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Interspecific Hybridization between Cherry (Small Fry) Tomato and Petomech for Resistance to Root-Knot Nematodes (Meloidogyne Species)

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Abstract:

Root-knot nematode, (Meloidogyne spp) is one of the important plant parasitic nematodes of tomato worldwide. The high cost of resources involved in controlling diseases and pests in vegetable crop production, the impact of agro-chemicals on the environment and human health has made the use of resistant or tolerant cultivars the most appropriate control technique of plant parasitic nematodes. This study looked at producing nematode resistant tomato variety. Two pot experiments and a field work were carried out separately at Kwame Nkrunmah University of Science and Technology (KNUST) and Crops Research Institute (CRI), KWADASO. This was done to identify resistant tomato genotypes and incorporate in breeding for root knot nematode resistance. The pot experiments were laid out in a completely randomized design. Crosses were however, done on the field and this was between Petomech (susceptible variety) and Small Fry (resistant variety). The heaviest fresh root weight was found in Petomech 7.9g.Tomato genotypes Small Fry and the F_2 obtained the least weights of 3.3g and 3.6g respectively. Petomech recorded the highest mean number of eggs (53.6). The mean number of juveniles recovered from the roots of the three tomato genotypes ranged from 18.6 to 33.6. Petomech recorded the highest number of juveniles which was significantly different from Small Fry and F_2.Moreover, Petomech was significantly different from Small Fry and F_2 regarding reproduction factor. Nevertheless there was no significant difference between Small Fry and F_2.The study established that a total of 1500 eggs/tomato plant was found as the optimum initial inoculum level for screening for resistance in tomato to Meloidogyne spp. The F_2 genotype with gall index (GI) >2 and reproduction factor (R_f)(1.12) was moderately resistant to root-knot nematodes with good horticultural attributes.

Keywords: *Root-knot nematode, reproduction factor, small fry, petomech, lycopersicon esculentum, gall index*

1. Introduction

Tomato (Lycopersicon esculentum) is the most important vegetable grown in the world, where it is cultivated in both tropical and temperate zones.The crop is, eaten on each day by every home (Awuah, 2006). Most households use tomato in preparing dishes, sauces, salads and drinks. Tomatoes grow well in moderately fertile soil with lots of organic matter. Loam and sandy loam soils are best for tomato production but these plants will grow in almost all soil types except heavy clay.Tomato production is a flourishing farming activity across the country but mostly in the savanna and forest-savannah. With about 70% of population into agriculture, tomato production serves a major source of income for some small-holder families (MOFA, 2013). The tomato sector has failed to reach its potential, in terms of attaining yields comparable to other countries, the ability to sustain processing plants and improving the livelihoods of those households involved in tomato production and the tomato commodity chain. The inability of some country to meet their domestic production needs is attributed to a number of challenges including, high illiteracy rate of farmers, disease and pest, unavailability of improved tomato varieties and unfavorable environmental conditions (Elizabeth et al., 2010).These factors combined to prevent farmers from producing tomato on a large scale. Most of the farmers do not use quality seeds of improved varieties because they use seeds from their own fields, friends and market women thereby compromising on quality which affect yield in the end (Elizabeth et al., 2010). . The most worrying production factor is the incidence of diseases and pests (Tweneboah, 1998).

About 1.2 billion tons of tomatoes are produced worldwide annually (Anonymous, 2006) of which nematodes have been estimated to cause about 40% yield loss (Banker, 1998) costing at least US$27 million in nematicides use alone (Nematech, 2007). Charchar et al., (2003) reported that tomato is one of the most susceptible vegetable crops and nematodes causes about 30 to 40% yield losses in tropical regions. The use of resistant varieties is promising method of controlling plant parasitic nematodes and the resistance is often managed by one or more genes in tomato cultivars (Ammati et al., 2005). They attack the plant roots, causing galls to develop, and reduce the size and efficiency of the root systems. Plant growth is stunted, fruit set is reduced and yields and quality of the crop is affected adversely (Tweneboah, 1998). A number of challenges have been identified as the cause of this in the tomato production and marketing chain. One of these challenges is disease and pest constraint in the tomato industry (Horna et al.,2006). Notable among them is the root-knot nematodes. Root-Knot nematodes, (Meloidogyne spp.) are sedentary endo-parasite nematodes and feed on a lot of crop plants making them one of the most damaging nematode pests to crops world-wide (Koenning et al., 1999). They are probably the most important eelworm affecting tomatoes production. Tomato farmers in many countries suffer major losses due to the effect of nematodes. This is because most of the varieties they grow are susceptible to nematodes while most of their soils are also infested with nematodes (Koenning et al., 1999). Evidence shows that the replacement of inbred lines by hybrid has remarkably increased yield while the genetic gain rate has been reduced due to low genetic diversity within cultivated tomatoes (Castagnone-Sereno, 2006). Therefore, host plant resistance will be an important component of controlling the impact of nematodes in tomato production. Therefore the objective of the study was to screen available germplasm to identify stable sources to produce a hybrid which can withstand the effect of nematodes.

