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ORIGINAL PAPER

Response of wheat to a Jordanian isolate of Mediterranean cereal cyst nematode (*Heterodera latipons*)

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Abstract A total of 52 wheat genotypes comprising landraces of hard (durum), common bread wheat cultivars, and synthetic hexaploid wheat were evaluated for their reaction against a Jordanian isolate of the Mediterranean Cereal Cyst Nematode *Heterodera latipons* (MCCN). Three genotypes obtained from International Center for Agricultural Research in the Dry Areas (ICARDA), the Australian bread wheat cultivars, Drysdale, Gladius, GS50A and Silverstar, and the synthetic hexaploids, Langdon*Aus18913, Langdon*Aus18964, Langdon*Aus181-1472, Langdon*CPI-110649 and Langdon*CPI-110756 showed resistance to MCCN. Further, 19 out of the 23 Australian synthetic hexaploid genotypes were also resistant. The characterisation of *Cre* genes showed that the synthetic haploids, Langdon*Aus18913, Langdon*Aus18964, Aus 34262, Aus 34423 and Aus 34448 genotypes, carrying *Cre*3 resistant gene, expressed high levels of resistance to MCCN while the genotypes CPI133859, CPI133872, Aus 10894 and Silverstar, carrying *Cre*1 resistant gene, varied in their levels of resistance to MCCN and ranged from moderately susceptible to resistant. The genotype, Frame carrying *Cre*8 was moderately resistant. We were not able to identify those wheat genotypes carrying *Cre*5 or *Cre*8 because of the lack of reproducibility of the PCR and the poor linkage of the markers associated with resistance to the two genes. On the other hand, some commercial cultivars and improved genotypes were resistant to MCCN and do not have *Cre*3 or *Cre*1. They may possess other uncharacterised *Cre* genes against MCCN and thus represent potential new sources of resistance genes that could be used for wheat improvement against *H. latipons*.

Keywords *Heterodera latipons* · Landraces · Cereal cyst nematode · Synthetic hexaploid

Introduction

Wheat is the most important cereal crop worldwide; the estimated annual global production in 2013/2014 is 717 million tonnes (FAO 2014). The Central West Asia and North Africa region (CWANA) is the central pillar of food security where wheat consumption per capita is the highest in the world (FAO 2014).

The most common nematodes attacking cereals and causing serious damage are cereal cyst nematodes (CCN) and root lesion nematodes (Nicol et al. 2003). Several species of CCN attack wheat (Rivoal and Cook 1993). The Mediterranean cereal cyst nematode (MCCN), *Heterodera latipons*, is one of the most damaging species causing yield loss of up to 55 % in winter wheat grown in Iran, 50 % in barley in Cyprus and ~24 % in barley and durum wheat in Syria (Philis 1988; Scholz 2001; Hajihasani et al. 2010). It is also reported to cause yield losses in other Mediterranean countries (Cohn and Ausher 1973; Sikora 1988). In Jordan, it caused moderate to severe yield losses with 100 % incidence in the Northern Mediterranean area (AL-Abed et al. 2004).

Due to the economic losses resulting from MCCN, this nematode should be managed so that population densities are below damage thresholds. Biological and cultural control

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and resistant cultivars are used to manage CCN (O'Brien and Fisher 1974; Kerry et al. 1999). The use of resistance genes against CCN is considered to be the most cost-effective and environmentally safe control method (O'Brien and Fisher 1974; Nombela and Romero 1999). A number of different resistance genes, designated as *Cre* genes, in wheat have been identified for controlling CCN (Slootmaker et al. 1974; O'Brien et al. 1980; Eastwood et al. 1991; Delibes et al. 1993; Paull et al. 1998; Romero et al. 1998; Taylor et al. 1998; Dundas et al. 2001; Jahier et al. 2001; Ogbonnaya et al. 2001a). Ogbonnaya et al. (2009) reported that 11 CCN resistance genes are now catalogued, most of which were incorporated into wheat from wild relatives of wheat. However, very few studies have investigated the resistance of wheat against the MCCN (Bekal et al. 1998; Rivoal et al. 2001; Nicol and Rivoal 2009).

Successful deployment of genetic resistance as a control measure necessitates the evaluation, characterisation and identification of potentially useful sources of resistance. Therefore, the objective of this study was to investigate the resistance of 52 wheat genotypes including landraces of hard (durum) wheat cultivars, common bread wheat cultivars, and synthetic hexaploids of wheat, to MCCN (*H. latipons*). Further, this study aimed to characterise genetic resources identified from the evaluation test for resistance against MCCN with molecular markers linked to previously identified CCN resistant genes. Such information will help understanding of the relationship between the resistances identified in the current study and those already present in common wheat, in particular the resistant genes *Cre*1, *Cre*3, *Cre*5 and *Cre*8.

Materials and methods

Plant material

A total of 52 wheat genotypes, including local cultivars and landraces, were assessed for resistance to MCCN (Table 1). Of these, one Jordanian bread (*Triticum aestivum*) and four Jordanian durum wheat cultivars (*Triticum turgidum* L. *durum*) were tested. Several ICARDA elite wheat germplasm, Australian wheat cultivars and synthetic hexaploid wheat genotypes (derived from crosses involving 10 durum and 11 accessions of *Aegilpos tauschii* parents) were included in this study (Table 1).

