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Investigation for Resistances to *Phelipanche Ramosa* l. Among research Tomato Genotypes

Rida DRAIE [abc]

[a] Faculty of Agriculture, Idleb University, Syria

[b] Research work carried out at Laboratoire de Biologie et Pathologie Végétales (LBPV), Université de Nantes, France

[c] Email: ridadraie@hotmail.com

Abstract

Invasion of tomato culture by *Phelipanche ramosa* is growing now. The broomrape is completely dependent on the host-derives and consecutively competes with the sink organs of infected plants. No capable and cost-effective manage means has been established to date. In this work, we screen fifteen research tomato genotypes for their resistance to branched broomrape. In the greenhouse conditions, we show that genotypes are different in the level of susceptibility to broomrape. Attachment number, emergence number, and fresh matter of parasites are affected by tomato genotype. A major influence of the parasitism onto the dry weight of all infected tomato genotypes with changeable scale is found. 1052791 that has fewer of attachment and emergence number and dry mass shows attractive for our purpose.

Keywords

Research Tomato Genotypes, Screening, Resistance, Tolerance, *Phelipanche ramosa*.

Introduction

Infestation of tomato fields by branched broomrape (*Phelipanche ramosa* L.) is rising in the world and specially in Mediterranean basin. Tomato is the mainly affected produce being the famous host of this parasite. Yield losses in tomato fields can reach to 75% in heavy-infested fields (Tóth and Cagán, 2003). The broomrape causes a significant reduction of tomato photosynthetic capacity, thus generating a significant loss of their aerial organs biomass (Mauromicale *et al.*, 2008). The risk of this parasite comes from the long viability of its tiny seeds in the ground, which exceeds at least the ten years, and of its very high rate of multiplication (Cubero, 1983). Moreover, the majority of the harm on the host is caused during the underground growth of the parasite and no practical methods to control it successfully (Gressel *et al.*, 2004).

Broomrapes are obligate parasitic flowering plants which depend entirely on their host plants for water and nutritional requirements (Parker and Riches, 1993). After connecting to the host root vascular system through a *haustorium* acting as a bridge for water and nutrient uptake, the parasite first undergoes a subterranean growth resulting in tubercle and subterranean stem development. This is followed by emerging above soil, flowering and producing a large number of seeds. Broomrape seed germination starts in response to stimulants secreted from roots of host plants (Barcinsky, 1934; Chabrolin, 1934). However seeds respond to stimuli only after being exposed to water and suitable temperatures for several days, corresponding to the conditioning period (Joel *et al.*, 1995).

Some combined methods based on cultural, biological, physical, and chemical means are proposed to control broomrape in processing tomato (Montemurro *et al.*, 2006). Certainly good results were obtained by solarization (Abu-Irmaileh, 1991) and by the integration of trap crops as they stimulate broomrape seed germination but are not infected, then reducing the broomrape seed bank of the soil. Biocontrol with either *Phelipanche*-pathogenic *Fusarium* strains or mycoherbicides is also promising (Boari and Vurro, 2004). Nevertheless the most successful method to control the parasitic weed broomrape in processing tomato is to apply sulfonylurea herbicides on tomato foliage and by injection

through the drip irrigation system (Eizenberg *et al.*, 2003). In parallel, there is an increasing market for organically grown tomatoes, where the use of chemical pesticides is not an option (Lopez-Pérez *et al.*, 2006).

The genetic approach getting resistance in tomato is unsuccessful until now as no immunity against broomrape was observed from the large-scale screenings in tomato germplasm (Dalela and Mathur, 1971; Abu-Gharbieh *et al.*, 1978; Foy *et al.*, 1987, 1988; Kasrawi and Abu-Irmaileh, 1989; Qasem and Kasrawi, 1995). The observed resistance is multifactorial since it rests on a limited number of broomrapes emerged by tomato plant, a very weak stimulative activity of the root exudates, an induction of necroses broomrapes fixed, a parietal reinforcements by lignification, and an occlusion of xylemian vessels (El-Halmouch *et al.*, 2006).

The research of resistance or tolerance in tomato remains a priority. Because genetic variability available at cultivated tomato is very limited, the search for sources of resistance to the broomrape at the wild species, which have a genetic diversity 10 times higher, can be an interesting solution (Miller and Tanksley, 1990; Causse *et al.*, 2000). The present work aimed to screen fifteen tomato genotypes against branched broomrape under artificial infestation in controlled environmental conditions, revealing their respective degree of resistance to the parasitic weed. Susceptibility was evaluated using the total number and dry weight (DW) of attached broomrapes per host plant and the number of emerged broomrape shoots per host plant as indicators. Resistance was estimated according to the impact of parasitism on DW biomass of the host plant.

Materials and methods

Plant materials

Fifteen tomato research genotypes from Vilmorin (1052723, 1052724, 1052727, 1052728, 1052761, 1052768, 1052775, 1052776, 1052778, 1052786, 1052788, 1052789, 1052791, 1052794, 1052796) were tested for their susceptibility to branched broomrape.

