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Impact of *Cre* and peroxidase genes of selected new wheat lines on cereal cyst nematode (*Heterodera avenae* woll) resistance

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Abstract

Cereal cyst nematode (CCN: *Heterodera avenae* Woll) causes a significant damage to wheat yield in the Kingdom of Saudi Arabia. Wheat cultivar Yecora Rojo and advanced lines derived from the cross between Yecora Rojo and local line (Sama) were sown in a field infected with CCN. Microsatellite markers linked to *Cre1, Cre3, Cre5, Cre8,* and *CreY genes* were used in this study. In addition, the presence of the peroxidase genes in wheat lines was investigated. Three groups of peroxidase genes (*TaPrx111-A, TaPrx112-D*, and *TaPrx113-F*) were found to be greatly induced by cereal cyst nematode in the resistance wheat line. In particular, parenchyma cells, where the nematode starts its feeding, were hyper-reactive to some probes belonging to (*TaPrx112-D*, and *TaPrx113-F*) groups. The field data showed significant differences in yield performance and CCN score. Four local lines were scored as resistant (KSU114, KSU118, KSU119 and L11-21).The highest grain yield was obtained from local lines KSU 110, KSU 118 and L11-21 compared to the check variety Yecora Rojo. Analysis of microsatellite markers linked with resistant genes revealed that there were significant differences in the presence of genes. Moreover, the results indicated that *Cre3* gene located on chromosome 2DL, provided high levels of resistance to CCN in wheat genotypes using *Xgwm301* marker. Also, there was a significant association between CCN resistance with *Cre1* locus which explained 12% of the phenotypic variation in CCN infection. Gene-specific primer pairs for amplification of peroxidase genes revealed the presence of the *TaPrx113-F* peroxidase gene in all wheat genotypes. Moreover, cultivar Yocora Rojo and advanced line KSU 119 had *TaPrx112-D* peroxidase gene. On the other hand, the other wheat lines lacked the presence of *TaPrx112-D* peroxidase gene. The advanced line KSU 119 was resistant to CCN and contained *Cre1*, which was absent in susceptible cultivar Yecora Rojo. Therefore, the presence of peroxidase genes alone did not explain differences among wheat genotypes for CCN resistance. Amplification conditions for *Cre3* and *Cre1* loci were optimized, and used in marker-assisted selection to identify CCN-resistant wheat in the Saudi wheat cultivars. Data of this study emphasized the potentiality of identifying a highly productive CCN resistant cultivar.

Key words: Wheat, Molecular markers, Peroxidase genes, Cereal Cyst Nematode, *Cre* genes, PCR, Marker Assisted Selection.
Abbreviations: CCN - cereal cyst nematode; PCR – polymerase chain reaction; SSR – simple sequence repeats; RAPD – random amplified polymorphic DNA.

Introduction

The Cereal Cyst Nematodes (CCN: *Heterodera avenae* Woll) have a global distribution and cause significant economic yield losses in many countries of the world (Nicol *et al.,* 2003). Developing high yielding cultivars with tolerance to abiotic and biotic stresses is one of the main challenges, and in light of the new information on CCN in Saudi Arabia, it is also necessary in some regions to identify, confirm and incorporate nematode resistance into new high yielding cultivars. Resistance is considered one of most appropriate control methods as it is cost-effective, environmentally friendly and achievable with collaboration of research groups around the world. The use of the former conventional method of screening for resistance involved in a biological assay, which was time-consuming, prone to inconsistencies and relatively expensive (Ogbonnaya *et al.,* 2001) is greatly reduced. Eight genes for resistance to CCN have been identified in hexaploid wheat and its relatives: Cre1 (Cereal root eelworm—locus 1) (*Triticum aestivum* 2B) (Williams *et al*. 1994), Cre2 (transferred to wheat from *Aegilops ventricosa*) (Delibes *et al.* 1993), Cre3 (2D, transferred from *A. tauschii*) (Eastwood *et al.* 1994), Cre4 (*A. tauschii*) (Eastwood *et al.* 1991), Cre5 (2A, VPM1 segment from *A. ventricosa*) (Jahier *et al.* 2001), Cre6 (*A. ventricosa* 5NV) (Ogbonnaya *et al.*, 2001), Cre7 (*A. truincialis*) (Romero *et al.*, 1998) and Cre8 (*T. aestivum* 6B) (Williams *et al.* 2003). CCN resistance genes have also been mapped in rye (6R, Taylor *et al*., 1998) and barley (2H, Kretschmer *et al.*, 1997). A linkage disequilibrium study (Paull *et al.* 1998) found an RFLP locus, *Xcdo347*, that was associated with the Festiguay-derived