Nematode culture

Cysts of the MCCN were obtained from soil samples collected from an infested wheat field located in the Northern Mediterranean region of Jordan in the Ramtha area (32°24′ N, 35°56′E, 641 m). Cysts were extracted from the collected soil samples using the flotation method (Shepherd 1970). The cysts were surface sterilised using sodium hypochlorite (0.1 %) for 5 min and then rinsed with sterile water. Eggs were obtained by crushing several cysts.

The second stage juveniles (J2) were obtained by incubating crushed cysts including the eggs for 16 days at 5 °C followed by an incubation at 10 °C for approximately 3 weeks (Al-Abed et al. 2004; Scholz and Sikora 2004). Freshly hatched J2 were used for (a) establishing cultures on the wheat cv Hourani nawawi, (b) conducting the reaction tests and (c) obtaining females and males from the inoculated Hourani nawawi roots for determining species identity. Females were excised from infected roots of cv Hourani nawawi while the males were recovered from the soil using a modified Baermann funnel method (Christie and Perry 1951).

The obtained females (white cysts), eggs, cysts, males, and hatched J2 were used for morphological identification. Temporary and permanent mounts were prepared following procedures of Hesling (1965) and Seinhorst (1959). All specimens were examined and photographed using a compound microscope equipped with a digital camera (MEIJI TECHNO ML2100, CK3800). Qualitative and quantitative morphological data were documented and used to identify the cyst nematode species. To validate the results obtained from the morphological identification of *H. latipons* the ITS regions including 5.8S plus flanking areas of the 18S and 28S rRNA genes and D2 and D3 expansion fragment of the 28S rDNA were sequenced and compared with those deposited in genebank (Rivoal et al. 2003; Subbotin et al. 2006). After several preliminary studies, the Jordanian durum wheat cv Hourani nawawi was chosen as a susceptible check in the screening test.

Response of wheat genotypes against the Jordanian isolate of MCCN

Seeds of each genotype were pre-germinated on 2.3 % water agar in Petri dishes incubated at 17±2 °C. One seedling of each genotype was planted in an open-ended electrical conduit tube (12.5×3 cm diameter) filled with a sterilised soil mix (83 % sand; 12 % clay; 5 % silt, pH 8.2). The tubes, placed in trays, were maintained in a growth chamber at 20 °C, and with a 16 h day, and 8 h night interval (Nicol et al. 2007). Temperature, relative humidity and light were monitored throughout the screening test using a Hobo data logger model U12 (Onset Corporation), to ensure the optimum conditions for cyst development. A completely randomised design was followed in this experiment; each genotype was replicated 12 times.

Three days after planting the pre-germinated seeds, each plant was inoculated with 100 J2 of *H. latipons* in 1 mL of water (Nicol et al. 2007). Plants were maintained by irrigation every 3 days for each tube and a solution of 100 ml mineral fertiliser (Super Fert 20-20-20+ T.E, ALWaleed Agri.Co. Ltd) at 1 g/L concentration was added periodically as a soil drench to each tube.

Three plants of each genotype were harvested at both 2 and 3 weeks after inoculation to ascertain the penetration and

Table 1 Wheat genotypes screened for resistance to Mediterranean cereal cyst nematode (*Heterodera latipons*), their pedigree and country or centre of origin