The broomrape seeds were collected from flowering spikes in infested rapeseed fields (pathovar C, Saint-Martin-de-Fraigneau, Vendée, France, 2005). Once cleaned, the seeds were stored in darkness at 25°C until use.

Germination tests

The ability of broomrape seeds to germinate was evaluated in the presence of the synthetic germination stimulant GR_{24}. Broomrape seeds (5 mg DW) were sterilized for 5 min in sodium hypochlorite (3.61%) and rinsed three times with sterile distilled water. The sterilized seeds were conditioned at 25°C for 1 week on filter paper moistened with 5 mL of sterile distilled water in a Petri dish (Ø 9 cm) covered with aluminum foil. Germination was then stimulated by addition of 1 mL of GR_{24} (1 ppm), a synthetic strigol analogue (natural stimulant). Four days after the conditioning period, seed germination rate was estimated by adding of 1 ml of pansso red, and then the germinated seeds were counted under a binocular microscope (Olympus SZX10).

Interactions tomato-broomrape in pot (Screenings in greenhouse)

Broomrape seeds (10 mg L^{-1} of soil) were mixed homogeneously with a 1:1:1 peat–sand–clay mixture in a pot of 3L (about 10000 seeds pot^{-1}). Cultures were managed in greenhouse conditions. Ten infested and ten non infested pots were equipped per tomato rootstock (n=10). The soil infestation by broomrape seeds then the mixture homogenization were carried out manually in each pot. The pots were watered and protected from the light with a black plastic film then maintained in this conditions for 1 week at 20-25°C (day-night temperature). Following broomrape seed conditioning, three tomato rootstocks seeds were sown directly in each pot. The plants grew under a photoperiod of 16h (300 µmol m^{-}

^{2} s^{-1} PAR) and at 20-25°C and 15-18°C (day-night temperature). Three weeks later seedlings were thinned to one per pot. The tomato rootstocks were sprinkled one time per week with a sterile solution of 50% Coïc neutrophile nutrient solution (Coïc and Lesaint, 1975). The tomato plants were addressed, propped and the greedy removed progressively with their development.

Two independent culture campaigns were performed. The first one was aimed to screen the fifteen research tomato genotypes. The second screening campaign was duplicated the first campaign. Nevertheless, from the results of the first campaign, the less interesting rootstocks were excluded from the second culture campaign.

Collecting of experimental data

Four month after sowing, tomato plants were gently uprooted from the soil. The broomrapes (attached and emerged) are collected, washed carefully then classified according to their stage of development (Labrousse *et al.*, 2001). stage 1: attachment of the germinated broomrapes to the host roots; stage 2: tubercle formation; stage 3: appearance of adventive roots on the parasite tubercle; stage 4: stem formation and development; stage 5: broomrape emerged in flowers and fructification; stage 6: faded flowers - mature capsules (Figure 1).

