtrate, or crude metabolite, was maintained at 4 ºC.

2.5 Preparation of 15% PEG for drought stress

In a 150 ml beaker, 15 g of PEG (polyethylene glycol - 6000) was weighed into 50 ml of sterile distilled water according to the procedure outlined by Abiala et al. (2023). The mixture was then heated to 45°C using hot plates and swirled progressively with a magnetic bead stirrer to maintain a homogeneous solution. The distilled water was gradually added until 100 mL was reached. After that, the 15% PEG solution was let to cool to room temperature in preparation for further use.

2.6 Effect of FOL spores and metabolites on germination of tomato cultivars under PEG-induced drought stress

Thereafter, pre-sterilized viable seeds of each tomato seed cultivar were separately inoculated with the freshly quantified inoculum (10^8 spore's mL^{-1}) and crude metabolite of FOL. Briefly, 10 FOL inoculated seeds were placed at equidistant positions in a 9 cm Petri dish over two layers of Whatman No1 filter paper moistened with 15% PEG and sealed with parafilm to minimize evaporation. The control solution was supplemented with sterilized distilled water. The experiment was in ten replicates for each tomato cultivar set up in a randomized complete block design (tomato alone - control; tomato + FOL; tomato + 15% PEG; tomato + FOL + 15%PEG; tomato + FOL metabolites; tomato + FOL metabolites + %15PEG). The control and the treated seeds were incubated in the growth conditions stated above. At day 7, seeds were considered to have germinated once the radicle protruded at least 2mm from the testa. Germination rate was calculated as the number of germinated seeds/total number of seeds and expressed as a percentage, according to Sharma *et al*. (2023).

2.7 Statistical analyses

Experimental treatments were compared using SAS software, version 9.2 (SAS Institute, Cary, NC, USA). For each experiment, replicated data sets were subjected to the analysis of variance (ANOVA). The mean comparison was carried out by Tukey's test.

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3. RESULTS AND DISCUSSION

3.1 Seed germination potentials

All the cultivars had germination of > 85% except NGB00715 (Fig. 2). The seed germination was carried out to establish the germination potential of each cultivar so as not to misinterpret poor germination for the effect of FOL separately or in combination with PEG-induced drought stress. This is because seed germination is an important stage in plant development playing a crucial role in seedling emergence and adaptation to environmental factors (Rezvani *et al*., 2021). Among the cultivars evaluated under 15%PEG-induced drought stress, NGB00725, followed by NGB00714 had good germination (Table 1) of ≥ 36%. The germination status of NGB00740 was not encouraging while that of NGB00715 was completely inhibited. According to Adetula (2017), 80% of most Nigeria tomato cultivars were lost during the dry season since shortage of water causes severe stress on tomato seeds and plants resulting in loss of leaves, then dehydration and death (Cantore *et al*., 2016).

