

Supplementary material

Protocol for DNA extraction of *Fusarium oxysporum*

For morphological and biological identification, infected root tissues of tomato were rinsed thrice with water and disinfected with 5% sodium hypochlorite solution (NaOCl) and washed with sterilized water, prior to cut aseptically into 2 cm pieces. Four pieces of samples placed into PDA medium plates and incubated for 7 days at 28 °C. After 7 days of incubation the isolate was investigated macroscopically (white to pinkish cottony growth of mycelium) and microscopically (sickle shape conidia with 3-4 septation) under microscope (Nikon Eclipse 80i equipped with NTS-Element F 3.0) following the key of identification of *Fusarium* spp [1].

For DNA extraction *Fo* mycelial was cultured on potato dextrose broth (PDB) ref and incubated for 48 hrs at 28 °C under shaking condition (200rpm). Mycelia was harvested and washed with sterilized water and ethylenediamine tetra acetic acid (EDTA). The DNA was extracted by cetyl trimethylammonium bromide (CTAB) method briefly; Frozen mycelia was grounded in sterilized pestle and mortar with 120 µl of CTAB. After fine grinding, powdered mycelia and 200 µl of CTAB added in 1.5mL eppendorf tubes were incubated at 65 °C for one hour. Tubes were centrifuged for 10 mins at 12000rpm and 800 µL, supernatant was taken in new 2mL tube with Chloroform:Phenol:Isomyl alcohol (24:24:1) and again centrifuged for 10 mins at 14000 rpm. In next step, 700 µL supernatant taken from the tube and add 700 µL of Chloroform:Isomyl alcohol (24:1) and again centrifuged for 10 mins at 14000rpm. This step was repeated three times. The 400 µL transparent supernatant was collected in tube with isopropanol and incubated for 1.5 hr at -20 °C. After incubation DNA pellet was precipitated at bottom by centrifugation and washed with 70 % ethanol. The washed DNA was suspended in 50 µL double distilled water and quantified with ThermoFisher Quawell Q3000 UV spectrophotometer.

Amplification of fungus DNA was performed in PCR by using ITS primer [58]. For the performance of PCR reaction a volume of 50 µl contained (5 µl 10X reaction buffer; 2.5 mM MgCl₂; 200 µM each of dNTP, 2.5 units of Taq DNA polymerase (CinnaGen, Tehran, Iran), 30 ng template DNA and 50 pmol of primer. The first denaturation step in a thermocycler was for 5min at 94 °C followed by 35 cycles at 94 °C for 30 sec, annealing was done at 56 °C for 1 min, and extension at 72 °C for 2 min and a final extension at 72 °C for 8 min. Amplified PCR products were electrophoresed on an agarose gel (1.5% w/v) in 1X TAE buffer at 100 V for 50 mins and stained with ethidium bromide [2] [3,4]. The ITS primer successfully amplified the DNA fragments. The Nucleotide was a blast on NCBI and confirmed its spp.

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Screening of Tomato cultivars

Tomato Genotypes

Twelve tomato F₁ commercial lines which are highly productive taken from CAAS China Vegetable seed Technology Co., Ltd. Beijing used in this experiment. Moreover, these lines are screened against *Fo* and *Mi*. Among them we choose one susceptible and one resistant advanced line for further analysis. The tomato seeds were surface sterilized with 5 percent NaOCl solution for ten seconds, after that washed twice with distilled sterilized water than dried for sowing in Nursery preparation.

Table S1. Tomato cultivars used for screening against *Fusarium oxysporum* and *Meloidogyne incognita*

S/N	Tomato Variety	Source of seeds	Origin	General Characteristics
1.	Zhongza 201	China Vegetable Seed Technology Co., Ltd. (Beijing)	China	Red round fruit, 95% germination rate, heat resistant
2.	Zhongza 109	China Vegetable Seed Technology Co., Ltd. (Beijing)	China	Round fruit, high yield, 95%germentation rate
3.	Zhongza 09	China Vegetable Seed Technology Co., Ltd. (Beijing)	China	Red round fruit, Heat resistant, Fusarium wilt resistance,
4.	Ji shi	China Vegetable Seed Technology Co., Ltd. (Beijing)	Israel	Hard fruit, Disease resistance, high quality & yield
5.	Gailing maofen 802	Xian jiaxin seed industry Co.,Ltd	Xian China	Wool type pink fruit, hard skin, strong resistance to wilting ToMV CMV & stress
6.	Zhongyan	Xian jiaxin seed industry Co.,Ltd	Beijing China	Pink color short, oblong cherry tomatoes, TY, Root knot nematode resistance
7.	Touch healthy	Beijing Zhongyanyinong Seedling Co., Ltd	Shang ai China	Highly disease and heat resistant with 85% germination rate super fruit quality
8.	Red fruit 808	Detian Co., Ltd	China	Top ranking quality and production particularly in south china, Fo wilt resistant
9.	Naite-3 F1	Beijing Zhongyanyinong Seedling Co., Ltd	China	Heat resistance and pink color fruit, heat resistant
10.	Cherry tomatoes	Beijing Zhongyanyinong Seedling Co., Ltd	China	TYLCV ToMv and wilt and heat resistance
11.	Maofeng 202	Baofeng Quality Seed Co., Ltd	China	Good quality seed, Heat and wilt resistant
12.	Xin Bite 2 F1	Baofeng Quality Seed Co., Ltd	China	Good germination rate, large fruit size

