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ORIGINAL ARTICLE

Screening of sugar beet pre-breeding populations and breeding lines for resistance to *Ditylenchus dipsaci* penetration and reproduction

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Abstract

Ditylenchus dipsaci is an economically important plant-parasitic nematode affecting European sugar beets. To date, no sugar beet cultivars carrying resistance against *D. dipsaci* are available to farmers. To find potentially resistant sugar beet lines restricting reproduction and penetration of *D. dipsaci*, three consecutive *in vivo* bioassays were carried out. The first experiment determined the penetration rate of *D. dipsaci* in 79 breeding lines and 14 pre-breeding populations. Based on these results, *D. dipsaci* penetration and reproduction resistance of eight genotypes was intensively investigated. It could be demonstrated that none of the genotypes showed resistance towards *D. dipsaci*. However, a high variation of the penetration rate by *D. dipsaci* was observed among the genotypes. The breeding line 'DIT_119' effectively reduced *D. dipsaci* penetration (34.4 ± 8.8 nematodes/plant at 22 days post-planting) compared to the susceptible control (109.0 ± 16.9) while ensuring a yield comparable to non-inoculated plants. However, the breeding line 'DIT_119' did not reduce *D. dipsaci* reproduction. The paternal line of the cultivar BERETTA KWS, demonstrating a high tolerance to *D. dipsaci* crown rot symptoms, did not reduce penetration and reproduction. Thus, no correlation can be established between reduced penetration rates, reproduction, and tolerance to *D. dipsaci*. This study provides an essential basis for the development of resistant sugar beet cultivars to *D. dipsaci*. The variations observed among genotypes now need to be confirmed with larger-scale screenings.

Keywords Breeding line · In vivo · Penetration · Pre-breeding population · Reproduction · Resistance breeding

Introduction

The stem and bulb nematode *Ditylenchus dipsaci* (Kühn 1857) Filipjev 1936 is a migratory endoparasite affecting up to 450 plant species worldwide (Duncan and Moens 2013; Seinhorst 1956). This nematode pest has emerged as an economically threatening plant-parasitic nematode in the European sugar beet (*Beta vulgaris* L.) production (Dewar and Cook 2006; Leipertz 2007; Subbotin et al. 2005). The penetration early in the growing season leads to swollen hypocotyls and distorted leaves and cotyledons (Griffin 1983). Later in the season, bacterial and fungal infection, such as *Rhizoctonia solani* (AG 2–2IIIB) and *Verticillium albo-atrum*, introduced by *D. dipsaci* leads to the crown's rotting (Hillnhütter et al. 2011; Vrain 1987). Since the withdrawal of the nematicide aldicarb, no effective direct management has been available for control of *D. dipsaci*. The broad range of host plants of *D. dipsaci* hinders crop rotation strategies for successful management of this nematode (Jones et al. 2013). The fungicide fluopyram, a succinate dehydrogenase inhibitor (SDHI), effectively reduced the fungal and bacterial infection introduced by the stem and bulb nematode (Storelli et al. 2020). However, no long-term effect on *D. dipsaci* population development was observed. Therefore, breeding for sugar beet cultivars' resistance is a sustainable management approach (Schomaker and Been 2013). Roberts (2002) described resistance as the plant's ability