2. Materials and Methods

The study was carried out at the plant house and pathology laboratory of the Department of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology (KNUST), and the Horticulture Division of the Council for Scientific Industrial Research (CSIR)-Crops Research Institute, Kwadaso, Kumasi. Twenty-two (22) tomato genotypes were collected from the CSRI-CRI, Kwadaso for evaluation of root knot nematodes (Meloidogyne species) resistance. The root-knot nematode inoculum was obtained from heavily infested tomato roots collected from vegetable farms around KNUST, Kumasi. Soils for nursery and pot experiment were sterilized using the barrel steam sterilization method. Top soil was mixed with river sand in the ratio 3:1 (v/v) and sterilized for three hours at 100^0C on fire was used for the pot experiment, after it has been cooled. The root-knot-nematode eggs were extracted using modified Hussey and Barker (1973) method. Root-knot nematode eggs were counted using a counting tray with the aid of a stereo microscope. Counting was done three times per entry. Juveniles were extracted from infested tomato roots, using modified Baerman tray method (Whitehead and Hemming, 1965). The 22 tomato genotypes were nursed separately in wooden seed boxes containing the sterilized top soil. Each pot of 2 l size was filled with 1.8 l of sterilized top soil mixed with river sand. Seedlings were transplanted to the pots three weeks after germination. The experimental design used was Complete Randomized Design (CRD) with three replications. Single seedling was planted per pot at a spacing of 60cm between rows and 40cm within rows. Two weeks after transplanting each of the potted seedlings was then inoculated with 1500 eggs. Three wells were made in a triangular form, 2cm each from the plant. The egg suspension of each level was homogenized by blowing air through the pipette and dispersed into the holes. Galling score on each root was done using the scale of 0-10 rating chart by Bridge and Page (1980). Nematode numbers in the roots from every pot were calculated and used to determine reproduction factors (Oostenbrink, 1966). Assessment of resistance, tolerance, susceptible responses of the tomato cultivars were assessed based on Canto-Saenz (1983) method. The results from the pot experiment were used to select Cherry (Small Fry) tomato which had a Reproduction Factor (Rf) of 0.98 as the resistant variety while Petomech with a Reproduction Factor (Rf) of (2.69) was used as the susceptible variety (Table 1).

Crosses were made between Petomech (Susceptible) and Cherry tomato (Resistant) on the field at CSIR-CRI, Kwadaso from the month of June to September 2013.

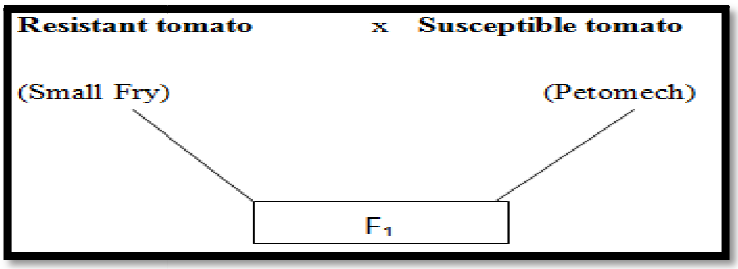

Figure 1: Illustration of Tomato Hybridization for F_1 Population

Nursery of the two parents (Small Fry tomato-Parent 1, Petomech-Parent 2) was staggered such that parent 1 which was used as male was transplanted two weeks earlier than the parent 2 (female). This was to ensure flowering synchronization. The F_1 seeds were sown on the 22nd September, 2013 and 30 seedlings were transplanted at 100cm x100cm three weeks after sowing to produce seeds for the F_2 population.

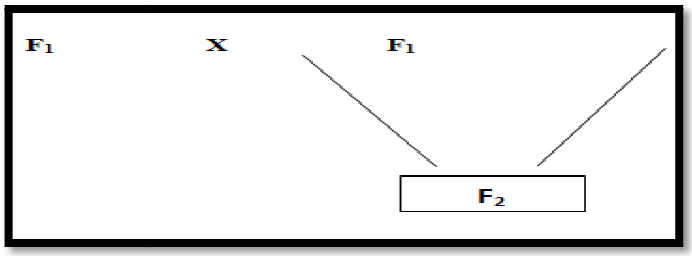

Figure 2: Illustration of Selfing F_1 to Generate F_2.