No	Country of origin or source[a]	Pedigree	Genotype or ID name	Species
1	Jordan	Tsi/Vee'S'	Amoon	*T. aestivum*
2	Jordan	YEMEN X Cr's'X PLc's'/'Gto's CM 18687-D85-D11	Der allah 6	*T. turgidum*
3	Jordan	Landrace	Hourani 27	*T. turgidum*
4	Jordan	Landrace	Hourani nawawi	*T. turgidum*
5	Jordan	Landrace	Noorseh	*T. turgidum*
6	Jordan	Landrace	Um-qais (UmRabi 6)	*T. turgidum*
7	ICARDA - Syria	GERARDO-VZ-469/3/JORI-1//ND-61-130/LEEDS	ACSAD 65	*T. turgidum*
8	ICARDA - Syria		Babaga-4	*T. aestivum*
9	ICARDA - Syria	PELICANO/RUFF//GAVIOTA/ROLETTE; PELICANO(SIB)/(SIB)RUFF//GAVIOTA(SIB)/(SIB)ROLETTE	Cham-1	*T. turgidum*
10	ICARDA - Syria	W-3918-A/JUPATECO-73	Cham-6	*T. aestivum*
11	ICARDA - Syria	JOPATICOCM67458-F-3/BLUEAY/VEE'S'-T-81(KAUZ)	Cham-8	*T. aestivum*
12	ICARDA - Syria	ICW92-0477-1AP-1AP-4AP-1AP-0AP	Hamam-4	*T. aestivum*
13	ICARDA - Syria	SW2*Cham-6	ICARDA-SW2	Synthetic hexaploid
14	Australia	HARTOG*3/QARRION	Drysdale (syn. QH-71-2)	*T. aestivum*
15	Australia	MOLINEUX/3*DAGGER	Frame	*T. aestivum*
16	Australia		Gladius	*T. aestivum*
17	Australia	Gatcher selection	GS50A	*T. aestivum*
18	Australia	AUS4930 6.5*GS50a	Aus4930 6.5/GS50a	*T. aestivum*
19	United States of America	YUMA,TR.DR/STEWART,TR.DR//CARLETON	Langdon	*T. turgidum*
20	Australia	Langdon*Aus18913	Langdon*Aus18913	Synthetic hexaploid
21	Australia	Langdon*Aus1896	Langdon*Aus18964	Synthetic hexaploid
22	Australia	Langdon*Aus181-1472	Langdon*Aus181-1472	Synthetic hexaploid
23	Australia	Langdon*CPI-110649	Langdon*CPI-110649	Synthetic hexaploid
24	Australia	Langdon*CPI-110756	Langdon*CPI-110756	Synthetic hexaploid
25	Australia	Langdon*CPI-110791	Langdon*CPI-110791	Synthetic hexaploid
26	Sweden	DIAMANT-II/2*KARNII; SVENNO/2*KARN-II	Prins	*T. aestivum*
27	Australia	PAVON-76(SIB)/TM-56	Silverstar	*T. aestivum*
28	Australia		6R(6D)	Secale cereale
29	Iran	Landrace	Aus 10894	*T. aestivum*
30	CIMMYT - Mexico	GARZA/BOY//AE.SQUARROSA (240)	Aus 30257	Synthetic hexaploid
31	CIMMYT - Mexico	DOY1/AE.SQUARROSA (293)	Aus 30258	Synthetic hexaploid
32	CIMMYT - Mexico	FALCIN/AE.SQUARROSA (389)	Aus 30265	Synthetic hexaploid
33	CIMMYT - Mexico	GREEN/AE.SQUARROSA (458)	Aus 30268	Synthetic hexaploid
34	CIMMYT - Mexico	SCA/AE.SQUARROSA (272)	Aus 30279	Synthetic hexaploid
35	CIMMYT - Mexico	CETA/AE.SQUARROSA (525)	Aus 30293	Synthetic hexaploid
36	CIMMYT - Mexico	DOY1/AE.SQUARROSA (526)	Aus 30294	Synthetic hexaploid
37	CIMMYT - Mexico	DVERD_2/AE.SQUARROSA (1027)	Aus 30299	Synthetic hexaploid
38	CIMMYT - Mexico	DOY1/AE.SQUARROSA (1027)	Aus 30300	Synthetic hexaploid
39	CIMMYT - Mexico	CETA/AE.SQUARROSA (1027)	Aus 30301	Synthetic hexaploid
40	CIMMYT - Mexico	CETA/AE.SQUARROSA (172)	Aus 30304	Synthetic hexaploid
41	CIMMYT - Mexico	CROC_1/AE.SQUARROSA (205)	Aus 34240	Synthetic hexaploid
42	CIMMYT - Mexico	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (205)	Aus 34241	Synthetic hexaploid
43	CIMMYT - Mexico	CROC_1/AE.SQUARROSA (224)	Aus 34261	Synthetic hexaploid
44	CIMMYT - Mexico	ALTAR 84/AE.SQUARROSA (224)	Aus 34262	Synthetic hexaploid
45	CIMMYT - Mexico		Aus 34408	Synthetic hexaploid

Table 1 (continued)

No	Country of origin or source[a]	Pedigree	Genotype or ID name	Species
		CPI/GEDIZ/3/GOO//JO69/CRA/4/AE. SQUARROSA (205)		
46	CIMMYT - Mexico	CROC_1/AE.SQUARROSA (224)	Aus 34422	Synthetic hexaploid
47	CIMMYT - Mexico	CROC_1/AE.SQUARROSA (224)	Aus 34423	Synthetic hexaploid
48	CIMMYT - Mexico	ALTAR 84/AE.SQUARROSA (224)	Aus 34424	Synthetic hexaploid
49	CIMMYT - Mexico	ALTAR 84/AE.SQUARROSA (205)	Aus 34448	Synthetic hexaploid
50	CIMMYT - Mexico	CROC_1/AE.SQUARROSA (205)	Aus 34453	Synthetic hexaploid
51	CIMMYT - Mexico	68.111/RUGBY//WARD/3/FLAMINGO/4/ RABICORNO) AUS24132 (SUBSP. STRANGULATA)	CPI133859 (synthetic syn. CIGM89.561)	Synthetic hexaploid
52	CIMMYT - Mexico	68.111/RUGBY//WARD/3/FLAMINGO/4/ RABICORNO)*A* AUS24199 (SUBSP. TAUSCHII VAR. TYPICA)	CPI133872 (synthetic syn. CIGM89.576)	Synthetic hexaploid

[a] Origin: *CIMMYT* International Center for Maize and Wheat Improvement, *ICARDA* International Center for Agricultural Research in the Dry Areas

development of *H. latipons*. The harvested roots were stained by acid fuchsin as described by Bybd et al. (1983) and examined using a dissecting stereomicroscope (Nikon, SMZ645). Another six plants of each wheat genotype were harvested 4 weeks after inoculation. This date of harvesting was chosen based on our preliminary tests to determine the best time for harvesting roots for the development white females and cysts (data not shown).

The roots of each plant were examined using a dissecting stereomicroscope (Nikon, SMZ645) for the presence of white females and cysts. The soil from each tube was transferred to a beaker and processed by washing in water five times. The cysts were collected after passing the cyst suspension through a 250 μm sieve (Shepherd 1970). The total number of recovered white females and cysts (soil and roots) for every wheat genotype was documented. Based on Özarslandan et al. (2010) the following scale was followed for determining the resistance and susceptibility of each wheat genotype; Resistant (R)=0–2; Moderately Resistant (MR) =3–4; Moderately Susceptible (MS) =5–8; Susceptible (S)=9–12; Very Susceptible (VS)≥13 white females and cysts/ plant.

