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THE EFFECT OF AMF (*GLOMUS CLARUM*) ON TOMATO RESISTANCE TO EARLY LEAF BLIGHT (*ALTERNARIA SOLANI*) ON TOMATO

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ABSTRACT

Tomato (*Solanum lycopersicum* L.), is an economic plant which belongs to Family Solanaceae and is widely consumed as food and other processed natural products. Tomato production areas are faced with economic losses due to early blight disease caused by *Alternaria solani*. Early blight disease reduce yield and renders fruit unmarketable, Cultural practices and fungicides are majorly adopted in the management of this disease. The use of tolerant varieties and *Glomus clarum* as alternatives which are environmental friendly necessitated this study. Therefore, this study investigated genetic resistance of tomato to early blight disease using *Glomus clarum* as revealed by SSR marker . Eight tomato accessions obtained from NACGRAB and Technoserve were evaluated, while Ojoo market accession served as check. The experimental treatments Control, *Glomus clarum* + Pathogen and Pathogen only were laid out in a complete randomized design with three replicates. The result showed that Rukuta Jubia and NGB00727 accessions had the highest mean values for stem girth (0.59cm) and leaf width (3.74cm) respectively, while Ojoo Market performed best for leaf length (6.78cm). *Glomus clarum* significantly ($p<0.05$) influenced the resistance of NGB00727 to *Alternaria solani* ,and enhanced the production of flowers (2.53) and mean weight of fruit per plant (0.94g). The NGB00727 accession showed the highest resistance to early blight disease, while NGB00754 accession was the most susceptible to *Alternaria solani*.

KEYWORD: Tomato, Early blight, *Glomus clarum*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) formerly *Lycopersicum esculentum* Mill is an economic tropical plant which belongs to one of the most important family Solanaceae. It includes a number of vegetables used in homes and also as processed food (Canene-Adams *et al*. 2005) Tomato fruit is widely consumed and used for refined products such as pastes, dice, form of juice, sauces and soup added to other food items.
Early blight caused by *Alternaria solani* Sorauer (FAOSTAT, 2005) is one of the most damaging diseases in many tomato production area worldwide (Sherf *et al.* 1986; Woudenberg *et al.* 2014). *Alternaria solani* destroys tomato plant by making its fruit unmarketable, reduce the yield and cause economic losses (Agrios, 2005). No genetic source of Early blight resistance is known within the cultivated tomato (Martin *et al*.1987; Foolad *et al.* 2000). Currently, early blight disease is primarily controlled through cultural practices and use of fungicides. However, there is need to identify resistant accessions within germplasm and wild species of tomato.
Arbuscular Mycorrhizal Fungi (AMF) are important component of plant root rhizosphere which form symbiotic association with root of over 90% of land plants (Smith *et al*. 2008). They are beneficial in plant ecosystem through improvement of plant establishment, growth and protection against environmental stresses such as salinity (He *et al*. 2007), drought (Auge. 2004; Olawuyi *el al*. 2014) and metal toxicity (Reuscher *et al*. 2013).
The enhanced nutrient supply to plants in the presence of AMFs is as a result of increase in absorption surface area of the root by fungal hyphae and high affinity of hyphal surface area for phosphate (Johri *et al*. 2015).
Therefore, this study was designed to investigate the resistance of tomato accessions to *Alternaria solani* using AMF (*Glomusclarum*).

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MATERIALS AND METHODS

Sources of Planting materials

A total of eight accessions of tomato were evaluated. This comprises of three tomato accessions obtained from National Centre for Genetic Resource and Biotechnology (NGB 0072, NGB 00752 and NGB 00754). Four tomato accessions were collected from Technoserve (Rukuta jubia, Dan eka mani, Rukuta mani and Dan eka jubia), while one Oojo market accession served as check.

Nursery Establishment, Experimental design and Treatments

Seedlings were raised at the nursery for 4weeks before transplanting in Screen house of the Department of Botany, University of Ibadan. A total of 72 perforated polythene bags were filled each with 5kg of dried sandy-loam soil and arranged in Complete Randomize Design with three replicates. The treatments were set up as: control (uninoculated), AMF only, AMF and Pathogen.

Application of Arbuscular Mycorrhizal Fungi and *Alternaria solani*

Five grams (5g) of *Glomus clarum* was applied to the tomato plant according to the method described by (Olawuyi *et al*. 2011) using ring method, while *Alternaria solani* pathogen was inoculated on some selected tomato plants according to the method described by (Arunakumar, 2006).