One thousand F_2 progenies were sown on the 14th of December, 2013 and planted at 60cm x 50cm three weeks thereafter to identify superior plant types. The F_2 plants which were identified to be of superior qualities were selected and screened together with the two parents in a pot experiment at the plant house of KNUST as described above.

3. Results

Table 1 presents the effect of root knot nematodes on number of eggs, root galling and Meloidogyne reproduction on 22 tomato genotypes eight weeks after inoculation from which Petomech and Small fry were selected as susceptible and resistant varieties respectively based on the reproduction factor calculated. There was a significant difference (P >0.05) between Small fry and Petomech on the mean number of eggs.

Tomato Genotype	Mean Number Of Eggs (Transformed*)	Mean Gall Score (0 -10)#	Reproduction Factor (Rf)	Reaction
CSIR-CRI 123	56.65	6.75	2.46	S
CSIR-CRI 115	43.70	7.00	2.58	S
CSIR-CRI 76	54.00	8.00	2.63	S
CSIR-CRI 48	51.20	5.50	2.33	S
CSIR-CRI 57	41.15	6.00	2.10	S
CSIR-CRI 153	38.35	7.00	2.14	S
CSIR-CRI 94	50.15	6.25	2.32	S
CSIR-CRI 102	57.00	5.25	2.47	S
CSIR-CRI 109	37.10	6.25	2.38	S
CSIR-CRI 83	53.15	7.00	2.19	S
CSIR-CRI 86	63.20	6.75	2.28	S
CSIR-CRI 54	41.25	6.00	2.52	S
CSIR-CRI 50	57.40	6.50	2.28	S
CSIR-CRI 74	38.00	7.00	2.61	S
CSIR-CRI 79	26.15	5.00	2.43	S
CSIR-CRI 154	56.80	8.25	2.51	S
CSRI-CRI 150	43.85	7.55	2.45	S
CSIR-CRI 23	50.00	5.75	2.50	S
Cherry tomato	19.15	3.25	0.98	R
Tomato Petomech CEE	45.65	8.00	2.69	S
Big Beef	23.60	4.50	1.36	M/R
Jet Setter	26.75	4.00	1.64	S
LSD (5%)	10.50			
CV (%)	16.87			

Table 1: The Effect of Root Knot Nematodes on Number of Eggs, Root Galling and Meloidogyne Reproduction on 22 Tomato Genotypes Eight Weeks after Inoculation
 # 0=No Knots on Roots, 10=All Roots Severely Knotted
**$\sqrt{X+0.5}$transformed X= Mean Egg Number*
® Rf= Final Egg Number/Initial Egg Number RF <1= No Reproduction, RF>1= Reproduction
R= Resistant, MS= Moderately Resistant and S= Susceptible

The means of gall index of the genotypes are recorded in Table 2. The greatest number of root galls was obtained in tomato variety Petomech (7.7). The lowest was however, recorded in Small Fry and F_2 tomato genotypes. Petomech was significantly different from Small Fry and F_2 tomato genotypes. The means of juveniles, gall index, nematode population are presented in table 1. The mean number of juveniles recovered from the roots of the three tomato genotypes ranged from 18.6 to 33.6. Petomech recorded the highest number of juveniles which was significantly different ($P < 0.05$) from Small Fry and F_2. However, Small Fry was not significantly different ($P > 0.05$) from the F_2. Similarly, there was a significant difference ($P < 0.05$) between Petomech and Small Fry on root galling. There was however, no significant difference ($P > 0.05$) between Small Fry and F_2. Moreover, Petomech was significantly different ($P < 0.05$) from Small Fry and F_2 regarding reproduction factor. Nevertheless there was no significant difference between Small Fry and F_2. All the tomato genotypes showed great variation in response to root knot nematode. Small fry and F_2 recorded a reproduction factor of about 1 and were resistant and moderately resistant respectively and Petomech however, recorded a reproduction factor greater than 1 and was therefore considered as susceptible(Table 2).

Tomato genotype	Mean number of eggs (Transformed*) / 5grams chopped root weight	Mean number of Juveniles (Transformed)*	Mean Fresh Root Weight (g)	Mean gall score (0 -10)#	Reproduction factor (Rf)	Reaction
Petomech	53.6	33.6	7.9	7.8	2.29	S
Small fry	24.6	18.6	3.3	2	1.01	R
F_2	28.6	20.7	3.6	2.4	1.12	M/R
LSD (5%)	10.6	10.6	0.9			
CV (%)	19.5	19.5	20.6			

Table 2: The Effect of Root Knot Nematodes on Three Tomato Genotypes Eight Weeks after Inoculation
 # 0=No Knots on Roots, 10=All Roots Severely Knotted
**$\sqrt{X+0.5}$transformed X= Mean Egg Number and Juveniles*
® Rf= Final Egg Number/Initial Egg Number Rf<1=No Reproduction, Rf>1=Reproduction
R=Resistant, MS= Moderately Resistant and S=Susceptible