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to suppress the development and consequently the reproduction of nematodes. The latter author further described tolerance as the plant's ability to compensate for nematode infection with little or no yield loss. To date, no sugar beet cultivar with resistance against *D. dipsaci* is although available. Kühnhold (2011) observed variations in *D. dipsaci* penetration and reproduction rates depending on the breeding line or cultivar tested. However, *D. dipsaci* penetration and reproduction rates in less susceptible genotypes were still high (Kühnhold 2011). Some sugar beet cultivars are tolerant to the fungal and bacterial infection introduced by *D. dipsaci* in the field (Leipertz and Valder 2020). Resistance towards *D. dipsaci* penetration may prevent nematode penetration and, consequently, the introduction of fungal and bacterial pathogens. However, resistance towards *D. dipsaci* reproduction may reduce nematode population development but does not avoid introducing fungal and bacterial pathogens. Monogenic or polygenic resistance towards the sugar beet cyst nematode *Heterodera schachtii* (Schmidt) is identified since a long time (Blok et al. 2018; Golden 1959; Savitsky 1975). Resistance towards *D. dipsaci* has been observed in clover (*Trifolium* spp.), lucerne (*Medicago sativa* L.), faba bean (*V. fabaea* L.), and oat (*Avena* spp.) cultivars (McDaniel and Barr 1994; Stanton et al. 1984; Starr et al. 2013). *Ditylenchus dipsaci* resistance is monogenic on lucerne, and polygenic on faba bean, wild oat (*A. ludoviciana* L.), and red clover (*T. pratense* L.) (Plowright et al. 2002). Resistant oat cultivars successfully reduced the reproduction of *D. dipsaci* but not the penetration of this nematode pest (Blake 1962; Griffiths et al. 1957). Plowright et al. (2002) reported high resistance against *D. dipsaci* in a faba bean line, which has been used to develop resistant cultivars for North Africa. Despite variations in the nematode reproduction rate among onion (*Allium cepa* L.) cultivars, Yavuzaslanoglu (2019) found no resistance towards *D. dipsaci*. Caubel et al. (1994) demonstrated a positive relationship between symptom expression at 3 weeks post-inoculation and *D. dipsaci* reproduction at 10 weeks post-inoculation on red clover. Cook and Evans (1988) reported no correlation between leaf size of white clover (*T. repens* L.) and tolerance towards *D. dipsaci* infection. The development of forage crops resistant to *D. dipsaci* was based on the characterization of symptoms on seedlings (Caubel et al. 1994). Variations in the resistance levels of lucerne species depending on the *D. dipsaci* population used were observed (Leclerq and Caubel 1991; Whitehead 1992). The resistance of a host plant to *D. dipsaci* is highly dependent on the geographic origin of the nematode population, which hinders any breeding programme. Our study aims were to identify among 14 pre-breeding populations and 79 breeding lines sugar beets with resistance towards *D. dipsaci* penetration and reproduction. A screening of a wide range of genotypes was first conducted to identify potential candidates for resistance towards *D. dipsaci* penetration. Based on this screening, a more in-depth investigation of the genotypes with potential resistance was conducted to determine their potential resistance to *D. dipsaci* penetration and reproduction. The tolerance of the sugar beet genotypes to *D. dipsaci* infection is determined by assessing plant survival and yield.

Materials and methods

Nematode inoculum

The *D. dipsaci* population used in all experiments was derived from three infested sugar beets tubers (cv. SAMUELA KWS) collected in the Seeland region (CH) in 2015 (47.058154, 7.275107). Nematodes were extracted by Oostenbrink dishes (European and Mediterranean Plant Protection Organization 2013). Fourth-stage juveniles (J4) and adult nematode stages were hand-picked after morphological identification of the tail, median bulb, stylet, and lip shape using an optical microscope at 40x magnification. After suspending the nematodes in an antibiotic solution containing 0.1% streptomycin sulphate (w/v) and 0.1% amphotericin-B (w/v) for 30 min, 50 nematodes were inoculated per surface sterilized (1% NaOCl) carrot disc (2.5 x 5 cm) and incubated for 45 days at 20 °C in the dark (Kühnhold et al. 2006; Storelli et al. 2021). The nematodes were extracted from the carrot discs, stored in the dark at 6–8 °C, and after 24 hr the sugar beet plants were inoculated. On average, *D. dipsaci* suspensions used for inoculation contained 43 ± 1.5% eggs, 28 ± 0% second- and third-stage juveniles (J2-3), and 29 ± 1.5% of J4s and adult stages.

Plant material

Investigated plant material included 79 breeding lines and 14 pre-breeding populations from the KWS gene pool (KWS SAAT SE & Co. KGaA, Einbeck, Germany). The pre-breeding populations, compared to breeding lines, were quite heterozygous and less advanced (KWS SAAT SE & Co. KGaA, personal communication). Currently, limited information on genetic variation for resistance or tolerance to *D. dipsaci* in sugar beet has been published (Kühnhold 2011; Leipertz and Valder 2020). The cvs. BELLADONNA KWS and BERETTA KWS, known to be susceptible and tolerant to the fungal and bacterial infection introduced by *D. dipsaci* in the field, respectively, were used in this study (Leipertz and Valder 2020). The paternal line 'DIT_006' of the cv. BELLADONNA KWS and the paternal line 'DIT_005' of the cv. BERETTA KWS were used as standards for the experiments.

