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Resistance screening in progenies of carrot wild relatives x carrot breeding lines to the northern root-knot nematode (*Meloidogyne hapla*) and histological characterization of plant-nematode interaction

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INTRODUCTION

Plant-parasitic root-knot nematodes of the genus *Meloidogyne* are an increasing economic risk causing significant yield and quality losses during carrot production. The most common species in Central and Northern Europe is the northern root-knot nematode (*Meloidogyne hapla* Chitwood). Extensive galling, forking of the taproot and enhanced lateral root formation can completely prevent commercial usage of the carrots.
Up to now, no *M. hapla* resistant carrot cultivars are available. In this study we tested wild relatives of carrot and progenies of carrot wild relatives x carrot breeding lines for resistance against the northern root-knot nematode. Additionally histological methods were used to detect differences in plant-nematode interaction.

Fig. 1 J_2-larva of *M. hapla* (**A**)*;* carrot root system with stained egg masses (**B**); forking of the taproots (**C**)

MATERIALS & METHODS

Resistance screening

- Screening of wild relatives and alloplasmic carrot breeding lines (8 experimental series in 2017 and 2018; altogether 1919 individual plants from 16x wild relatives and 59x alloplasmic backcross progenies)
- Cultivation in a climatic chamber (16 h 22 ˚C; 8 h 20 ˚C; 10 klx; 60 % relative humidity) for four weeks
- 30 plants per accession in plastic boxes with a sandy substrate
- Inoculation with 400 *M. hapla* J_2 larvae
- Staining of the egg masses with Cochenille A
- Counting of egg masses and galls
- Recultivation of plants with 0 to 3 egg masses in the greenhouse

Sowing Day 1 → Separation after 2 weeks → Inoculation after 4 weeks → Evaluation after 14 weeks

Histological methods

- Galls, distinguished according to their morphological characteristics, were collected, fixed and embedded.
- Sections of the specimens were stained with toluidine blue and observed under a light microscope.

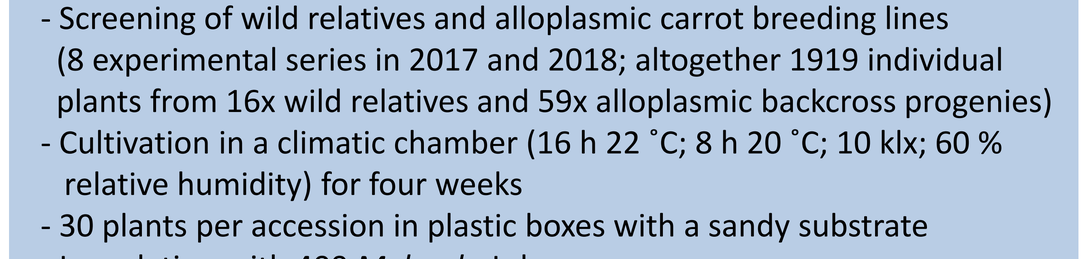

Fig. 2 Resistance screening. Box with carrot seedlings (**A**); inoculation with *M.hapla* (**B**); staining with Cochenille A (**C**); counting of egg masses and galls under the light microscope (**D**); seed production of selected plants (**E**).

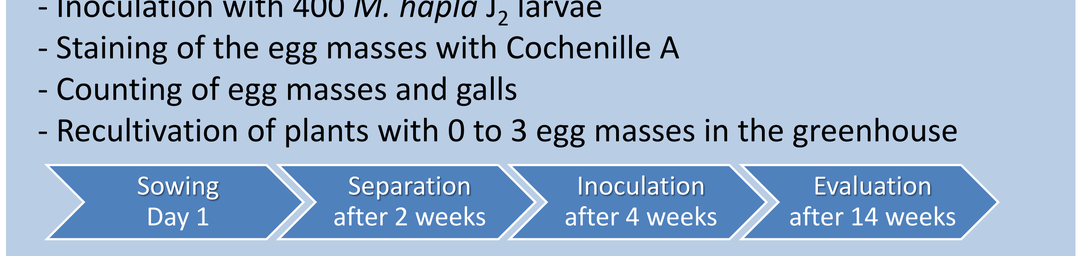

Fig. 3 Classification of gall specimens (**A**); embedding of galls in epoxy resin (**B**); sections of a specimen (**C,D**).

Fig. 4 Root shape expression and coloration of the carrot wild relative *Daucus carota azoricus* (**A**) and a corresponding alloplasmic breeding line of BC_2F_3 generation (**B**).