Disease resistance and severity assessment

The resistance and susceptibility pattern of tomato to early blight disease caused by *Alternaria solani* using the rating scale described by Pandey *et al*. (2003).

Morphological Statistical Analysis

Morphological data was analyzed using SAS 9.3 software while difference in means were separated using Duncan Multiple Range Test (DMRT) at $P < 0.05$.
Variation observed among the quantitative traits were established using Pearson Correlation coefficient and Principal Component Analysis (PCA)

RESULTS

Mean square variance of *Glomus clarum* and *Alternaria solani* on growth and yield characters of Tomato

The result in table 1 shows the mean square variance of growth characters in tomato. The treatment produced significant effect ($p<0.01$) on stem girth, but highly significant effect for leaf width, plant height, number of leaves. The accessions produced significant effect ($p<0.001$) on stem girth, leaf width and plant height. Weeks after transplanting tomato shows significant effect ($p<0.001$) on stem girth, leaf width, plant height, number of leaves and leaf length. The result of the mean square variance on yield characters presented in Table 1 shows that the accessions, weeks and interaction of accessions*weeks is significantly ($p<0.001$) higher on the number of fruit and number of flower produced per plant, while the treatment had highly significant effect ($p<0.001$) on number of fruit produced per plant. The first order of interaction for accession * replicate, accession * treatment and the second order of interaction accession * treatment * replicate had significant effect ($p<0.05$) on disease severity, number of flowers and fruits produced by tomato accessions.
The second order of interaction for accessions * treatment* replicate, treatment* weeks* replicate, accession * weeks* replicate, accession * treatment * weeks significantly ($p<0.05$) influenced the number of flowers dropped by tomato due to Early blight.

Table 1:Mean square interaction of *Glomus clarum* and *Alternaria solani* on growth and yield characters of tomato (*Solanum lycopersicum*)

Source of Variation	Df	Stem girth	Leaf width	Plant height	No of leaves	Leaf length	No of Flower per plant	No of fruit per plant
Replicate	2	0.15***	1.11**	2801.12***	23.90*	11.66***	24.54**	0 72*
Treatment	2	0.07**	6.21***	5660.39***	2566.24***	18.81***	50.34***	1.39*
accessions	7	0.27***	6.40***	22988***	3301.15***	21.35***	46.69***	10.43***
Weeks	11	2.14***	18.99***	97526.60***	3680.85***	88.42***	67.95***	3.49***
Treatment*Replicate	4	0.01*	1.11**	467.31*	184.20**	0.92*	5.02*	0.27
accessions*Replicate	14	0.05***	3.20***	2817.58***	276.48***	9.95***	9.52**	1.18**
Week*Replicate	22	0.02**	0.76**	542.81**	64.52*	0.98*	5.65*	0.31
accessions*Treatment	14	0.03**	0.75**	1161.17***	223.24**	3.39***	7.65*	1.47**
Treatment*weeks	22	0.01*	0.43*	531.35**	233.85***	1.28*	4.46	0.71*
accessions*weeks	77	0.01*	0.54**	851.93***	276.05***	2.10***	10.36***	1.18***
accessions*Treat*Rep	28	0.031***	0.79***	1413.28***	168.93**	3.05***	7.81**	1.04**
Treatment*Weeks*Rep	44	0.01*	0.26*	356.52*	46.79	0.70	4.58*	0.45
accessions*Weeks*Rep	154	0.01*	0.59**	511.46***	87.31*	1.25**	4.58*	0.45
accessions*Treat*weeks	154	0.01*	0.37*	311.15*	79.78*	0.93	4.18	0.64*
Corrected total	863							

* $p<0.05$ ** $p<0.01$ *** $p< 0.001$

Mean square variance of disease severity on *Solanum lycopersicum*

The result of mean square variance on disease severity, growth and yield of tomato in Table 2, shows that treatment, accessions, accessions*replicate, accessions*treatment, treatment * week, accessions * weeks, accession * treatment * replicate, accessions * weeks * replicate, accessions * treatment * week had highly significant effect (p<0.001) on the disease severity. The first order of interaction for accession * replicate, week * replicate, accession * treatment had significant effect (p<0.01) on disease severity and the second order of interaction which shows that accessions * treatment * replicate, treatment * weeks * replicate, accession * weeks * replicate, accession * treatment * weeks had significant effect (p<0.05) on number of flowers dropped per tomato plant due to early blight disease of tomato. (Table 2)

Table 2: Mean square interaction of *Glomus clarum* and *Alternaria solani* on disease severity of tomato