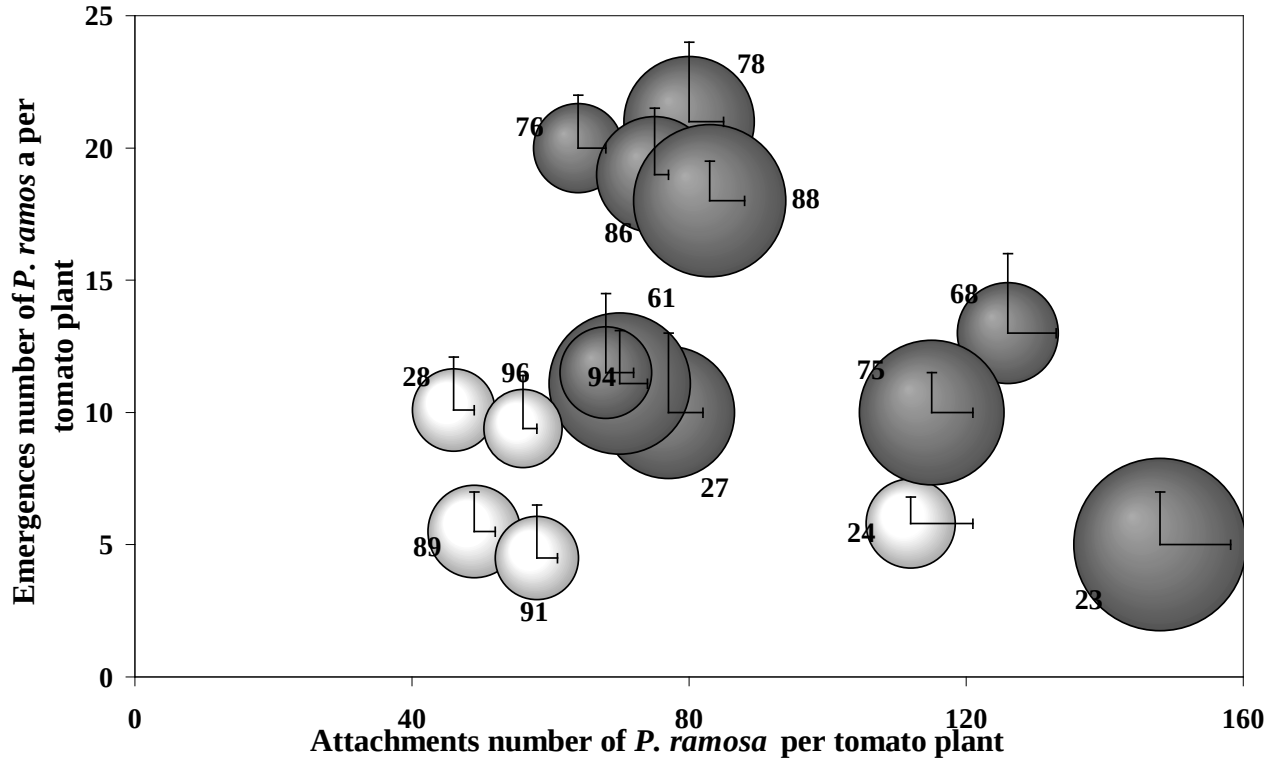

Figure 1. Evaluation of the sensitivity to the branched broomrape of various research rootstocks (Vilmorin, 10527XX).
The values are the averages ± SE (ANOVA, SNK, P≤0.05, n=10). The size of the circles corresponds to the total dry mass of the broomrapes fixed by tomato plant. **In gray**: the rootstocks least sensitive to the broomrape. The behavior of these genotypes will be revalued during the screening campaign of second series. **In white**: rootstocks very sensitive to the broomrape. They will not be revalued in second series.

The degree of rootstocks susceptibility was evaluated by the total number of fixed broomrapes per plant (Sillero *et al.*, 1996b), by the number of emerged broomrapes per plant (Gil *et al.*, 1984; Gil *et al.*, 1987; Cubero, 1991; Snelder *et al.*, 1994; Qasem and Kasrawi, 1995; Sillero *et al.*, 1996a), and by the total dry biomass of broomrapes per plant (El-Halmouch, 2004). In parallel, the impact of parasitism on the rootstocks was estimated by measurement of the fresh and

dry aerial parts and roots biomass of the infected and healthy plants. The biomasses were measured after drying of the samples at 80°C for 72h (El-Halmouch, 2004; Abbes, 2007).

Statistical analysis

Statically analyses were performed using the SigmaStat software (version 3,5). The comparisons of average were based on the test of Student Newman Keuls (ANOVA, SNK, P≤0,005, n=10).

Results and Discussion

The grafting of tomatoes onto vigorous rootstocks made it possible to improve the yield considerably (Augustin *et al.*, 2002; Pogonyi *et al.*, 2005). Moreover, the tomato grafting onto abiotic stresses tolerant rootstocks (extreme temperatures, salinity, moisture…) was also effective to reduce the damage caused by these environmental constraints (Black *et al.*, 2003; Fernandez-Ballester *et al.*, 2003; Ruiz *et al.*, 2005; Colla *et al.*, 2006). In the same way, the grafting onto resistant rootstocks became a very effective technique to manage the telluric parasites such as fungi, bacteria, viruses and nematodes (Scheffer, 1957; Pavlou *et al*., 2002; Giannakou and Karpouzas, 2003; Rivero *et al*., 2003; Bletsos, 2005).

The attacks of broomrape constitute a major factor limiting tomato productivity in the Mediterranean basin. Several methods (chemical, biological, farming…) were proposed to manage this parasite, but the majority among prove to be insufficient to control this adventitious parasite. To our knowledge, the interest of the grafting to manage the broomrape was not evaluated.

Thus, this study proposes to evaluate the behavior of many rootstocks resistant to the telluric parasites with respect to the branched broomrape. therefore, the problems posed are to know if the resistances carried by the rootstocks are effective against the broomrape. In this part, the impact of parasitism on the development of the rootstocks will be also given.

All the tomato research genotypes (provided by Vilmorin Company) were screened in greenhouse for their resistance to branched broomrape during two programs independent of screening.

First series of screening

After four months of culture, the various genotypes of research (1052723, 1052724, 1052727, 1052728, 1052761, 1052768, 1052775, 1052776, 1052778, 1052786, 1052788, 1052789, 1052791, 1052794 and 1052796) were uprooted. Their sensitivity to the broomrape was evaluated according to next indices: the total number of attachments, the total number and percentage of emerged broomrapes, the broomrape dry-weight per tomato plant, and the reduction of roots and vegetative part in dry matter of tomato plant in response to the parasitic attack.