Tomato seeds of each variety was surface sterilized with (1.0% NaOCl for 1 min) and sown in pot containing 1kg autoclaved peat moss. After 30 days of seedlings germination each variety was inoculated with 1500 J2

of *Mi* by making three holes around the roots at the same distance except control. Same varieties were also screened against *Fo* by inoculation spore suspension 1×10^5 CFU mL⁻¹ in soil around the roots except control. The experiment was repeated 4 times and each treatment have 20 replicates. Data was recorded after 35 days of *Mi* inoculation.

For screening against *Mi* galls on each root system were scored on a scale of 0–10 (no damage to severe damage), using the severity rating chart by Bridge and Page [5]. The mean gall scores (GSs), egg count per gram of root, and reproductive factors (Rfs) obtained were used as the basis to evaluate the susceptible/resistant status of the rootstocks. Rootstocks were classified as resistant when their root GS < 2 and Rf < 1, tolerant when GS < 2 and Rf ≥ 1, or susceptible when GS ≥ 2 and Rf > 1 [6]. A screening test was also performed to assess susceptible or resistant varieties of tomato against *Fo*. Disease rating scale was made on the basis of disease incidence and disease severity. To calculate the Fusarium wilt disease index we adopted a grade scale, ranging from 0 to 4 where: HR= plants with no symptoms, R = 1 to 25 % leaves shows symptoms of wilting or yellowing, MR = 25 to 50 % leaves with intense wilting or yellowing, MS = 50 to 75 % severely wilted plants, associated with leaf yellowing and necrosis, S = 75 to 100 % leaves shows severe wilting and necrosis, HS = complete plant death. Disease incidence was observed as the symptoms appeared on the plant, and calculate by this formulae:

Disease Incidence (%)= Number of plants showing disease symptoms/ Total number of plants × 100

Results

Table S2. Screening of tomato cultivars against *Fusarium oxysporum* and *Meloidogyne incognita*

S/N	Tomato Variety	<i>Fusarium oxysporum</i>	<i>Meloidogyne incognita</i>
1.	Zhongza 201	Highly susceptible	Susceptible
2.	Zhongza 109	Highly susceptible	Susceptible
3.	Zhongza 09	Resistant	Susceptible
4.	Ji shi	Moderately susceptible	Susceptible
5.	Gailing maofen 802	Moderately resistant	Susceptible
6.	Zhongyan	Susceptible	Susceptible
7.	Touch healthy	Highly susceptible	Susceptible
8.	Red fruit 808	Moderately susceptible	Susceptible
9.	Naite-3 F1	Moderately susceptible	Susceptible
10.	Cherry tomatoes	Resistant	Resistant
11.	Maofeng 202	Susceptible	Susceptible
12.	Xin Bite 2 F1	Highly susceptible	Susceptible