CCN resistance of the wheat cultivars Molineux, Frame and Barunga. Williams *et al.* (2003) used this RFLP as a starting point to genetically locate, with RFLP markers, the gene *Cre8* which provides CCN resistance (tolerance) in the cultivar Molineux. *Cre1* confers resistance to several European *H. avenae* pathotypes as well as the Australian pathotype, albeit with varying levels of nematode reproduction in different genetic backgrounds of the host. Comparison of *Cre1* with the nematode resistance gene *Cre3*, derived from the diploid D genome progenitor of wheat, *Aegilops tauschii*, showed that both provide resistance to the Australian pathotype, but differ in their specificity to European and Middle Eastern pathotypes (Ogbonnaya *et al.,* 2001). *Cre1* and *Cre3* are located on the long arms of chromosomes 2B and 2D, respectively (Eastwood *et al.* 1994; Williams *et al.* 1994).

Resistant plants react to nematode infection by activating a number of inducible responses that are thought to be disease resistance related. Incompatible interaction between H-93-8 or TR-3531 lines and the CCN induces hypersensitive response (HR) with previous formation of syncitial cells and active oxygen species (AOS). Plants possess both enzymatic and nonenzymatic antioxidant defense systems to counteract AOS generated under stress conditions (Delibes et al., 2008). The antioxidant enzymes include peroxidase (PER, EC 1.11.1.7), esterase (EST, EC 3.1.1.2) and superoxide dismutase (SOD, EC 1.15.1.1). Isoelectrofocusing (IEF) isoenzyme analysis, four and seven days after infection, revealed that PER, EST and SOD activities increased with time in roots of lines H-93-8 and TR-3531, carrying *Cre2, Cre5* and *Cre7* genes, respectively, in comparison with the susceptible control (Andrés *et al*., 2001; Montes *et al.*, 2004). Recently, twenty wheat peroxidase genes were shown to fall into seven groups (*TaPrx108* to *TaPrx114*) (Simonetti *et al*., 2009). The objectives of the present investigation were to: (1) study the genetic variation in yield, yield components, and resistance to CCN in a field infected with CCN, (2) screen wheat lines for resistance genes using SSR and RAPD markers developed for known resistance genes (*Cre* genes), and (3) investigate the presence of the peroxidase genes in wheat lines by PCR analysis.

Materials and methods

Field experiment

Field experiment was conducted in a CCN infected wheat field at NADC Hail project, Saudi Arabia, during the growing seasons 2007/2008 and 2008/2009. Eight selected bread wheat lines and adapted susceptible cultivar were sown on 20th December 2007 and 24th December 2008 with a seeding rate of 140 kg/ha. These included cultivar Yecora Rojo as well as 8 advanced lines (F10) selected from the wheat breeding program at the Plant Production Department, College of Food and Agriculture Sciences, King Saud University (Table 1). The plot size was 4 rows, 3 m long with row to row spacing of 20 cm. The recommended fertilizer requirements of wheat in Hail region were 200, 200 and 100 kg/ha NPK, respectively. A randomized complete block design with three replicates was used. One month before harvest, plant samples were carefully collected by selecting an area of 0.6 m^2 within each plot. The selected plants were carefully removed. Soil around the roots was gently washed away with a stream of water by using a 60-mesh sieve. The washed root systems were then examined for the presence of immature white cysts. The number of white cysts/plant were then counted. Plants having more than three white cysts/plant were designated as susceptible (Mathur *et al.*, 1974; Ireholm, 1994), while those having up to three cysts/plant were designated as "unknown" until they would be re-evaluated again under controlled rigid regime to verify their reaction.