None of the research rootstocks is immune to the parasite. These genotypes separate in several groups (ANOVA, SNK, P≤0.05, n=10). The first group comprises only rootstock 1052723 (150 attachments). The second group is composed of rootstocks 1052724, 1052768 and 1052775 with 115 attachments per plant on average. The rootstocks remaining set up five overlapping groups, with a number of attachments per plant going from 46 to 83 attachments. To simplify, the research rootstocks seem to be able to line up in three groups of sensitivity to the broomrape (thresholds: 150, 115 and 65 attachments on average). The great majority of the rootstocks make the group with 65 attachments per plant on average, (Figure 1).

In the same way, all the research rootstocks make it possible the parasite to emerge. Nevertheless, some appear ready to support the development of the parasite in post-fixing than of others. Thus, the percentage of emergences per plant discriminates five groups of rootstocks (ANOVA, SNK, P≤0.05, n=10). The first group is only made up of the rootstock 1052723 which is characterized by a very small percentage of emergences (3%). The second group comprises rootstocks 1052768, 1052789, 1052724, 1052791 and 1052775 (9% on average). The third group is composed of rootstocks 1052727, 1052761, 1052796 and 1052794 (16% on average). The fourth group comprises rootstocks 1052788, 1052728, 1052786 and 1052778 (23%). Lastly, the last group is consisted of the rootstock 1052776 (31%). Overlapping exist between groups 2 to 5, (Figure 1).

The total dry mass of the fixed broomrapes also discriminates five groups of rootstocks, with a biomass of broomrape going on average from 0.58 to 1 g MS per tomato plant, (Figure 1).

From the whole of these results, rootstocks 1052628, 1052689, 1052691 and 1052696 are not very sensitive (low number of broomrapes fixed per plant) and do not support the emergence of the parasite (reduced percentage of emergences). Their behavior will be revalued at the time of the last screening campaign. It will be the same for the rootstock 1052624 which increases on the contrary a very great number of broomrapes whose great majority do not have emerged after 12 weeks of culture.

Second Series of screening

Evaluation of the sensitivity degree of the selected rootstocks

This revaluation campaign of the sensitivity degree of the research rootstocks (selected genotypes) is based on the same indicators of sensitivity as previously. Moreover, the measurement of fresh and dry roots and aerial parts masses of the tomato plants after sampling of the broomrapes will contribute to a finer characterization of the tomato genotypes tested. Realized on plant control not infested, these measurements will also make it possible to evaluate the impact of parasitism on the development of the rootstock.

Initially, this second screening campaign confirms the respective sensitivity of the research rootstocks selected for this revaluation (Figure 2). By taking account of the attachments number per plant, the collation of the rootstocks is actually respected compared to the first series of screening. On this whole of research rootstocks, the index "number of broomrapes fixed per plant" discriminates two groups of rootstocks (ANOVA, SNK, P≤0.05, n=10). The first group is composed of 1052728, 1052789, 1052791 and 1052796, with an average 67 broomrapes fixed per plant. The second group comprises only of 1052724, with an average of 138 attachments per plant.