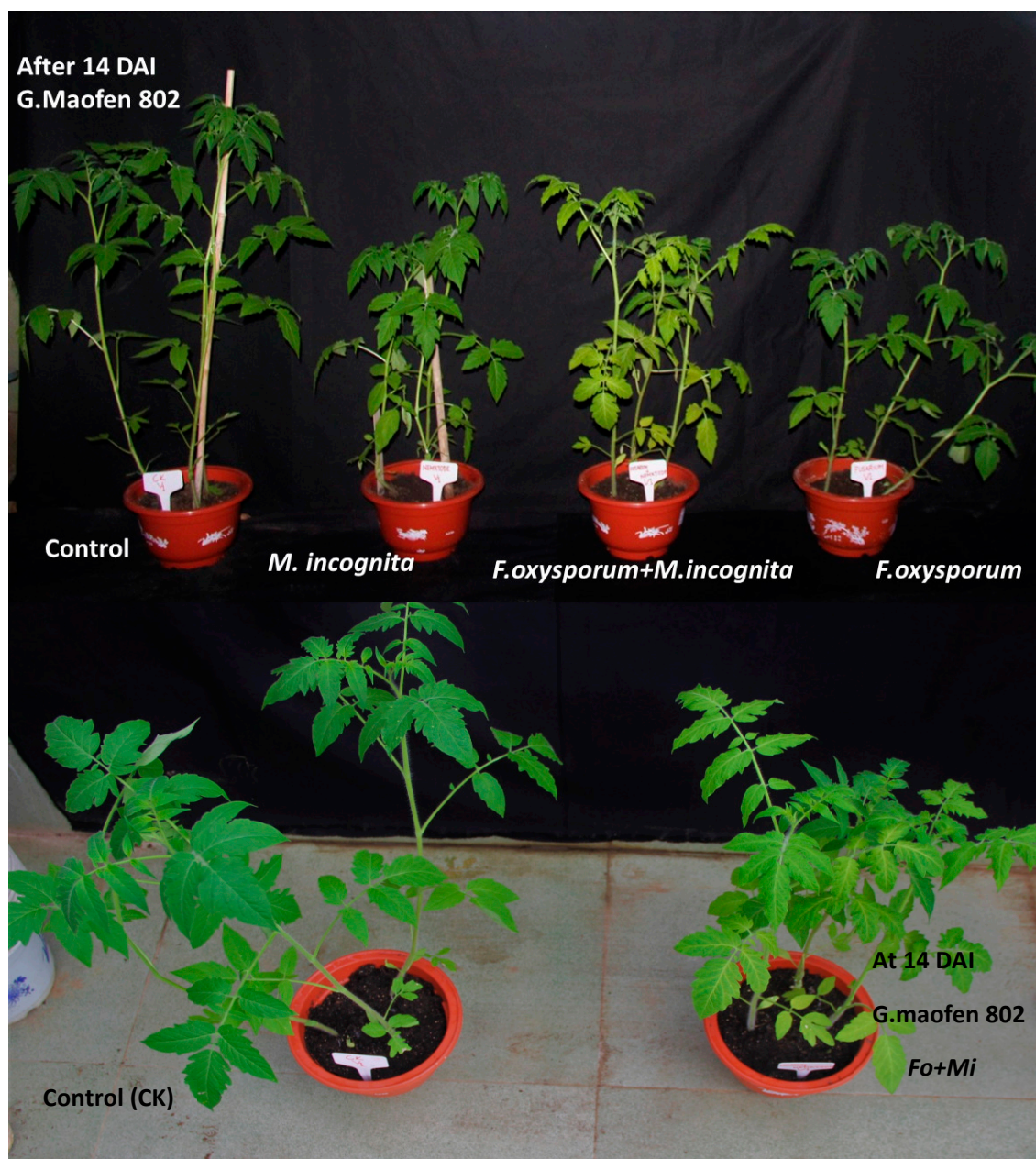


Figure S1: Effect of *Fusarium oxysporum* and *Meloidogyne incognita* on foliar part of G.maofen 802 at 14 DAI.

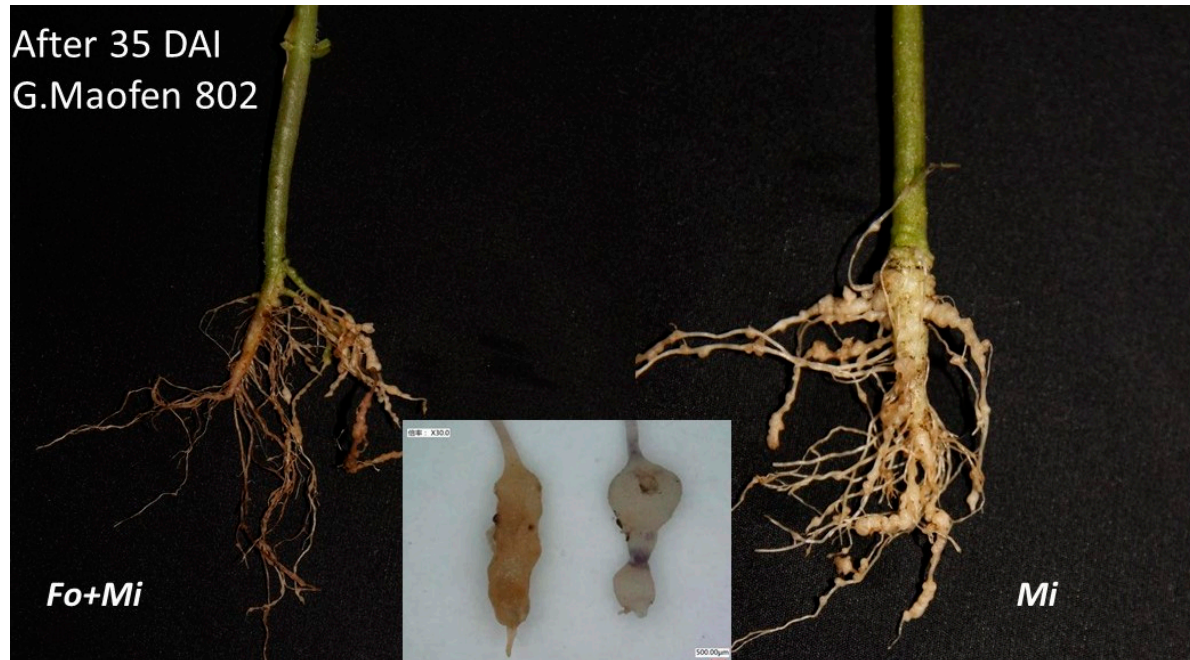


Figure S2: Effect of *Fusarium oxysporum* and *Meloidogyne incognita* single and combine application on roots of *G. maofen 802* after 35 DAI.