At maturity, measurements were made on grain yield (GY), biological yield (BY), plant height (PH). Grain and biological yields were determined from the 4 rows and converted to grain yield per hectare. Harvest index (HI) was calculated as grain yield/ biological yield.

DNA extraction

Frozen young leaves (500 mg) were ground to a powder in a mortar with liquid nitrogen. The DNA extraction was done using CTAB method (Sagahi-Maroof *et al.* 1984).

SSR and RAPD markers for Cre genes

A number of primers were used to amplify segments of various *Cre* genes from wheat genotypes. The amplified *Cre* genes, primer sequences and PCR cycling required for these primers are shown in Table 2. The SSR markers used in this study included Crecon, Xgwm301-2D, Xgwm140 and Xgwm147-6B. The PCR reactions were performed as described by Röder *et al*. (1998) using a thermal cycler (Thermolyne Amplitron). RAPD marker for *CreY* gene was detected using arbitrarily primer (OPY16) as described by Williams *et al*. (1990) and Barloy *et al*. (2007). The PCR products were separated by electrophoresis in 1.5% agarose using TBE buffer and detected by ethidium bromide staining.

Specific PCR amplification for peroxidase genes

Gene-specific primer pairs for amplification of the peroxidase genes (*TaPrx111-A, TaPrx112-D*, and *TaPrx113-F*) are shown in Table 3. These primers were designed on the basis of the published sequence (Simonetti *et al.* 2009). Amplification was carried out in 25 µL reaction volumes, containing 1X Taq polymerase buffer (50 mM KCl, 10 mM Tris, pH 7.5, 1.5 mM MgCl2) and 1 unit of Taq polymerase (Pharmacia Biotech, Germany) supplemented with 0.01% gelatin, 0.2 mM of each dNTPs (Pharmacia Biotech, Germany), 25 ρmol primer, and 50 ng of total genomic DNA. Amplification was performed in a thermal cycler (Thermolyne Amplitron) programmed for 1 cycle of 30 s at 94˚C; and 40 cycles of 1 min at 94˚C, 1 min at 56˚C, and 1 min at 72˚C, followed by 5 min at 72˚C. An aliquot of 10 µL from each reaction product was resolved by electrophoresis on 1.5% agarose gel in 1X TAE buffer, stained with ethidium bromide, and visualized with UV light.

Statistical analysis

Field data (grain yield, biological yield, plant height, harvest index and number of white cysts/plant) were statistically analyzed by using a randomized complete block design with three replicates according to SAS (1992). The least significant differences (LSD) test was used to compare means at the 5% level. Only differences significant at $P \leq 0.05$ were considered in the text. Regression analysis was employed to detect the effects of RAPD and SSR markers linked to the total phenotypic variation for cereal cyst nematode resistance according to Moreno and Gonzalez (1992).

Table 1. List of 9 genotypes tested at NADC wheat farms at Hail infested with cereal cyst nematode.

NO	Name	Pedigree	Origin
1	KSU 102	Sama\ Yecora Rojo-5	plant production dept.KSU
2	KSU 118	Sama\ Yecora Rojo-10-1	plant production dept.KSU
3	Ksu 110	Sama\ Yecora Rojo-11-6	plant production dept.KSU
4	L11-8	Sama\ Yecora Rojo-11-8	plant production dept.KSU
5	L11-17	Sama\ Yecora Rojo-11-17	plant production dept.KSU
6	L11-21	Sama\ Yecora Rojo-11-21	plant production dept.KSU
7	KSU 114	Sama\ Yecora Rojo-11-23	plant production dept.KSU
8	KSU 119	Sama\ Yecora Rojo-10-4	pant production dept.KSU
9	Yecora Rojo	US cultivar	USA

Table 2. PCR primers information and PCR cycling conditions for the amplification of specific *Cre* genes.