4. Discussion

The greater number of egg masses per root system was obtained in tomato genotype Petomech followed by F_2. The minimum was however, recorded in tomato genotype Small Fry. This shows that Petomech which had the highest mean number of eggs was susceptible to root knot nematode. Lawrence and Clark (2009) observed that the number of females, root-galls and egg masses were increased on susceptible cultivar inoculated with M. incognita.Similar results have been reported by Darban et al. (2003) and Pathan et al. (2004), while artificially inoculating the different tomato varieties with M. incognita under pot and field conditions. This observation is in agreement with El-Sheriff et al. (2007) who studied the effect of population densities of M. incognita race 1 on the yield of tomato and found that maximum number of nematode juveniles were recorded at moderate population density and at very high population densities, the reproduction potentials of root-knot on the plant declined, because the population in the root system reached its peak and could not support further reproduction. Genotypes which gave the least number of eggs could be attributed to the fact that their root exudates exerted a suppressive effect on root-knot nematode reproduction as reported by Vicente and Acosta, 2007. The highest fresh root weight can be attributed to damage caused by root-knot nematode. This is in accordance with what El-Sherif et al. (2007), who reported that root-knot nematode increases root weight for the most vulnerable cultivar compared to resistant cultivar. This is because root-knot functions as metabolic sinks related to an emerging fruit as nutrients formed in the leaves are re-distributed rapidly to the roots and into the bodies of the nematodes. According to Hunt et al. (2005), root-knot nematodes establish specialized feeding cells in roots, redirecting photosynthate produced in the leaves to supply the energy demands of the nematode in the roots.

Root weight of susceptible cultivar as a result of nematode parasitism increases whereas shoot weight declines, affecting the root shoot balance (Roberts, 2004). The presence or absence of root galls on tomato plants indicates, whether a variety is resistant or susceptible to root-knot nematodes. This was evident in tomato genotype Petomech which gave the largest mean gall score. Variations in the number of galls present on roots can be attributed to different levels of susceptibility. Thus, the above observation is in accordance with Khan (2004) who indicated that the development of galls on plant roots increased significantly on the susceptible genotypes compared with resistant genotypes thereby affecting plant performance. The presence of galled roots leads to modification in absorption of water and nutrients from soil and their translocation to foliage, resulting in foliage chlorosis and stunting of vegetative growth (Bala, 2004). The arrested root system could not be able to fully explore the soil for water and nutrients (Clark et al., 2003). However, root galling is not a

satisfactory indicator of the durability of root-knot nematode resistance (McClure et al., 1994); Root-knot nematodes juvenile reproduction on the three tomato genotypes varied from each other. The occurrence of this variation among the three tomato genotypes might be due to genetic differences (Brow et al., 1997; Ehlers et al., 2002). The highest number of juveniles which was found on tomato genotype Petomech is in accordance with El-Sheriff (2007), who indicated that roots of susceptible genotypes are found to be more favorable to root-knot nematode activities and promote reproduction and survival of juveniles. The highly susceptible genotypes supported the greatest number of juveniles that penetrated and completed their development to maturity as shown by high gall numbers and egg masses present, while in tolerant and resistant cultivars limited numbers of juveniles were able to penetrate, develop to maturity and lay egg masses. Therefore, more juveniles were identified on susceptible genotypes compared to the resistant genotype. Furthermore, Chen and Dickson (2004) reported that the susceptibility of a plant to root-knot nematodes depends on the ability of nematode juveniles to penetrate the roots of the plant and cause the formation of huge cells which appear as galls on the roots. This was buttressed by Karssen and Moens (2006) who described that highly susceptible host plants allow the juveniles to enter the roots reach maturity and produce many eggs while the resistant plants suppress their development and thus, do not allow reproduction. Khan, (1994) also reported that root knot nematode juveniles develop poorly on the resistant accessions as compared to susceptible accessions. The study established that a total of 1500 eggs/tomato plant was found as the optimum initial inoculum level for screening for resistance in tomato to Meloidogyne spp.Petomech recorded the highest number of juveniles, eggs and also scored the maximum gall index while Cherry (Small Fry) tomato recorded the least number of juveniles and eggs.The F_2 genotype with GI>2 and R_f (1.12) was moderately resistant to root-knot nematodes with good horticultural attributes. Financial and time constrains could not allow for further backcrosses to be done on the F2 until full resistance is achieved ,therefore further crosses on the F_2 with the recurrent parent should be carried out until nematode resistance is completely achieved.Tomato genotype Small fry can be incorporated into other breeding programmes for root- knot nematode resistance.

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