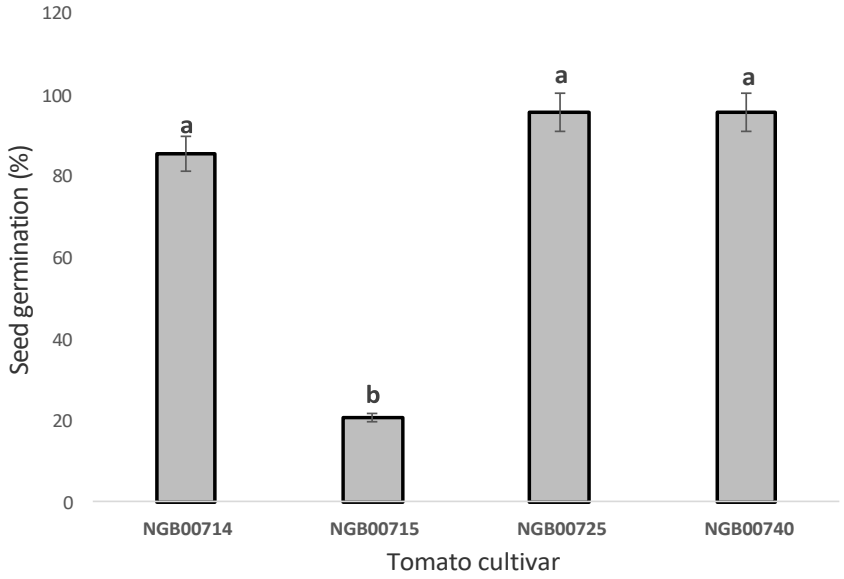

Figure 2 – Germination (%) potential of each tomato cultivar. Means followed by the same letters are not significantly different ($P \leq 0.05$; Tukey's Test. The results shown are mean ± standard error (n = 10).

3.2 *In-vitro* effect of FOL and PEG-induced drought stress on tomato cultivars

When the cultivars were subjected to FOL stress, it was observed that FOL crude metabolites had more effect than the FOL spores on the germination of each tomato cultivar. FOL crude metabolites reduced the germination of all the cultivars (Table 1) except for NGB00725 which maintained good germination. When 15%PEG was used to induce drought under FOL stress, the seed of NGB00715 died completely, that is, there was no sign of germination till day 7 (Table 1, Fig. 3). Technically, during drought, increased cell permeability normally leads to nutrient linkage into the apoplast which can facilitate multiplication of some pathogens (Ahluwalia *et al*., 2021; Pierre *et al*., 2023) such as FOL known to be destructive to tomato. FOL has the potential to progress swiftly and by the time the tomato plant shows outward signs of infection, it is already too late and the tomato plant wilts and dies (Ajibola and Babalola, 2013; Abiala *et al*, 2021; Pierre

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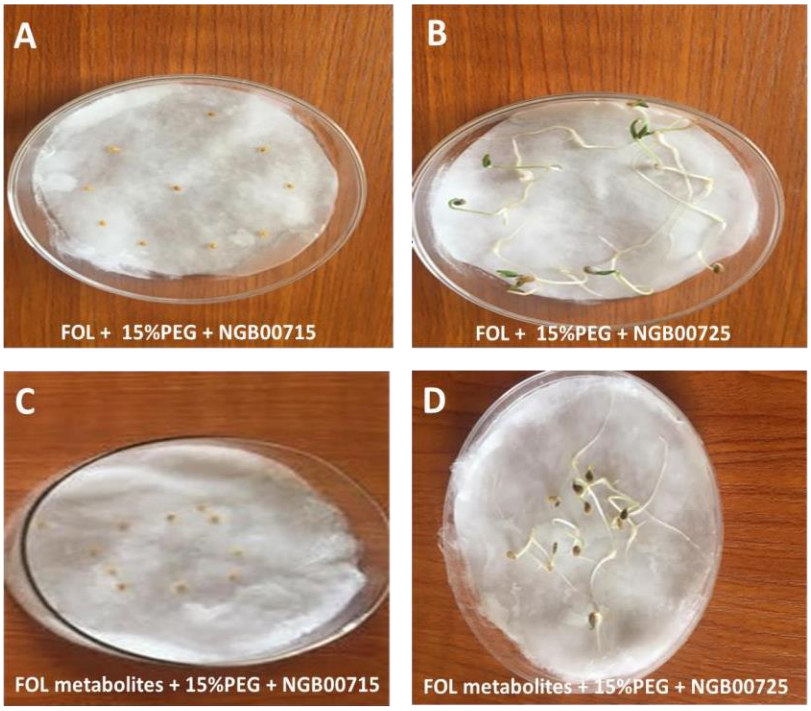
et al., 2023). In our study, it was observed that before the germination commenced, FOL may have colonized it, taken advantage of the PEG-induced drought, thereafter, death of NGB00715 cultivar.