General methods

The sugar beet seeds were sown in 200-ml plastic pots filled with a 180-ml non-sterile sieved loess soil: compost mixture (1/1) (v/v). The loess soil originated from Einbeck (Germany). Per pot, three seeds of pre-breeding populations or two seeds of breeding lines were sown to compensate for the partially low sugar beet germination rate. After the emergence of the first plant, all following emerging plants were removed each day to ensure only one seedling per pot remained. Due to the large variation of growth rates among genotypes, nematode inoculation was split into two inoculation time intervals. At 8 and 11 days post-planting (dpp), approx. 1.5 cm from the centre of the pot, 500 nematodes (of mixed life stages) were inoculated in 500 µl into two 1-cm deep holes (diam. 3 mm), resulting in 1000 *D. dipsaci* individuals/plant. The plants were regularly watered to maintain a suitable soil moisture allowing nematode movement during the entire experiment. The resistance of the genotypes towards *D. dipsaci* penetration was determined by the number of nematodes in the whole plant at 22 dpp. The sugar beet seedlings were removed from the pots at 22 dpp, gently washed, transferred to a plastic beaker containing a 0.1% acid fuchsin/lactic solution, and boiled twice in a microwave oven for 1 min (Kühnhold et al. 2006). The stained seedlings were then rinsed to remove the staining solution. The total number of nematodes per seedling was counted using a stereomicroscope at 10x magnification after maceration of the whole plant (6500 RPM) in 30 ml tap water using an Ultra Turrax blender (T25 basic/S25 N - 18 G, IKA Labortechnik, Germany). The resistance of the genotypes towards *D. dipsaci* reproduction was determined by the number of nematodes in the whole plant at 60 post-inoculation (dpi). The sugar beet plants were removed from the pots at 60 dpi, washed, weighed (whole fresh plant), and the whole plants cut into 0.5-cm pieces. Nematodes were extracted from the sliced plant material using Oostenbrink dishes for 24 h (European and Mediterranean Plant Protection Organization 2013). The number of *D. dipsaci* individuals per sugar beet plant was determined by counting 3 × 1 ml aliquots from 15 ml total volume using an optical microscope at x40 magnification. The effect of the genotypes on the nematode incidence (%) at the harvest time point (number of plants containing nematodes/number of harvested plants*100) and on the incidence of the symptoms (Fig. 1) (number of swollen plants at 14 dpi/number of germinated plants at 14 dpi*100) was determined in experiments 2 and 3. The number of harvested sugar beet at 60 dpi in relation to the number of emerged plants at 22 dpp determined plant survival (%).

Fig. 1 *Ditylenchus dipsaci* infected sugar beet seedling at 14 days post-inoculation (dpi) showing swollen leaf-axil

Experiment 1: screening of sugar beet lines for *Ditylenchus dipsaci* penetration

To estimate the genetic variation of genotypes belonging to breeding lines (79) or pre-breeding populations (14), they were screened for their response concerning *D. dipsaci* penetration rates. 'DIT_006' was used as the susceptible standard to determine the relative (%) penetration susceptibility (number of nematodes in inbred line 'DIT_xxx'/number of nematodes in 'DIT_006'*100). The experiments were set up at 15 max/8 min °C temperature range and a photoperiod of 18/6 hr day/night and conducted twice in a glasshouse. The investigation of breeding lines and pre-breeding populations was performed with 10 and 15 replicates, respectively.

Experiment 2—effect of sugar beet genotypes on *D. dipsaci* penetration potential

Genotypes selected for their response after inoculation with *D. dipsaci* in the first screening experiment were investigated for their potential to reduce penetration rates of this nematode pest. Only the sugar beet genotypes showing the lowest and the highest *D. dipsaci* penetration values in experiment 1 were further investigated in experiment 2 with a greater replication number. The experiment was set up as described above regarding the temperature range and photoperiod and conducted twice in a growth chamber (KBWF 720, Binder GmbH, Germany), with 20 replicates.

Experiment 3—effect of sugar beet genotypes on *D. dipsaci* reproduction potential

Genotypes selected for their response after inoculation with *D. dipsaci* in the first screening experiment were investigated for their potential to reduce reproduction of the species. The experiment commenced in a growth chamber (KBWF 720, Binder GmbH, Germany) under the same temperature and photoperiod conditions as indicated above for experiments 1 and 2. For optimal growth of the sugar beets, the plants were transferred, at 22 dpp, to a glasshouse where a temperature range of 22 max/15 min °C and a photoperiod of 18/6 hr day/night prevailed. The experiment was performed with 10 replicates and conducted twice. For each genotype, 2 x 10 replicates of non-inoculated plants were used as control.

Data analyses

The investigation of the effect of the breeding lines on the aggressiveness of *D. dipsaci* in experiment 1 was performed in a randomized complete block design. The investigation of pre-breeding populations in experiment 1 and investigations in experiments 2 and 3 were performed in a complete randomized design. In experiment 1, a Friedman rank-sum test was performed to determine the effect of the breeding lines on *D. dipsaci* penetration rate in the whole sugar beet seedlings. The effect of the pre-breeding populations on *D. dipsaci* penetration rate in experiment 1 was determined by using a Kruskal–Wallis rank-sum test. In experiments 2 and 3, problems with normal distribution led to using a Kruskal–Wallis rank-sum test to determine the effect of the genotype on *D. dipsaci* penetration and reproduction rate, respectively. Dunn's multiple comparison tests were performed as post hoc tests. In experiment 3, the effect of *D. dipsaci* inoculation on the fresh biomass of each beet plant at 60 dpi was determined by performing a Wilcoxon signed-rank test to compare data for inoculated and non-inoculated plants. Statistical analyses and figures were performed using the software R.