Characterisation of genetic resistance against MCCN in wheat genotypes

All wheat genotypes screened for resistance against MCCN were molecularly characterised to ascertain the presence or absence of *Cre* genes, that had previously been identified in resistant wheat plants against *H. avenae* or *H. filipjevi*, using linked or diagnostic markers (*Cre*1*, Cre*3*, Cre*5*,* and *Cre*8). These markers are deployed in marker assisted selection (MAS) in wheat and included *Cre*1 marker (M19Cre1) (de Majnik et al. 2003). *Cre*3 marker (Cre3sp) (Ogbonnaya et al. 2001b). *Cre*5 marker (Xgwm140) (Röder et al. 1998) and *Cre*8 marker (Xgwm147-6B) (Williams et al. 2006).

Plant DNA of wheat genotypes was extracted according to the protocol previously published in Ogbonnaya et al. (2001b). The extracted genomic DNA from all wheat genotypes were used in PCR reactions for detecting the presence of previously identified *Cre* resistance genes. Four PCR cocktail reactions were carried out for the different *Cre* gene markers and each reaction was performed according to Ogbonnaya et al. (2001b). Röder et al. (1998) and Williams et al. (2006).

The PCR of *Cre* genes markers were performed in a Biorad PCR machine (Bio-RAD, My Cycler). Each PCR product for all wheat genotypes of *Cre*1 marker was run on a 1 % (*w/v*) TAE buffer agarose gel, while the *Cre*3, *Cre*5 and *Cre*8 markers were run on a 1.5 % (*w/v*) TAE buffer agarose gel, in a cell of electrophoresis at 120 V. The gels were stained with ethidium bromide, photographed and analysed using Alphaimager™2200 gel documentation system (Alpha Innotech, USA). The molecular weights of the amplified products for each *Cre* gene were estimated by using BioLabs DNA ladder marker (100 bp). Amplification of all *Cre* genes markers were performed twice for all wheat genotypes.

Data analysis

All data were subjected to analysis of variance (ANOVA) and the means separated using least significant difference (LSD) at $P=0.05$. All statistical analyses were performed using GenStat 16 (VSN International 2013).

Results

Morphological and molecular identification of *Heterodera latipons* local isolate

All morphometrics and qualitative characters of J2, males, females and cysts confirmed the identity of cyst nematode used in our experiments as *Heterodera latipons* as described by Franklin (Franklin 1969). The analyses of the sequences of

both the D2-D3 expansion fragment of the 28S rDNA and the ITS regions of J2 of the isolate confirmed the identity as being *H. latipons*.

Reaction of wheat genotypes to MCCN

Juveniles were observed inside the roots of all tested wheat genotypes 2 weeks post-inoculation (2 wpi) (Fig. 1, Supplementary Table 1). The numbers of vermiform J2 (VJ2) that penetrated the roots ranged from three for the synthetic hexaploid Aus 30268 to 48 for ACSAD65 (Fig. 1) Swollen J2 (SJ2) two wpi were observed inside the roots of most genotypes except in ICARDA SW2 and AUS 30293 genotypes (Fig. 1). Whereas J3/J4 were seen in only 20 genotypes and were greatest in the Prins genotype. Juvenile stage 4 (J4) males were observed only in Prins, Aus 30265 and Aus 30268 genotypes (Fig. 1, Supplementary Table 1). The Hourani nawawi and Prins genotypes supported the development of J4 females (Fig. 1).

Subsequent examination of stained roots, 3 weeks post-inoculation (3 wpi) showed that vermiform J2 (VJ2) were still present in all but one genotype, with significantly high numbers in GS50A, Babaga-4 and Aus 34448 (17–21 VJ2) (Fig. 2, Supplementary Table 1). The development of swollen J2 (SJ2) to further juvenile stages (J3 /J4, J4 females, J4 males) was faster in 21 wheat genotypes (Fig. 2). Further, adult males were recovered from 47 genotypes. The number of recovered males ranged from zero for Drysdale to seven for Hourani nawawi (Fig. 2).

The development of white females and cysts 4 weeks after inoculation revealed that numbers of cysts varied among the wheat genotypes (Fig. 3, Supplementary Table 1). There was a