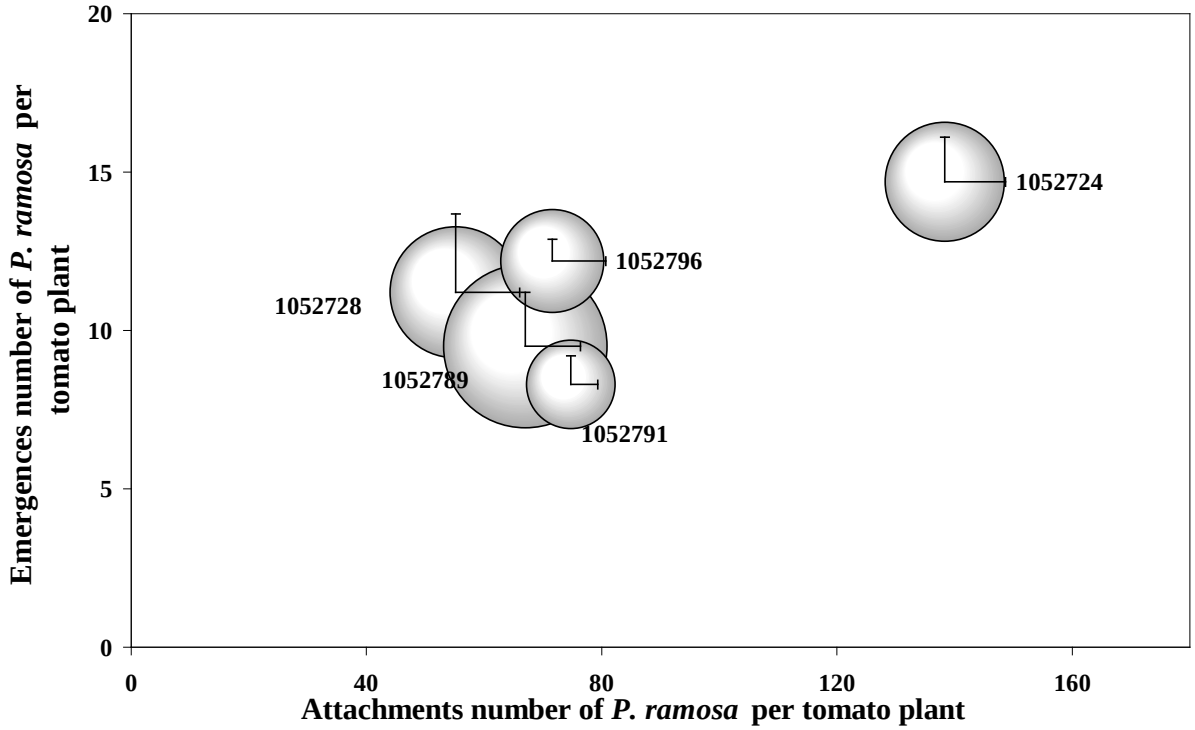

Figure 2. Revaluation of the degree of sensitivity of selected research rootstocks (Vilmorin).
The values are the averages ± SE (ANOVA, SNK, P≤0.05, n=10). The size of the circles corresponds to the total dry mass of the broomrapes fixed per tomato plant.

In the same way, this second screening campaign confirms the behaviors characteristic of the research rootstock 1052724 in term of "emergence of the parasite". Thus, the index discriminates clearly this rootstock (15 emergences; ANOVA, SNK, P≤0.05, n=10) of the other rootstocks. The latter are distributed in only one group, with 10 broomrapes emerged per tomato plant.

1052724, 1052728 and 1052789 are the rootstocks having the highest mass of broomrapes (1 g MS per plant). Thus, a strong proportion of the broomrapes fixed on this rootstock is emerged, from where a significant weight of broomrapes. The other rootstocks (1052791, 1052796) line up in a second group (0.6 g MS; ANOVA, SNK, P≤0.05, n=10).

Estimation of the two screening campaigns

The sensitivity degree of the rootstocks can be given by realizing the results of the two screening series (Table 1).

Table 1. Average values of the various sensitivity indices of the rootstocks calculated following two screening campaigns.
The values are the averages of twenty plants per rootstock (n=20). By index, the values carrying the same letter are not significantly different (ANOVA, SNK, P≤0.05, n=20)

Rootstock	Attachments Number	Emergences Number	Dry Matter of Broomrapes (g MS)
1052724	125,9[a]	10,3[a]	0,7[a]
1052728	50,6[b]	10,7[a]	0,7[a]
1052789	58,0[b]	7,5[b]	0,8[a]
1052791	66,4[b]	6,4[b]	0,6[a]
1052796	63,8[b]	10,8[a]	0,6[a]

Among all research rootstocks tested, 1052724 is the rootstock least sensitive to the broomrape, with an average of 126 broomrapes fixed per plants of which 8% an average emerged after four months of culture. Consequently, the total mass

of broomrapes carried by this genotype is low (0,7 g DM), from where an average mass of 6 mg DM per broomrape. In addition, this rootstock of research is distinguished from the four other genotypes of research. The latter shows characteristics significantly indifferent: an average 60 broomrapes fixed per plant, 9% of emergences, 11 mg DM per broomrape (0,7 g DM broomrape per plant).

Influence root mass of rootstocks on their degree of sensitivity to the broomrape

The root mass of rootstock tends to condition the number of fixed broomrapes (Figure 3, R^2=0.5). Thus, these rootstocks present an average 25 broomrapes fixed per g MS of roots. The size of the root system influences at the same time the surface of contact with the parasite and probably the intensity of production of the germination stimulants of seeds broomrape.