Results

Experiment 1: screening of sugar beet lines for *Ditylenchus dipsaci* penetration

No significant difference of *D. dipsaci* penetration into sugar beet seedlings was observed among the breeding lines ($P > 0.05$, Fig. 2). The average number of nematodes penetrating sugar beet seedling at 22 dpp varied from 7.5 to 105.2 nematodes per plant. The standard 'DIT_006' showed an average of 80.5±19.1 nematodes per plant at 22 dpp. 'DIT_119' showed the lowest (10.6%) and 'DIT_144' the highest (129.2%) relative susceptibility to *D. dipsaci* penetration. The breeding line 'DIT_005' showed 60.4±15.3 nematodes per plant at 22 dpp. The pre-breeding populations significantly affected *D. dipsaci* penetration into sugar beet seedlings ($P < 0.05$, Fig.3). The pre-breeding populations 'DIT_207' showed the lowest number of nematodes per plant (12.2 ± 20.9) at 22 dpp.

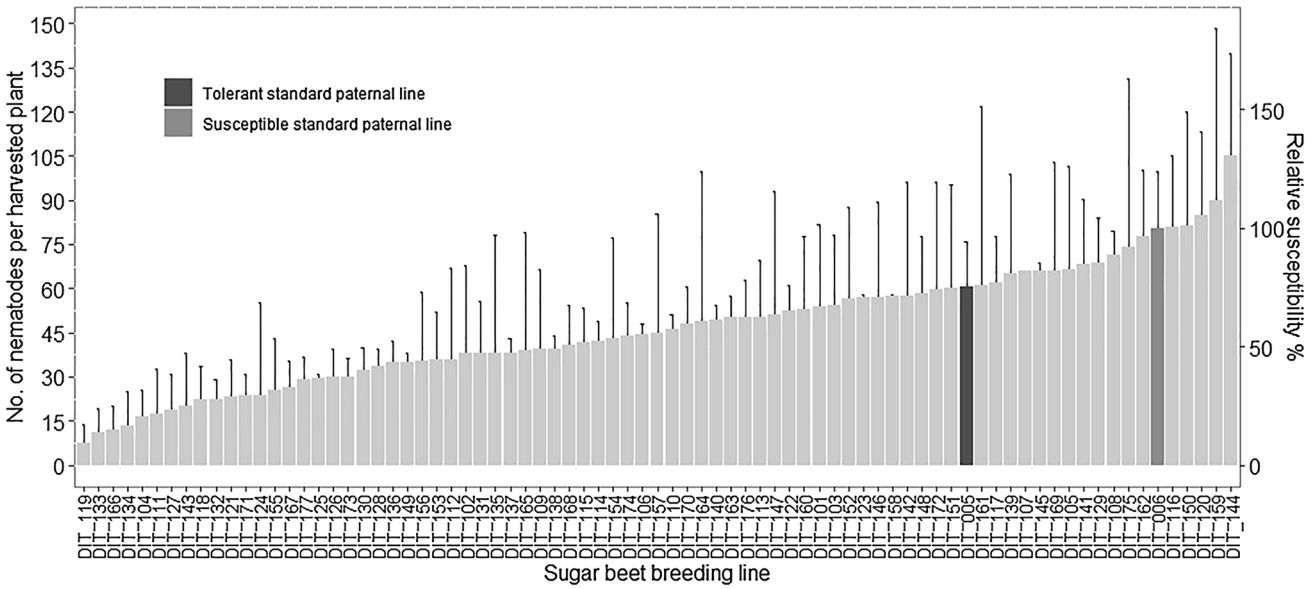

Fig. 2 Effect of sugar beet breeding lines on *Ditylenchus dipsaci* number per harvested plant at 22 days post-planting (dpp), and their relative susceptibility (%) to *D. dipsaci* (average number of nematodes per plant/average number of nematodes in the inbred line DIT_006) in a glasshouse trial. No significant differences among breeding lines according to Friedman rank-sum test (n = 10)