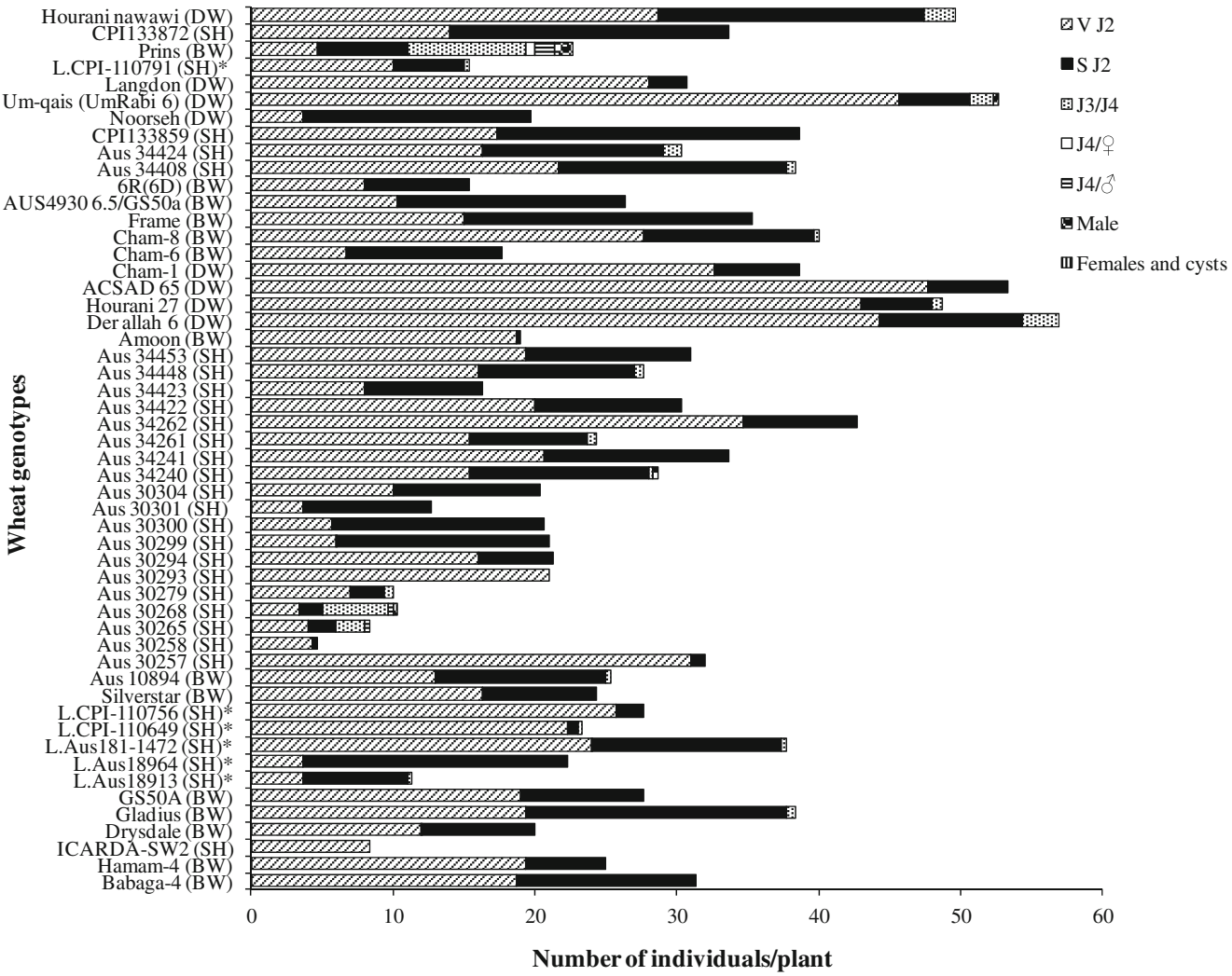

Fig. 1 Developmental stages of a Jordanian isolate of *Heterodera latipons* 2 weeks post inoculation in wheat genotypes comprising landraces of hard (durum) (DW) and common bread wheat cultivars (BW), and synthetic hexaploid wheat (*L.: Langdon*)(SH). Vermiform second stage juveniles (VJ2), swollen second stage juveniles (SJ2), third or fourth stage juveniles (J3/J4), fourth stage female juveniles (J4/♀), fourth stage male juveniles (J4/♂), males and females. Means of three plants are presented; LSD ($P=0.05$) for VJ2=6.98, SJ2=6.98, J3/J4=1.63, J4/♂=0.39