Source of Variation	Df.	Dead Flower per plant	Disease symptoms on leaves
Replicate	2	0.19	5.09
Treatments	2	0.12	2277.24***
accessions	7	0.33	121.21***
Weeks	11	0.68**	531.0.2
Treatment *replica	4	0.08	4.53
accessions*Replicate	14	0.44*	40.29***
Week*Replicate	22	0.20	14.40**
Variety*treatment	14	0.37	37.10***
Treatment*week	22	0.32	138.16***
accessions*weeks	77	0.35	41.67***
Variety *treatment*replicate	28	0.46*	13.82***
Treatment*weeks*replicate	44	0.44*	7.18
Variety*weeks*replicate	154	0.41*	17.82***
Variety*treatment*weeks	154	0.39*	15.27***
Corrected total	863		

* p<0.05 ** p<0.01 *** p< 0.001

Growth characters of *Solanum lycopersicum*

There were variations in accessions with respect to the mean value of growths characters in Table 3, Rukuta Jubia is significantly higher for stem girth (0.59cm), NGB00727 recorded the highest value of leaf width (3.74cm), plant height (107.18cm) and number of leaves (38.82) in Table 3,However, Dan eka jubia had the least value of plant height (67.57), while Oojo accession is significantly higher for leaf length (6.78cm).

Table 3: Genotypic effect on growth characters of tomato under the interactions of *Glomus clarum* and *Alternaria solani*

Accessions	stem girth (cm)	leaf width (cm)	plant height (cm)	no of leaves	leaf length (cm)
Dan Eka Jubia	0.47[de]	3.11[e]	67.57[e]	26.47[d]	5.75[d]
Rukuta mani	0.45[d]	3.31[de]	77.85[d]	27.53[d]	6.04[c]
Dan eka mani	0.48[c]	3.10[e]	69.24[e]	29.00[dc]	5.74[d]
Rukuta Jubia	0.59[a]	3.48[bc]	86.82[c]	34.42[b]	6.64[a]
NGB 00727	0.52[b]	3.74[a]	107.18[a]	38.82[a]	6.72[a]
NGB 00752	0.53[b]	3.45[c]	96.75[b]	34.42[b]	6.33[b]
Market acc	0.48[c]	3.64[ba]	101.16[b]	30.80[c]	6.78[a]
NGB00754	0.44[d]	3.17[de]	83.06[c]	21.09[e]	5.82[dc]

Means with the same letter on the same column are not significantly different at p<0.05 according to Duncan multiple range test (DMRT).

Effect of Early Blight Disease on the Yield Characters of *Solanum lycopersicum*

The result in Table 4, showed that NGB00727 had the highest number of fruit produced per plant (0.94), number of flowers per plant (2.53) which is significantly different from other accessions. Dan Eka Jubia, Rukuta Mani, Dan Eka Mani, Rukuta Jubia, NGB00752, Oojo Mar and NGB00754 are not significantly different from one another.Rukuta Mani and Oojo Market had the highest number of dead flowers per plant (0.14).The highest disease severity was observed in NGB00754.

Table 4 : Yield and early blight disease traits of tomato accessions

Accessions	Number of flowers.	Number of fruit	Dead flowers	Disease on leaves
Dan Eka Jubia	1.60^{b}	0.06^{c}	0.11^{a}	3.45^{c}
Rukuta Mani	0.51^{d}	0.02^{c}	0.00^{a}	3.81^{bc}
Dan Eka Mani	0.85^{cd}	0.06^{b}	0.03^{a}	2.47^{d}
Rukuta Jubia	0.81^{d}	0.04^{c}	0.00^{a}	2.28^{d}
NGB00727	2.53^{a}	0.94^{a}	0.05^{a}	2.00^{d}
NGB00752	1.89^{b}	0.38^{b}	0.12^{a}	2.27^{d}
Market acc	1.58^{b}	0.16^{c}	0.14^{a}	4.27^{ba}
NGB00754	1.47^{cb}	0.09^{c}	0.09^{a}	4.81^{a}

Means with the same letter on the same column are not significantly different at $p<0.05$ according to Duncan multiple range test (DMRT).

Growth characters of *Solanum lycopersicum* affected by *Glomus clarum and Alternaria solani*

Variations exist on growth And yield performance of tomato enhanced by *Glomus clarum* and suppression of disease severity caused by *Alternaria solani* of *Solanum lycopersicum* as shown in Table 5.Control (untreated plant) is significantly higher ($p<0.05$) for stem girth (0.51cm), leaf width (3.51cm), plant height (91.21cm), number of leaves (33.73cm) and leaf length (6.51cm), while AMF+Pathogen treatment had higher mean value than Pathogen treatment for stem girth(0.49cm), leaf width (3.39cm) ,number of leaves (28.72cm) and leaf length (6.16cm). The pathogen treatment had higher plant height (84.65cm) compared with AMF+Pathogen treatment (84.65cm).