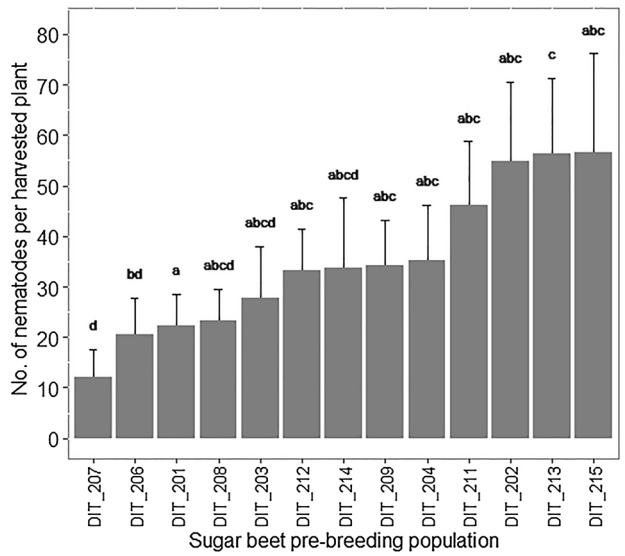

Fig. 3 Effect of sugar beet pre-breeding lines on *Ditylenchus dipsaci* number per harvested plant at 22 days post-planting (dpp) in a glasshouse trial. Different letters above the bars indicate significant differences between pre-breeding lines at $p < 0.05$, according to Dunn's multiple comparison test (n = 15)

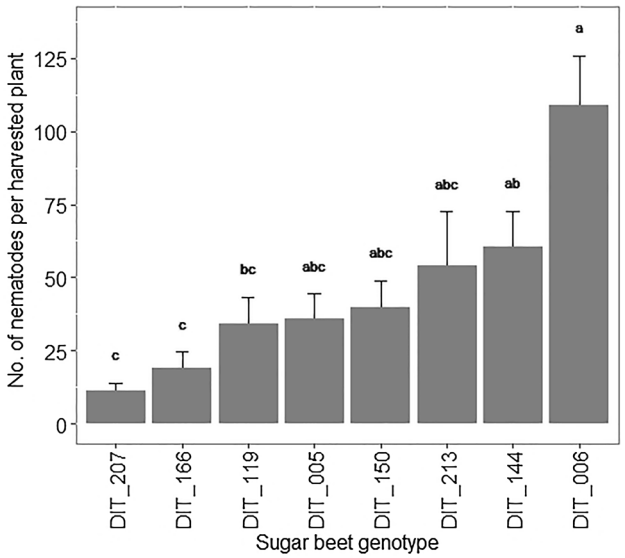

Fig. 4 Effect of sugar beet genotypes on *Ditylenchus dipsaci* number per harvested plant at 22 days post-planting (dpp) in a growth chamber trial. Different letters above the bars indicate significant differences between genotypes at $p < 0.05$, according to Dunn's multiple comparison test (n = 20)

In contrast, 'DIT_213' contained the highest number of nematodes per plant at 22 dpp (56.7 ± 75.6).

Experiment 2—Effect of sugar beet genotypes on *D. dipsaci* penetration potential

In contrast to experiment 1, the genotypes tested significantly influenced *D. dipsaci* penetration into sugar beet seedlings ($P \leq 0.0001$, Fig. 4). At 22 dpp, the susceptible line 'DIT_006' contained an average of 109 ± 16.9 nematodes per plant, significantly higher than 'DIT_207', 'DIT_166', and 'DIT_119' with 11.2±2.8, 19.2±5.3, and 34.4±8.8 nematodes per plant, respectively. The pre-breeding population 'DIT_207' did not significantly reduce the number of penetrated nematodes into sugar beet seedling at 22 dpp compared to the pre-breeding population 'DIT_213' (54.1±18.3). All seedlings of the breeding lines 'DIT_005', 'DIT_006', 'DIT_144', and 'DIT_150' contained nematodes at 14 dpi (Table 1). The breeding line 'DIT_166' showed the lowest incidence, with 74% seedlings containing *D. dipsaci* individuals. Concerning the incidence of the symptoms at 14 dpi (Table 1), the breeding line 'DIT_119' and the pre-breeding population 'DIT_207' showed the lowest percentage of swollen hypocotyls (13%). The pre-breeding population 'DIT_213' contained the highest percentage of swollen hypocotyls (73%).