Table 1: *In-vitro* effect of FOL and PEG separately and in combination on tomato cultivars

Tomato cultivar	Tomato alone	Tomato + FOL	Tomato + 15%PEG	Tomato + FOL + 15%PEG	Tomato + FOL metabolites	Tomato + FOL metabolites + 15%PEG
NGB00714	85.40 ± 5.25a	70.00 ± 11.40a	66.66 ± 12.52a	62.00 ± 4.89b	58.00 ± 8.00b	56.00 ± 2.45c
NGB00715	20.80 ± 9.93b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00d
NGB00725	95.83 ± 4.18a	90.00 ± 5.65a	88.36 ± 5.65a	84.00 ± 2.45a	88. 00 ± 4.89a	88.00 ± 3.74a
NGB00740	95.80 ± 2.42a	84.00 ± 2.45a	76.68 ± 10.35ab	72.00 ± 3.74ab	70.00 ± 5.48ab	67.60 ± 1.94b

Apart from tomato cultivar NGB00725, the effect of FOL or FOL metabolites with or without drought stress induced by 15%PEG significantly ($P<0.05$) affects all the tomato cultivars. NGB00715 died completely, while that of NGB00725 significantly ($P<0.05$) germinated. Means followed by the same letters within a column are not significantly different ($P \leq 0.05$; Tukey's Test. The results shown are means ± standard error (n = 10). *Fusarium oxysporum lycopersici* – FOL; polyethyleneglycol – PEG at 15% concentration.



Figurer 3 – The effect of FOL or FOL metabolites under drought stress induced by 15%PEG on tomato cultivars NGB00715 and NGB00725. When FOL spores were used with 15%PEG, the seeds of NGB00715 died completely (A), while that of NGB00725 germinated (B). Also, when FOL metabolites were used with 15%PEG, the seeds of NGB00715 died completely (C), while that of NGB00725 germinated (D). *Fusarium oxysporum lycopersici* – FOL; polyethyleneglycol – PEG at 15% concentration.

FOL is a sophisticated adaptable fungus that adapts quickly to changes in the environment (Gupta and Senthil-Kumar, 2017). This implies that the FOL can interfere with the resistance of NGB00715 and may have attacked its water conductivity vessels. Moreso, the seed of NGB00715 could not withstand the threat of FOL and PEG-induced drought knowing fully well that the seed of NGB00715 poorly germinated during our seed germination bioassay, before imposing stress

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(Fig. 2), thus, this may have led to its death (Fig. 3).

Our study further revealed that FOL metabolites demonstrated a greater impact on seed germination under drought stress. FOL metabolites are primarily released when the seed or plant has been attacked due to a shortage of water and a weakened defense system (Osakabe *et al*., 2014). Both NGB00740 and NGB00714 were partially threatened (Table 1). On day 7, the young seedlings of NGB00740 and NGB00714 had slight symptoms of premature abscission of cotyledons, brown lesions that girdle the hypocotyl (Srinivas *et al*., 2019). This indicates that the combined effects of FOL and drought have the potential to threaten tomato production in Nigeria. Thus, this could be linked to why many small holder farmers in Nigeria are skeptical about growing tomatoes due to these challenges (PwC, 2018; Ogunsola and Ogunsola, 2021). Of interest, NGB00725 exhibited significant ($P < 0.05$) resistance to FOL spores and its metabolites (Table 1). In fact, when drought was imposed on the seed of the tomato cultivars through PEG, the resistance of NGB00725 was more significantly ($P < 0.05$) pronounced in comparison to other tomato cultivars. The resistance of NGB00725 to FOL under drought stress may be associated with some biomolecules such as melatonin (Altaf *et al*., 2022) as well as the expression of some resistant traits in NGB00725.

4. CONCLUSION

Tomato cultivar NGB00725 tolerated FOL and/or its metabolites under drought stress and germinated successfully. Apart from good germination of NGB00725, its radicle was not affected as well as the hypocotyl. Based on this remarkable preliminary evidence, we intend to carry out further study at the greenhouse and field conditions to affirm the stress tolerance status of tomato cultivar NGB00725 for cultivation under the threat of FOL and drought stress.

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