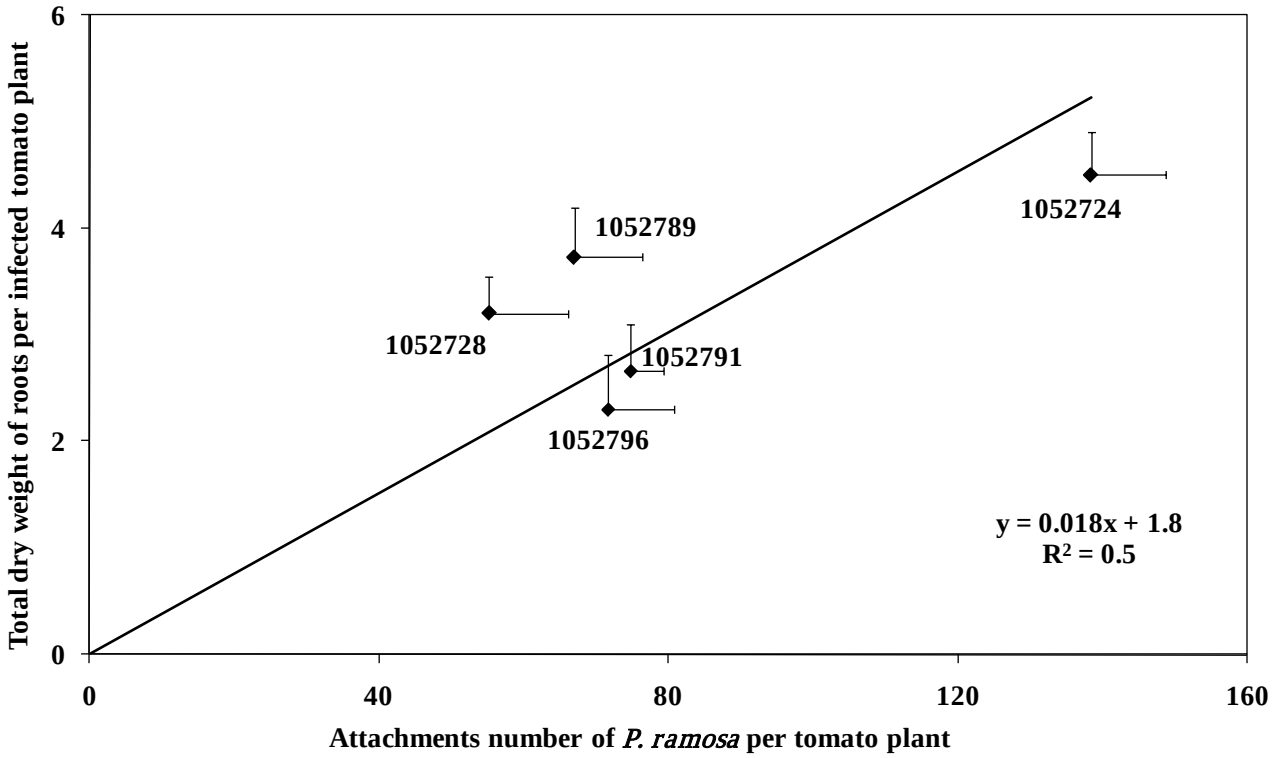

Figure 3. Relation between the total dry mass of the roots of the tomato rootstocks and the number of broomrapes fixed per plant. The data correspond to the averages ± SE (n=10).

Impact of parasitism on rootstocks development

The negative impact of parasitism on the total dry weight of rootstocks tested in second screening campaign is presented in Figure 4. It appears that the total loss of dry weight of a rootstock is correlated positively with the total dry weight of the fixed broomrapes. Thus, the highest loss of dry weight is observed for the 1052728 genotype. An average, 1g of broomrape dry weight causes a loss of 23g dry weight for the rootstock. These results confirm that the broomrapes act like additional wells for the rootstocks and show that none of the rootstocks tested compensates for the diversion of dry weight by the parasite.