Experiment 3—effect of sugar beet genotypes on *D. dipsaci* reproduction potential

The genotype tested significantly influenced *D. dipsaci* reproduction in sugar beet ($P \leq 0.001$, Fig. 5). 'DIT_207', 'DIT_150', and 'DIT_006' led to the highest *D. dipsaci* reproduction with 6,097±1,863, 8,255±1,091, and 8,670±3,429 nematodes per harvested plant at 60 dpi. The effect of

Table 1 Effect of the genotype on the incidence of the symptoms induced by *Ditylenchus dipsaci* penetration into sugar beet at 14 days post-inoculation (dpi) (number of swollen plants at 14 dpi/number of germinated plants at 14 dpi*100) and on the *D. dipsaci* incidence (number of plants containing nematodes/number of harvested plants*100 at 14 and 60 dpi) in experiments 2 (n =20) and 3 (n=10)

Line	Symptoms incidence (%)		*D. dipsaci* incidence (%)	
	Experiment 2	Experiment 3	Experiment 2 (14 dpi)	Experiment 3 (60 dpi)
'DIT_005'	70	50	100	100
'DIT_006'	33	58	100	100
'DIT_119'	13	30	91	100
'DIT_144'	30	80	100	100
'DIT_150'	40	70	100	100
'DIT_166'	30	40	74	89
'DIT_207'	13	90	88	100
'DIT_213'	73	100	96	100

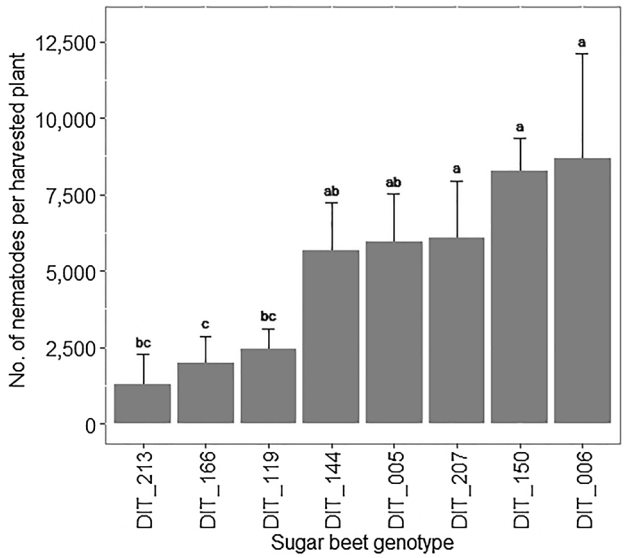

Fig. 5 Effect of sugar beet genotypes on *Ditylenchus dipsaci* number per harvested plant at 60 days post-inoculation (dpi). Different letters above the bars indicate significant differences between genotypes at $p < 0.05$, according to Dunn's multiple comparison test (n = 10)

Table 2 Effect of *Ditylenchus dipsaci* inoculation on the sugar beet fresh weight (g) at 60 days post-inoculation (dpi) and survival (%) (number of harvested plants at 60 dpi/number of germinated plants at 22 days post-planting) in a glasshouse trial

Line	Plant weight (g)[a]		Survival (%)
	Inoculated	Non-inoculated	
'DIT_005'	7.6 (± 2.3)	9.3 (± 1.1)	80 (± 14.1)
'DIT_006'	6.4 (± 4.3)	12.3 (± 3.3)**	60.7 (± 15.2)
'DIT_119'	11.3 (± 4.2)	13 (± 4.6)	95 (± 7.1)
'DIT_144'	4.8 (± 3.4)	10.1 (± 0.8)**	50 (± 28.3)
'DIT_150'	8.7 (± 3.5)	10.3 (± 3.9)	75 (± 21.2)
'DIT_166'	9.5 (± 2.8)	12.5 (± 3.1)*	73.3 (± 9.4)
'DIT_207'	7.5 (± 3)	12.7 (± 3.5)**	75 (± 21.2)
'DIT_213'	9.6 (± 2.6)	9.3 (± 3.2)	25 (± 0)

[a]Wilcoxon signed-rank test *$P < 0.05$, **$P \leq 0.01$; (mean ±SD)

'DIT_207', 'DIT_150', and 'DIT_006' on *D. dipsaci* reproduction significantly differed from 'DIT_213', 'DIT_166', and 'DIT_119'. At 60 dpi, the number of nematodes per harvested plant was 1,298.5±985.8, 1,978±895.5, and 2,437.5±687.6 in the genotypes 'DIT_213', 'DIT_166', and 'DIT_119', respectively. The breeding line 'DIT_166' was the only genotype showing plants without nematode at 60 dpi with a nematode incidence of 89% (Table 1). Concerning the incidence of the symptoms at 14 dpi (Table 1), the breeding lines 'DIT_119' (30%) and 'DIT_166' (40%) showed the lowest percentage of swollen hypocotyls. The pre-breeding population 'DIT_213' contained the highest percentage of swollen hypocotyls (100%). For the genotypes 'DIT_006', 'DIT_144', 'DIT_207', and 'DIT_166', after inoculation with *D. dipsaci*, the fresh plant weight was significantly reduced after 60 days ($P \leq 0.05$, Table 2). Whereas, *D. dipsaci* inoculation did not significantly reduce the sugar beet fresh plant weight of 'DIT_005', 'DIT_119', 'DIT_150', and 'DIT_213' compared to the non-inoculated plants. The breeding line 'DIT_119' showed the highest survival rate, with 95% harvestable at 60 dpi (Table 2). In contrast, 25% of germinated plants of 'DIT_213' were harvestable at 60 dpi. All *D. dipsaci* non-inoculated plants (100%) survived until harvest (60 dpi).