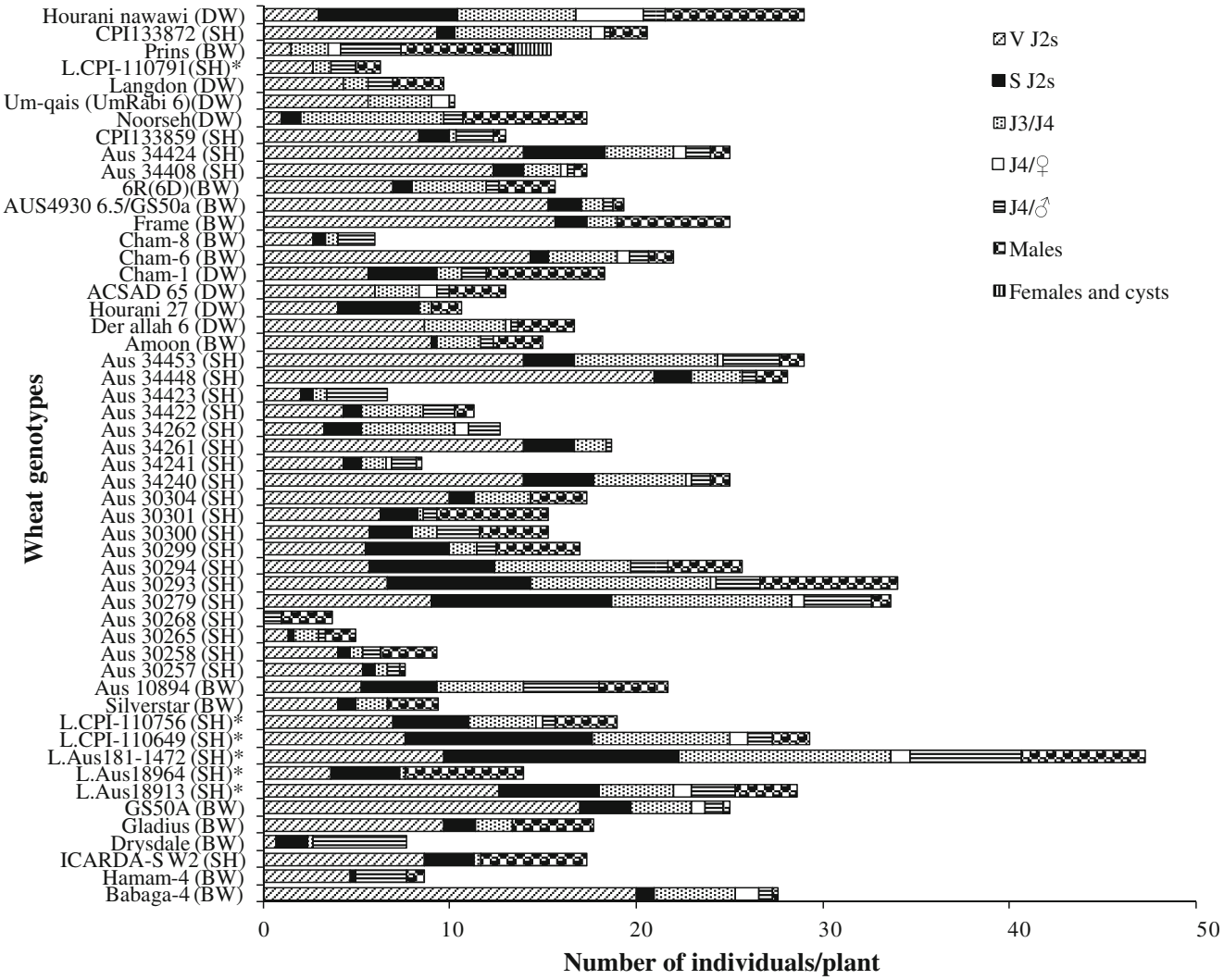

Fig. 2 Developmental stages of a Jordanian isolate of *Heterodera latipons* 3 weeks post inoculation in wheat genotypes comprising landraces of hard (durum)(DW) and common bread wheat cultivars (BW), and synthetic hexaploid wheat (*L.: Langdon*)(SH). Vermiform second stage juveniles (VJ2), swollen second stage juveniles (SJ2), third or fourth stage juveniles (J3/J4), fourth stage female juveniles (J4/♀), fourth stage male juveniles (J4/♂), males and females. Means of 3 plants presented; LSD ($P = 0.05$) for VJ2=6.44, SJ2=3.97, J3/J4=4.20, J4/♀=1.085, J4/♂=3.06, Males=2.81

high percentage (64 %) of resistant genotypes, 23 % were moderately resistant, 11 % were moderately susceptible, and only one was susceptible (2 %). The Jordanian durum cv Hourani nawawi was the only susceptible cultivar with nine cysts per plant ($P<0.05$). The two Jordanian durum wheat cvs Der allah 6 and Hourani 27 were moderately resistant, whereas the Jordanian durum wheat cvs Noorseh and Um-qais were moderately susceptible. The Jordanian bread wheat cv. Amoon was moderately resistant.

The three genotypes obtained from ICARDA, Babaga-4, Hamam-4, and ICARDA-SW2, were resistant to the MCCN. The improved bread wheat genotype Hamam-4 was highly resistant compared with the other two genotypes with an average of one cyst (range of 0 to 2) per plant. The genotypes Cham 1, Cham-6 and Cham-8 were moderately resistant to the MCCN.

The Australian bread wheat cvs Drysdale, Gladius, GS50A, Silverstar were resistant to MCCN and ranged from one cyst/plant in Gladius to two cysts/plant in Drysdale. The other two Australian wheat cvs Frame and Prins were moderately resistant and moderately susceptible, respectively. The durum cv. Langdon was moderately susceptible. However, when this cultivar was used to produce synthetic hexaploids (SHW) in crosses involving CCN resistant lines of the diploid *Ae. tauschii*, the resulting SHW namely: Langdon*Aus18913, Langdon*Aus18964, Langdon*Aus181-1472, Langdon*CPI-110649, Langdon*CPI-110756, were resistant to MCCN with an average number of cysts of 1–2 cysts/plant (Fig. 3). The genotype Langdon*CPI110791 was moderately susceptible.