Table 5 Growth characters of tomato under the treatments of *Glomus clarum* and *Alternaria solani*

Treatment	Stem girth(cm)	leaf width(cm)	plant height(cm)	no of leaves	leaf length(cm)
AMF+Path	0.49^{b}	3.39^{b}	82.76^{b}	28.72^{b}	6.16^{b}
Pathogen	0.48^{b}	3.22^{c}	84.65^{b}	28.42^{b}	6.02^{b}
Control	0.51^{a}	3.51^{a}	91.21^{a}	33.73^{a}	6.51^{a}

Mean with the same letter in the same column are not significant at $p<0.05$ according to Duncan Multiple Range Test

Keys : AMF -*Glomus clarum* Pathogen – *Alternaria solani*

Correlation Coefficient among the growth, yield related characters and disease severity of Tomato *(Solanum lycopersicum)*

The correlation result in Table 6 shows that Stem girth is positively correlated with growth
Characters viz: Plant height (r=0.69), Leaf length(r=0.57) and Weeks(r=0.60). Leaf width had strong positive correlation with Leaf length (r=0.77). The number of leaves is positively associated with leaf length(r=0.52).

Table 6:Correlation coefficients among the growth related characters of *Solanum lycopersicum*

	SG	LW	PH	NL	LL	FL	FR	DF	DL	VA	TR	WEK	REP
LW	0.48												
PH	0.69**	0.40											
NL	0.48	0.45	0.40										
LL	0.57*	0.77**	0.47	0.52 *									
FL	0.29	0.28	0.33	0.35	0.31								
FR	0.14	0.14	0.30	0.23	0.13	0.25							
DF	0.02	0.04	0.06	-0.01	0.02	0.01	-0.01						
DL	0.21	-0.02	0.31	-0.14	0.02	-0.03	-0.02	0.05					
VA	0.01	0.10	0.20	-0.00	0.10	0.10	0.08	0.04	0.05				
TR	0.05	0.06	0.08	0.15	0.09	0.12	0.06	-0.02	-0.30	0.00			
WEK	0.60	0.14	0.74	0.11	0.17	0.15	0.21	0.07	0.43	0.00	0.00		
REP	0.09	0.03	0.06	0.02	0.05	0.09	-0.04	-0.03	0.02	0.00	0.00	0.00	

- **KEYS**:**SG**: Stem girth,**PH**:Plant height ,**NL**:no of leaves,**LL**:Leaf length, **FL**:Flower,**FR**:Fruiting,**DF**:Dead flower, **DL**:Disease on leaves,**VA**:Variety,**TR**:Treatment,**WEK**:Weeks,**REP**:Replicate

DISCUSSION

The presence of *Glomus clarum* suppressed the damaging effect of *Alternaria solani* on tomato. This is similar to the observation of (Harrier *et al.* 2004; Pozo *et al*. 2005; Bi *et al.*, 2007; Zhou *et al*. 2015; Oyewole *et al*. 2017) which shows that *Glomus clarum* + *Alternaria solani* treatment suppressed disease severity due to early blight when compared with *Alternaria solani* treatment alone. NGB00727 showed higher tolerance to early blight disease of tomato and when combined with *Glomus clarum* treatment, it enhanced the performance of plant height, number of leaves, number of flower per plant and number of fruit produced per plant. Mycorrhizal associations benefit not only plant nutrient absorption, but also plant resistance to diverse abiotic stresses and soil borne fungi pathogen. The NGB00754 had the highest disease tolerance on leaves and fruit. Rukuta jubia had the least mean value for number of dead flower per plant. The findings also revealed significant yield differences in Tomato accessions. The NGB00727 had highest mean value for number of flowers and fruits produced per plant.

CONCLUSION AND RECOMMENDATION

Glomus clarum enhanced tomato production with higher mean cumulative number of flowers and fruits. Accession NGB00727 performed best for growth, yield and disease resistance. Therefore, it could be selected for improvement of other tomato accessions. Primers LETaa001 and LECaa001 are highly polymorphic. Therefore, they could be considered for further molecular breeding of tomato.

CONFLICT OF INTEREST STATEMENT

The Authors declare that there is no conflict of interest

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