Discussion

This study demonstrated the genetic variation of sugar beet genotypes at reducing *D. dipsaci* penetration into seedlings. The high variation among the genotypes is encouraging from the perspective of finding resistance to *D. dipsaci* penetration. A lower *D. dipsaci* penetration rate in experiment 1 than in experiment 2 suggests a lower inoculation success. Indeed, 'DIT_119' seedlings contained 4.5 times less *D. dipsaci* individuals in experiment 1 than in experiment 2. The low inoculation success in experiment 1 may explain the high variation observed within the genotypes. Despite the lack of significant differences among the breeding lines, some genotypes, such as 'DIT_119' and 'DIT_166', were superior in terms of their low variance of *D. dipsaci* number penetrating sugar beet seedlings. After considering the results from experiment 1, tolerance to *H. schachtii* does not involve resistance to *D. dipsaci* penetration. Due to cross-pollination, the pre-breeding populations consist of a mixture of homozygous and heterozygous individuals (Bos and Caligari 2008), explaining the high variation observed. The second experiment confirms the higher level of resistance of 'DIT_207', 'DIT_119', and 'DIT_166' towards *D. dipsaci* penetration. In contrast, the susceptible breeding line 'DIT_006' attests to its low resistance to *D. dipsaci* penetration. The second experiment suggests that the increase in repetitions (n= 20) significantly improves the probability of observing differences among the genotypes. In the third experiment, nematode reproduction was observed in the eight genotypes tested. However, the number of produced nematodes varied among the genotypes. 'DIT_119' and 'DIT_166' validated their higher resistance level towards *D. dipsaci* infection. Based on the values obtained in the second and third experiments (number of nematodes at 22 dpp and at 60 dpi), the nematode population in each genotype increased up to 100-fold, suggesting that the mechanisms responsible for resistance occurred during the penetration. *Ditylenchus dipsaci* proved to have a rapid population growth (Abolfazl et al. 2017; Kühnhold et al. 2006; Storelli et al. 2020). The few nematodes that penetrated sugar beet