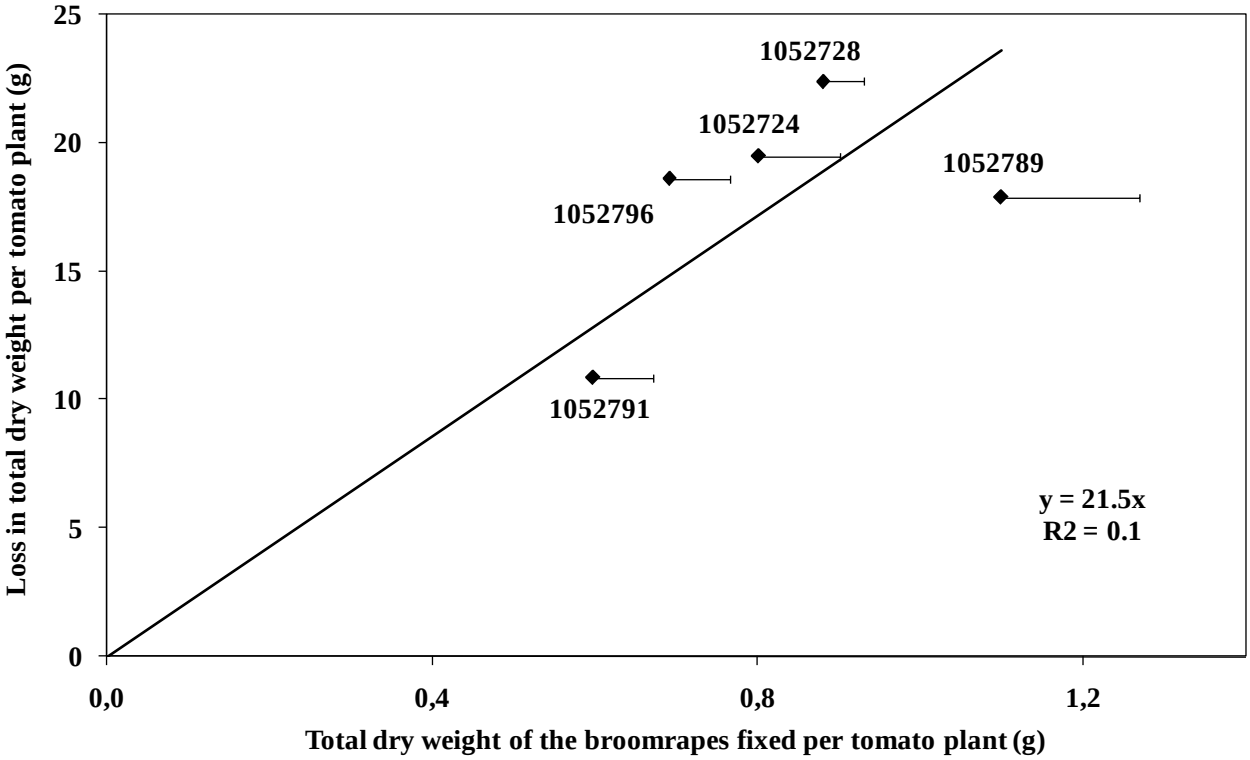

Figure 4. Correlation between the loss in total dry weight of different rootstocks and the total dry weight of the fixed broomrapes. The data correspond to the averages ± SE (n=10).

Thus, the total dry weight of the rootstocks is strongly reduced in response to the broomrape. The percentage of total reduction of the ms varies from 57% for 1052791 to 68% for 1052796 (Table 2). Whatever the rootstock, the impact of parasitism is stronger on the development of the vegetative aerial parts than on that of the root system (66% and 45% respectively). Consequently, the infestation impacts negatively on the ratio aerial-parts/root of the rootstocks (Figure 5), attesting a modification of the plants allometry in consequently a preferential allowance of the dry matter towards the underground organs. The most significant modifications are observed for 1052728 rootstocks.

Table 2. Impact of parasitism on the development (dry weight) of different rootstocks.
The values are the averages of ten plants per rootstock. By parameter, the values carrying the same letter are not significantly different (ANOVA, SNK, P≤0.05, n=10). R: roots; AP: vegetative aerial part.

Rootstock	Non-parasited		Parasited		% Reduction		
	R	AP	R	AP	R	AP	Total
1052724	7,55^{a}	24,40^{b}	4,50^{a}	7,96^{b}	40	67	61
1052728	4,82^{bc}	33,10^{a}	3,20^{ab}	12,32^{a}	34	63	59
1052789	6,23^{ab}	22,47^{b}	3,73^{ab}	7,09^{b}	40	68	62
1052791	5,5^{ab}	13,63^{c}	2,66^{bc}	5,60^{b}	52	59	57
1052796	5,6^{ab}	21,57^{b}	2,30^{bc}	6,33^{b}	59	71	68