Cre gene *Cre1*	Primer pair	Forward and reverse PCR primer sequence 5′-3′	Chromo-somes	PCR cycling
	Crecon	F: ATCTGATCAACTTGCGGCAT R: ACTCTGACTCCGATTCCAAG	2B,2BL	1×95°C 30″ 38×(94°C 30″; 50°C 30″; 72°C 1′30″) 1×72°C 10′ 1×10°C hold
Cre3	Xgwm301-2D	F: GAG GAG TAA GAC ACA TGC CC R: GTG GCT GGA GAT TCA GGT TC	2DL	1×95°C 30″ 38×(94°C 30″; 58°C 30″; 72°C 1′30″) 1×72°C 10′ 1×10°C hold
Cre5	Xgwm140	F: ATG GAG ATA TTT GGC CTA CAA C R: CTT GAC TTC AAG GCG TGA CA	2AS	1×95°C 30″ 38×(94°C 30″; 55°C 30″; 72°C 1′30″) 1×72°C 10′ 1×10°C hold
Cre8	Xgwm147-6B	F: CAAACAAGGTGGGTTCACTG R: TTTTTGAGTTCAACGGAGAC	6BL	1×95°C 30″ 38×(94°C 30″; 50°C 30″; 72°C 1′30″) 1×72°C 10′ 1×10°C hold
CreY	OPY16	GGGCCAATGT	3BL	1×95°C 3′ 45×(94°C 1′; 36°C 1′ ; 72°C 1′30″) 1×72°C 10′ 1×10°C hold

Table 3. Primer pairs for amplification of the *peroxidase* genes

Gene	Forward and reverse PCR primer sequence 5′-3′	
TaPrx111-A	F: GGATCTACGAGAAATATGCCG	R: GAATTCGTTACACATGTGGACAG
TaPrx112-D	F: AGCTGTGTCCTATCTAACAAGCT	R: CCACCAAGAAATTAAGTACGG
TaPrx113-F	F: AAGAAGTGCAGGTAGCTAACCA	R: CATACGTATAGTGTTCAGCATTCAG

Table 4. Mean values for agronomical traits of 9 genotypes under infected field in NADC wheat farms at Hail during the growing season 2007/08.

Genotype NO	Name	Grain yield Ton/ha	Biological yield Ton/ha	Harvest index %	Plant height Cm
1	KSU 102	7.98	22.28	0.36	84.33
2	KSU 118	8.49	19.91	0.43	85.67
3	KSU 110	7.70	16.50	0.48	61.33
4	L11-8	7.13	18.98	0.38	69.67
5	L11-17	7.16	20.24	0.36	70.00
6	L11-21	8.21	20.34	0.40	75.33
7	KSU 114	8.05	22.27	0.36	67.67
8	KSU 119	6.23	18.98	0.33	92.00
9	Yecora Rojo	7.27	15.80	0.46	68.00
	LSD 5%	1.45	3.98	0.09	11.15