seedling produced a high number of nematodes at 60 dpi. The low *D. dipsaci* number at 60 dpi observed in the pre-breeding population 'DIT_213' was not the effect of resistance towards *D. dipsaci* reproduction. The remaining plants of 'DIT_213' presented rotten hypocotyls at harvest. As *D. dipsaci* is an obligate plant parasite (Duncan and Moens 2013), the rotting of its feeding site led the nematodes to leave the plant before the time point of plant harvest. Abolfazl et al. (2017) showed the inability of *D. dipsaci* to reproduce on fungi. The low survival and germination of infected plants compared to the non-infected plants suggest a high sensitivity of the pre-breeding population 'DIT_213' to *D. dipsaci* infection. The earlier emergence of 'DIT_119' may explain the lowest *D. dipsaci* penetration into sugar beet seedlings. Indeed, it has been observed in the present study that 'DIT_119' emerged earlier compared to the other genotypes. The period of susceptibility of sugar beets to *D. dipsaci* penetration occurs at the emergence of the plant (Storelli et al. 2021). The rapid growth of 'DIT_119' may then reduce this period of susceptibility. It may also explain its lowest amount of swollen hypocotyls and its higher survival rate. Nematode penetration occurs later when the seedlings are already better developed to head to swellings of the hypocotyl. Griffith et al. (1997) indeed reported that symptoms initiation might occur before petioles are fully differentiated. A screening of a large-scale population of early emerging sugar beet genotypes to penetration and infection by *D. dipsaci* may help to validate this statement in the future. The absence of swollen hypocotyls allowed 'DIT_119' to grow with well-developed leaves until 60 dpi. In contrast, a higher proportion of damaged leaf-axils in the other genotypes did not allow for a proper development of the hypocotyl, which later forms the beet. Similar plant biomass measurements between inoculated and non-inoculated plants suggest a higher tolerance of 'DIT_119' to *D. dipsaci* at 60 dpi than other genotypes. However, *D. dipsaci* damages may occur later during the beet storage (Schomaker and Been 2013). The increased amount of nematodes found in the whole plant tissue of 'DIT_119' at 60 dpi suggested no strong resistance towards *D. dipsaci* reproduction. Many non-swollen hypocotyls contained nematodes at 14 and 60 dpi. These results suggest that *D. dipsaci* can survive in plant tissue without exhibiting symptoms (Cook and Evans 1988). However, this statement is not valid for each *D. dipsaci* host plant. Caubel et al. (1994) positively correlated the lack of symptoms with the absence of nematode in red clover tissue. Resistance can be thus determined by characterizing the symptoms on seedlings, which facilitates the screening of a wide range of plant genotypes (Plowright et al. 2002). However, resistance determination based on expressed symptoms does not work on sugar beet interacting with *D. dipsaci*. The presence of a low *D. dipsaci* number is sufficient to trigger abnormal morphogenesis (Griffith et al. 1997). In contrast, a high amount of *D. dipsaci* in sugar beet seedling does not automatically lead to a swollen hypocotyl. Leipertz and Valder (2020) reported tolerance of BERETTA KWS towards the fungal and bacterial infection introduced by *D. dipsaci* in the field. The high susceptibility of 'DIT_005', the paternal line of BERETTA KWS, suggests no direct relation between the resistance of one hybrid component towards *D. dipsaci* development and tolerance of a respective hybrid to the fungal and bacterial infection introduced by *D. dipsaci*. The nematode inoculation success depends on environmental conditions and the viability of the inoculated nematode population (Storelli et al. 2021). This was also demonstrated by the experiments shown here. Thus, nematode aggressiveness towards sugar beet is highly heterogeneous across the experiments and within the cultivars (Kühnhold 2011; Westphal 2013). When evaluating inoculation experiments, a possible inoculation failure must be taken into account in any case. After an inadequate inoculation or if the inoculation fails, the subsequently determined infection level may be low, but the plants are not necessarily resistant (Francis and Luterbacher 2003). Additionally, the existence of a few susceptible plants may bias the resistant pre-breeding population's observation since they were not genetically homogeneous (Scholten et al. 2001). This study did not succeed to find complete resistance in sugar beet genotypes towards *D. dipsaci* penetration or reproduction under glasshouse conditions. However, partial resistance to *D. dipsaci* penetration was observed in some genotypes, where a proportion of plants was less or not affected (Bovien 1955). The lowest incidence of *D. dipsaci* presence in sugar beet seedlings was observed for the breeding line 'DIT_166' suggesting potential candidates with resistance within this line. The pre-breeding population 'DIT_207', with its particular low heterogeneity and low number of *D. dipsaci* per seedling, is a potential candidate for a source of resistance towards *D. dipsaci* penetration, despite the high incidence and reproduction rate of the nematode. The initial inoculum level used in the experiments was up to 1000-fold higher than an initial natural density occurring in the field at sowing (Storelli et al. 2020). An initial density of 10 *D. dipsaci* individuals per 500 g soil may cause severe damage to onions. The damage was nearly 100% when the initial density of 25 *D. dipsaci* individuals per 500 g soil was reached (Seinhorst 1956). With an increasing initial density of *H. schachtii*, the yield ofresistant sugar beet cultivars decreased (Heijbroek et al. 2002). Further trials under field conditions and at lower nematode density may determine the investigated genotypes' real resistance potential. Khanam et al. (2018) reported a similar penetration of *D. angustus* in the resistant and susceptible rice cultivars, suggesting that host attraction is not linked to resistance. However, *D. dipsaci* damages to sugar beet seedlings encourage resistance development through penetration (Cottage and Urwin 2013).

Therefore, screening a large number of pre-breeding populations is recommended as they represent a source of genes for resistance (Tanksley and McCouch 1997). Much of the available germplasm resources remain to be characterized for resistance to nematodes (Starr et al. 2002). Due to the lack of resistant cultivars, high-yielding cultivars, tolerant to the fungal and bacterial infection introduced by *D. dipsaci*, are now the only measure to avoid economic damage. As different fungal and bacterial organisms are introduced by *D. dipsaci*, a case-by-case study is recommended to grow specific cultivars tolerant to the pathogen introduced by the nematode. In a field infested with *R. solani*, the use of *R. solani* resistant cultivars will prevent high yield reduction (Hillnhütter et al. 2011). The use of resistant crops, such as oat, in the rotation to reduce the nematode population has to be further investigated. Thus, tolerant sugar beet cultivars and resistant crops may probably help to maintain a sustainable sugar production in *D. dipsaci* infested fields while resistant cultivars are urgently needed for growers.

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Availability of data and material The data that support the findings of this study are available from the corresponding author.

Declaration

Conflicts of interest None.

Consent for publication All authors give their consent for the publication of the manuscript in the Journal of Plant Diseases and Pests.

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