The bread wheat landrace Aus 10894, the source of cereal cyst nematode resistance gene, *Cre*1 was resistant to the

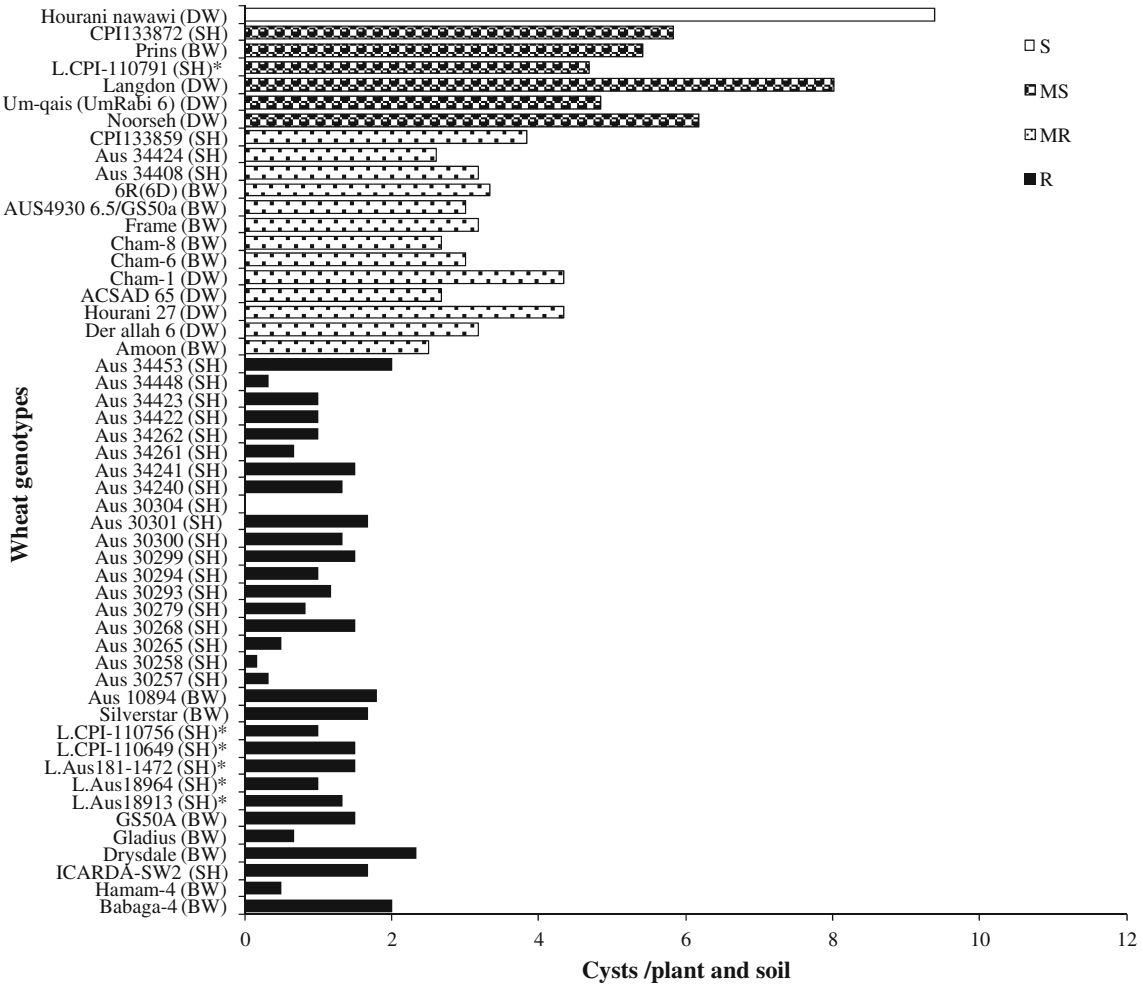

Fig. 3 Cysts of a Jordanian isolate of *Heterodera latipons* recovered from plants and soil 4 weeks post inoculation in wheat genotypes comprising landraces of hard (durum)(DW) and common bread wheat cultivars (BW), and synthetic hexaploid wheat (SH); (*L.: Langdon*). Resistant (R)=0–2, moderately resistant (MR) =3–4, moderately susceptible (MS) =5–8, susceptible (S)=9–12, very susceptible (VS)≥ 13 cysts/ plant and soil. Means of 6 plants presented; LSD ($P=0.05$)= 2.39

MCCN. The bread wheat cv. 6R(6D), in which the 6D chromosome in bread wheat was substituted with a rye chromosome, was moderately resistant to MCCN.

Nineteen of the other 23 SHW genotypes were resistant to MCCN. The genotype Aus 30304 was highly resistant because no cysts were recovered from any of the six replicates. Two synthetic hexaploids, Aus 34424 and Aus 34408 were moderately resistant with an average of three cysts/plant. The synthetic wheat genotypes CPI133859 and CPI133872 were moderately resistant and moderately susceptible, respectively.

Characterisation of genetic resistance of MCCN in wheat genotypes

The result of the amplification of the marker associated with the *Cre*1 gene located on the chromosome 2BL showed that the genotypes, CPI133859, CPI133872, Aus 10894 and Silverstar possessed a similar sized fragment corresponding to that of the presence of CCN resistance gene, *Cre*1 (1 Kb). Similarly, the amplification of the 52 genotypes with the Cre3sp marker associated with CCN resistance gene, *Cre*3, located on chromosome 2D resulted in a band size of approximately 750 bp and was detected in only the following genotypes: Langdon*Aus18913, Langdon*Aus18964, Aus 34262, Aus 34423 and Aus 34448 (data not shown).

The marker Xgwm140 was not reliable for detecting the genotypes carrying *Cre*5 genes since the expected 230 bp band was present in most wheat genotypes similar to the positive genotype (Milan). In addition, extra bands were present in the studied wheat genotypes. The marker of *Cre*8 was also not reliable for detecting the presence of *Cre*8 genes in wheat genotypes, but in this case the *Cre*8 marker did not give the expected band size (242 bp) in the positive controls (cvs. Frame and VP5053). Instead, two higher bands were detected in most genotypes even in some *Aegilops* species. A lower band (approximately 200 bp) was detected on synthetic hexaploid genotypes Aus 34241, Aus 34261, Aus 34262, Aus 34408 and Aus 34422 (data not shown).