Result and discussion

Screening Wheat Genotypes for Resistance to CCN

Data presented in Tables (4 and 5) showed the mean values for agronomical traits and CCN count of 9 genotypes in a field infected with CCN in NADC wheat farms at Hail during the growing seasons 2007/08 and 2008/09. Most of the selected lines had higher grain yield than the check cultivar Yecora Rojo. The highest grain yields were obtained from genotype KSU 118 and KSU 110 (8.49 and 9.31ton/ha) in the growing seasons 2007/08 and 2008/09, respectively. Also, the advanced line L11-21 gave higher grain yield (8.21 and 8.68 ton/ha) compared to the check variety Yecora Rojo (7.27 and 6.50 ton/ha) in both seasons, respectively. From the above mentioned results, it could be concluded that wheat lines KSU 118, KSU 110, and L11-21 achieved one of our goals in order to develop high yielding varieties. Concerning biological yield, the average yields for genotypes ranged from 15.80 to 22.28 ton/ha and 14.25 to 21.82 ton/ha in the growing seasons 2007/08 and 2008/09, respectively. The highest values of biological yield were obtained from genotypes KSU 102 (22.28 ton/ha) and L11-21 (21.82 ton/ha) in the growing seasons 2007/08 and 2008/09, respectively. Among wheat lines, KSU 110 gave the highest harvest index (0.48 and 0.44) in both seasons, respectively. However, the lowest harvest indices were calculated for KSU 119 (0.33) and L11-7 (0.36) in the growing seasons 2007/08 and 2008/09, respectively. Plant height ranged from 61.33 cm in the genotype KSU 110 (the shortest genotype) to 92.00 cm in the genotype KSU 119 (the tallest genotype) in the first season. Apparently, short stature plant type is desired because it is associated with resistance to lodging. Five genotypes (KSU 118, L11-8, L11-21, KSU 114 and KSU 119) could be scored as resistant to CCN (0.0, 3, 2.75, 1.75, and 2.25) and (1.14, 2.38, 0.66, 1.4, and 0.92) in both seasons, respectively (Table 6). The advanced lines KSU 118 and L11-21 were the most resistant genotypes, in the growing seasons 2007/08 and 2008/09, respectively according to Mathur *et al.* (1974) and Ireholm (1994). On the other hand, cv. Yecora Rojo, which is the principal cultivar grown in the Saudi wheat fields, recorded 17 and 35.11 white cysts/plant in the growing seasons 2007/08 and 2008/09, respectively (Table 6). Previous reports supporting the ability of *H. avenae* to reproduce on wheat cultivars worldwide are numerous (Meagher, 1977; Dhawan, 1988; Holdeman and Watson, 1977; Ibrahim, 1989). In Saudi Arabia, Al-Hazmi *et al*. (1994) had also reported the susceptibility of eight barley and wheat cultivars, including cv. Yecora Rojo, to CCN. The ability of this nematode to infect and reproduce on all the tested wheat cultivars and lines, herein, especially cv. Yocora Rojo, make the search for resistant or tolerant wheat cultivars is of great necessity. It should be noted that the performance of lines KSU 118, L11-21 and KSU 114 (selected from wheat breeding program at plant production department) were good examples of promising lines, productive (high grain yield) and could be scored as resistant to cereal cyst nematode, which should be considered for cultivation.