RESULTS

In some populations of carrot wild relatives was observed a significantly reduced infestion in comparison to the susceptible control. Also various progenies of carrot wild relatives x carrot breeding lines showed a significantly reduced number of egg masses and galls. 126 plants with 0-3 egg masses (resistance criterion) were selected and recultivated in the greenhouse for inbreed and backcross seed production.
127 specimens of galls were fixed, 28 of them were sectioned so far. The sections show a normal development of nematodes and characteristic induced giant cells.

Tab. 1 Average number of egg masses of four wild relatives and the corresponding alloplasmic breeding lines in comparison to the susceptible control cv. Rotin

Accession	Gen.	Ø EM	SP	Accession	Gen.	Ø EM	SP
D.c.azoricus	Pop.	1.0	8	*D. capillifolius*	Pop.	0.4	7
D.c.azo x D.c.sat	BC_2F_3	1.6	3	*D.cap x D.c.sat*	F_4	10.3	0
D.c.azo x D.c.sat	BC_3F_2	1.4	5	*D.cap x D.c.sat*	F_5	1.0	0
cv. Rotin	Pop.	5.3	0	cv. Rotin	Pop.	7.9	0
D.c.commutatus	Pop.	4.4	2	*D.c.halophilus*	Pop.	0.6	9
D.c.com x D.c.sat	F_3	2.1	9	*D.c.hal x D.c.sat*	BC_1F_2	1.1	2
D.c.com x D.c.sat	BC_1F_2	7.9	2	*D.c.hal x D.c.sat*	BC_1F_4	1.3	5
cv. Rotin	Pop.	5.5	0	cv. Rotin	Pop.	6.1	0

Gen. – pedigree generation; Ø EM – average of estimated egg masses; SP – number of selected plants without egg masses and galls

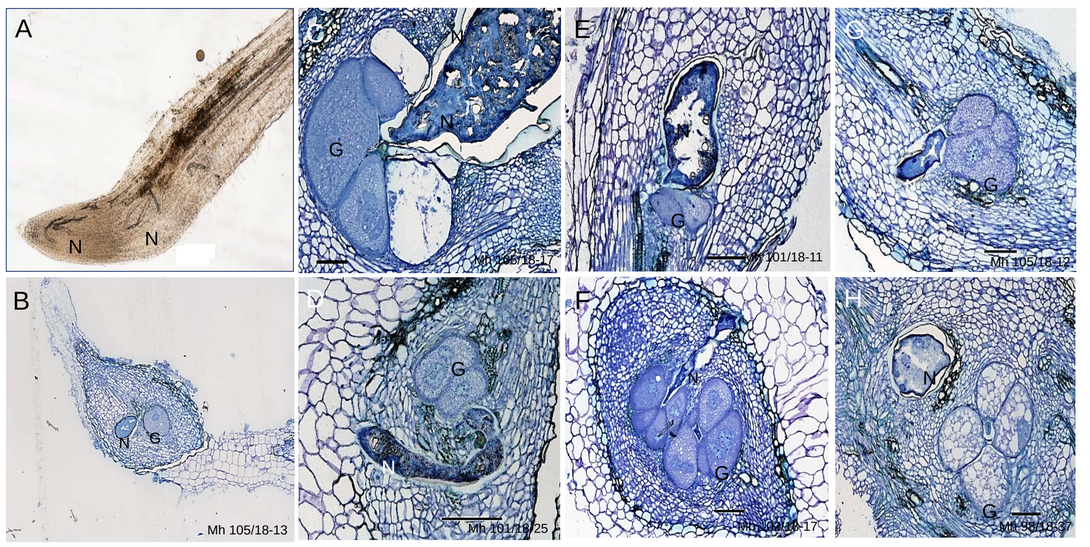

Fig. 5 Cross sections showing plant-nematode interaction in plants of cv. Rotin. Nematodes in the root tip (**A**); Cross section of a juvenile gall (**B**); Cross sections of galls showing the nematode (N) and the development of giant cells (G) (**C-H**).

CONCLUSIONS

- Nematode resistance was confirmed for various carrot wild relatives.
- Potential resistance was observed in the alloplasmic breeding material.
- Histological method has been established for the differentiation of less and highly susceptible genotypes.

PERSPECTIVES

- Genetic analysis of resistance after verification
- Use of resistant plants for breeding of new varieties
- Investigation of the resistance mechanisms

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