Discussion

Morphological and molecular characterisation confirmed the species identity of the Jordanian isolate of *H. latipons* used in this study. Further studies to determine whether this isolate is a pathotype of *H. latipons* may be needed (Rivoal et al. 2001).

Observations were made of roots harvested two and three wpi to ascertain the penetration and whether further stages (swollen J2, J3, J4, males, females) of cyst nematodes were developed in both susceptible and resistant genotypes. The presence of relatively high numbers of J2 inside the roots of most genotypes at 2 weeks after inoculation indicated the viability of the inoculum. Similarly, several other studies have reported the penetration of J2 in resistant and susceptible plants of CCN (O'Brien and Fisher 1978; Williams and Fisher 1993). The presence of different developmental stages of the nematode were higher in number and developed faster in the susceptible and moderately susceptible wheat genotypes (cvs Hourani nawawi, Um-qais, and Prins) than in the resistant ones. In some cases, the young females appeared in the second week after inoculation in the susceptible cultivar Hourani nawawi and in the moderately susceptible cultivar Prins. Sağlam et al. (2009) reported that development of *H. filipjevi* was faster in the susceptible winter wheat cv Bezostaya compared with the resistant winter wheat cv Katea and spring wheat cvs Milan, and Silverstar.

It was noticed that not all penetrated J2s developed to further developmental stages and thus resulted in very low number of cysts in wheat genotypes carrying the *Cre*3 resistance gene compared to those genotypes carrying *Cre*1 and *Cre*8 resistant genes. These findings suggest that different pre- and post-penetration resistance mechanisms may be associated with the differential response of the genotypes. In the present study, in some resistant cultivars the development of immature stages were to males. Lelivelt and Hoogendoorn (1993) demonstrated that the resistance to beet cyst (*Heterodera schachtii*) nematode in cruciferous crops was correlated with an increased male to female nematode ratio in resistant cultivars compared to susceptible cultivars.

Our results showed that several wheat genotypes were resistant and moderately resistant to the Jordanian isolate of MCCN. This result might be expected because most of the chosen wheat genotypes in this investigation either have *Cre* genes or express some level of resistance against CCN (*H. avenae* and *H. filipjevi*) (Bekal et al. 1998; Nicol et al. 2008; Nicol et al. 2009).

The Jordanian durum genotypes were either susceptible or moderately susceptible to MCCN, and these reactions might be due to the adaptation of the Jordanian isolate to the Mediterranean region where durum wheat has been cultivated for long periods, emphasising the necessity to search for new sources of resistance to MCCN.

Our results revealed that some genotypes tested in this study responded to MCCN similarly to those reported to *H. avenae* or *H. filipjevi* while others varied in their reactions. These findings suggest that the previously identified cereal cyst nematode resistance genes (*Cre*1, *Cre*3 and *Cre*8) confer resistance also to the Jordanian isolate of *H. latipons* used in this study. The *Cre*3 was superior to *Cre*1 and *Cre*8 against MCCN. The five genotypes having *Cre*3 genes were resistant to MCCN while of those carrying *Cre*1 two were resistant, one was moderately resistant and one was moderately susceptible. The cv Frame known to have *Cre*8 was moderately resistant to MCCN. We were not able to confirm the presence or absence of *Cre*5 in the tested genotypes. Recently it has been reported that Xgwm140 is a poor marker since it was not detected in the positive parental wheat that carries the *Cre*5 (Dreisigacker 2010).

In this study the cv. 6R (6D), which has *Cre*R, showed moderate resistance to MCCN. This cultivar was also moderately resistant against *H. filipjevi* (Turkish isolate), but was resistant to a Chinese isolate of *H. filipjevi* (Nicol et al. 2008; Lei et al. 2012). On the other hand other commercial cultivars and improved genotypes were resistant and do not have *Cre*3 or *Cre*1. They might have other *Cre* genes or uncharacterised *Cre* genes and thus represent potentially new sources of resistance genes that could be used for wheat improvement against *H. latipons*.

The fact that several species of CCN such as *H. avenae, H. latipons and H. filipjevi* could occur sympatrically in the regions cropped with durum wheat, suggests that pyramiding different resistance genes into a single genotype will be needed to obtain durable resistance (Eastwood et al. 1991; Safari et al. 2005). It has been recorded that the level of resistance of the pyramided wheat genotype carrying *Cre*X and *Cre*Y was significantly higher than that of the single introgression genotypes (Barloy et al. 2007).

This is the first report of screening wheat against a Jordanian isolate of *H. latipons*. Our results contribute to the international wheat improvement and global aims to determine the effectiveness of resistant germplasm against species of CCN and pathotypes in other countries (Nicol et al. 2009). The decision whether to grow resistant genotypes in Jordan or in other Mediterranean countries where *H. latipons* is present is dependent on traits preferred by farmers and whether these cultivars suit these environments. Thus more investigations and field experiments are required to successfully deploy genetic resistance as a control measure. We recommend that the Ministries of Agriculture should include these cultivars in their national trials and select cultivars that suit farmers in specific locations.

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