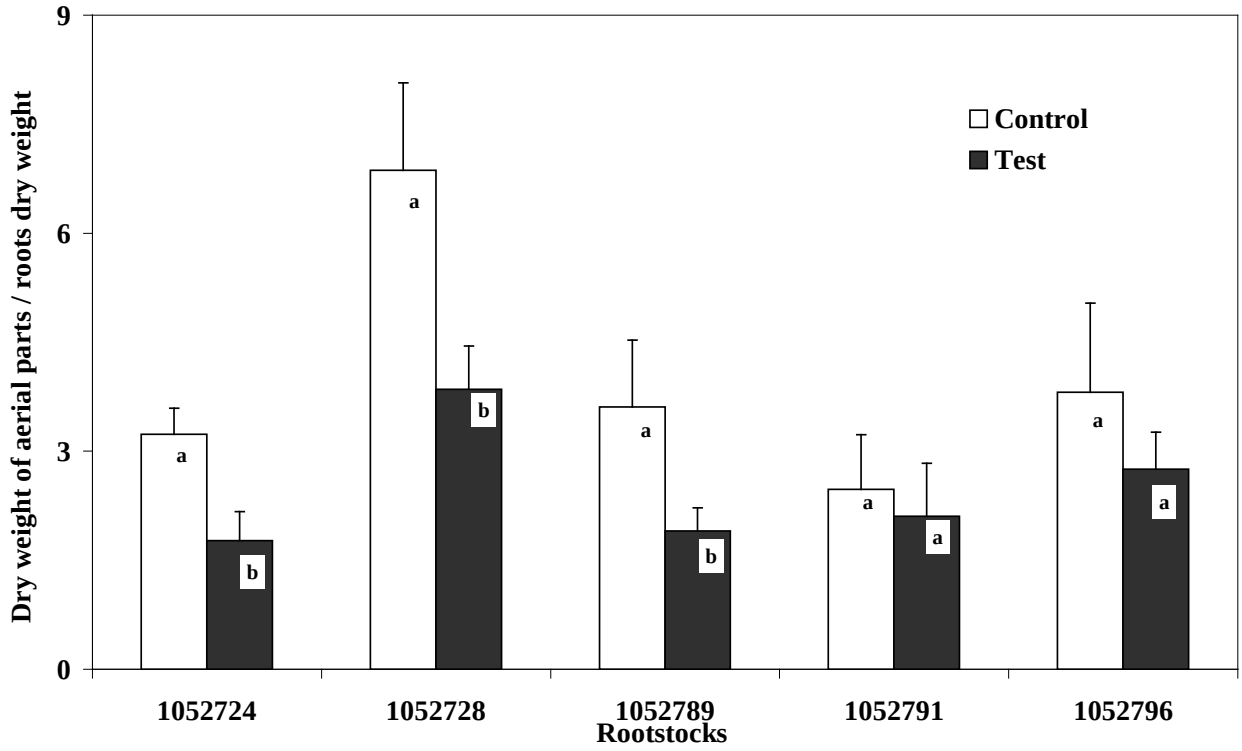

Figure 5. Effect of parasitism on the ratio: dry weight of the aerial parts/roots dry weight of the different rootstocks.
Control: not infested rootstocks, Test: rootstocks infested by the broomrape. The values represent the averages ± SE. By rootstock, the values carrying the same letter are not significantly different (ANOVA, SNK, P=0.05, n=10).

These results join those of Press (1995) and of Barker *et al.*, (1996) which underline the reduction in the photosynthetic capacity and the ratio aerial-parts/roots tomato plants parasitized by the species *O. aegyptiaca*. Such an impact seems to be a common response to the plants parasitized by the broomrape (Graves, 1995; Barker *et al.*, 1996). Nevertheless, the degree with which the parasite affects the mass and the allometry of the plants depends on biotic factors (genotypes of the plant host and the parasite) and abiotic (precocity of the parasitic attacks, conditions of culture.), like that was shown for *Striga*, another plant root-parasitize (Pieterse and Verkleij, 1991; Cechin and Press, 1993).

Conclusions

This study did not reveal any source of resistance to the branched broomrape among the many rootstocks tested. All the genotypes tested are sensitive and show a consequent loss of biomass under infestation.

This work allowed nevertheless an evaluation of the genetic variability of part of the material of research of the Vilmorin Company according to the sensitivity to the branched broomrape. This variability appeared low, from where interest extremely limited of this material for the development of new genotypes to interesting behavior against branched broomrape. The increased sensitivity of these rootstocks could come from the relative *S. hirsutum* whose several accessions were shown like very sensitive to the branched broomrape (El-Halmouch, 2004). This study also showed the degree of sensitivity of the rootstocks is conditioned by its root biomass. Thus, this result suggests that the lowest sensitivity is explained by a weaker surface of contact with the parasite seeds and/or more limited production of germination stimulants. Thus, an interesting prospect from this work would be to evaluate the stimulating capacity of the rootstocks root exudates to the germination of seeds of branched broomrape and to compare them with the degree of sensitivity of these genotypes. It would also be advisable to understand why 1052724 has a density of parasites

definitely higher on its roots than the other rootstocks. For this reason, an analysis of the dynamics of infestation of the rootstocks by branched broomrape in conditions of *in vitro* culture would make it possible to appreciate complementary indicators of sensitivity, such as the percentage of seeds germinated near the roots and the ratio of germinated seeds which succeed in being fixed at the hosts roots. In fact, the number of fixed broomrapes depends at the same time on the germinated seed rate of broomrape and on the rate of germinated broomrapes which manage to penetrate the host root cortex and to fix itself at conducting tissues (Fernandez-Aparicio *et al.*, 2007).

This study is added to several other studies aiming to investigate of sources of resistance to the broomrape among very many tomato genotypes (Dalela and Mathur, 1971; Abu-Gharbieh *et al.*, 1978; Foy *et al.*, 1988; Qasem and Kasrawi, 1995; Avdeyev *et al.*, 2003). The study of the most significant width is that of Foy *et al.*, (1998) which evaluated the sensitivity of 1361 genotypes of *Solanum*, without identifying resistant genotypes. As at many other cultivated species, resistance to the branched broomrape is thus rare, even very rare, at tomato. To date, the most promising line for its resistance to branched broomrape was obtained in Russia (Pzu-11) (Avdeyev and Scherbinin, 1977), and is used in selection to introduce resistance to the broomrape into varieties of tomato intended for the production in the South of Russia (Avdeyev *et al.*, 2003). For as much, this resistance seems ineffective in other areas of production (Foy *et al.*, 1987). The work of El-Halmouch, (2004) and of Qasem and Kasrawi, (1995) agrees to record levels of resistance raised among some wild tomato accessions. This resistance rests on the weak stimulative activity of their root exudates touching the germination of seeds of the parasite (El-Halmouch, 2004; El-Halmouch *et al.*, 2006). Those are thus proposed like sources of resistance for the programs of selection. In parallel, the effort must be dedicated on the screening of wild accessions for the identification and the characterization of resistances. Finally, to increase the genetic variability of tomato, a great number of tomato mutants were also created by mutagenesis EMS (ethyl methane sulfanate) then screened in the field or under artificial infestation for their resistance to the branched broomrape (Kostov *et al.*, 2007). Thus, some mutants characterized by a number of emergences per plant definitely more reduced than that of the parental lines could be obtained (Hershenhorn, 2006; Kostov *et al.*, 2007). The implied mechanisms of resistance are not characterized (or available) to date.

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