Screening wheat genotypes for Cre genes

Microsatellite 5markers linked to *Cre1, Cre3, Cre5, Cre8,* and *CreY* genes were used in this study. The results showed that wheat lines KSU 118, L11-8, L11-17 and L11-21 possessed all the tested *Cre* genes (Table 7). However, *Cre3* gene located on chromosome 2DL (Eastwood *et al*., 1994; Lagudah *et al*., 1997) and identified by gene-based SSR marker (*Xgwm301*), exhibited the most significant association explaining 45% and 56% of the phenotypic variation in CCN resistance amongst the wheat genotypes in the growing seasons 2007/08 and 2008/09, respectively (Table 8). In addition, there was a significant association between CCN resistance with *Cre1* locus which explained 12% of the phenotypic variation in the second season. The cultivar Yecora Rojo lacked both *Cre3* and *Cre1* genes, while the advanced line KSU 110 lacked the *Cre1* gene and both genotypes were categorized as susceptible to CCN. Safari *et al.* (2005) concluded that *Cre3* had the largest impact on reducing the number of female cysts, followed by *Cre1* and *Cre8*. Martin *et al.* (2004) optimized the amplification conditions for the *Xgwm301* marker and applied it in marker-assisted selection to identify *Cre3* CCN-resistant wheat in wheat cultivars. Moreover, Ogbonnaya *et al.* (2001) concluded that the gene-based STS marker, *Cre3sp* on chromosome 2DL showed the most significant association explaining 23% of the phenotypic variation in CCN resistance. The results indicated that neither *Cre1* or *Cre3* markers were found in the genetic background of Yecora Rojo. However, five advanced lines (KSU 118, L11-8, L11-17, L11-21, KSU 114) selected from the wheat breeding program, King Saud University were found to test positive with the *Cre1* and *Cre3* primers. Combining the *Cre1* and *Cre3* primers in one assay would be highly cost- and time-effective, however that requires more detailed studies. This study clearly showed that markers can be optimized and utilized for characterizing germplasm (Akar *et al*. 2009). These markers will be applied in a marker assisted selection of *Cre* genes in early generations which are otherwise complex and expensive to phenotype within a breeding program. As indicated by Ogbonnaya *et al*. (2001), the traditional screening method presents a high degree of variation, and efforts are underway to improve the assay accuracy, to further validate the current findings. Considering preliminary evidence implicating CCN as a major biotic constraint for cereal production in Saudi Arabia, efforts are currently underway to introgress the globally known *Cre* resistant genes including *Cre1, Cre3, Cre5, Cre8,* and *CreY* into wheat germplasm, in addition to identifying new sources.

Molecular analysis of peroxidase genes (TaPrx111-A, TaPrx112-D, and TaPrx113-F) in wheat genotypes

The peroxidase genes (*TaPrx111-A, TaPrx112-D,* and *TaPrx113-F*), which are responsible for the resistance to *Heterodera avenae*, were amplified from wheat genotypes (Fig. 1). The PCR product of the *TaPrx113-F* peroxidase gene was present in all wheat genotypes. PCR amplification by using genome-specific primers revealed the presence of *TaPrx112-D* peroxidase gene in cultivar Yecora Rojo and advanced breeding line KSU 119. The other wheat lines lacked the presence of *TaPrx112-D* peroxidase gene. Moreover, PCR analysis using genome-specific primers did not detect *TaPrx111-A* peroxidase gene in the studied wheat genotypes. *TaPrx112*, and *TaPrx113* were induced by nematode infection in resistant and susceptible wheat genotypes but with differing magnitude and timing. *TaPrx112* and *TaPrx113* groups increased more in resistant than in susceptible lines (Simonetti *et al.*, 2009). Andres *et al*. (2001) reported that peroxidases, esterases and superoxide dismutase might represent products of genes whose expression is correlated with the resistance response to CCN. In this study,

Table 5. Mean values for agronomical traits of 9 genotypes under infected field in NADC wheat farms at Hail during the growing season 2008/09.

Genotype NO	Name	Grain yield Ton/ha	Biological yield Ton/ha	Harvest index %	Plant height Cm
1	KSU 102	6.31	16.96	0.372	81.33
2	KSU 118	8.45	21.68	0.403	93.00
3	KSU 110	9.31	21.13	0.442	70.00
4	L11-8	7.36	20.29	0.364	76.00
5	L11-17	7.14	19.60	0.362	68.00
6	L11-21	8.68	21.82	0.398	77.67
7	KSU 114	7.75	19.32	0.409	74.00
8	KSU 119	6.96	19.04	0.365	81.00
9	Yecora Rojo	6.50	14.25	0.456	63.00
	LSD 5%	2.00	4.95	0.076	14.30

Table 6. Mean values for CCN count of 9 genotypes under infected field in NADC wheat farms at Hail during the growing season 2007/ 2008 and 2008/09.

Name	No.CCN/ Plant 2007-2008	No.CCN/ Plant 2008- 2009	Reaction
KSU 102	7.75	91.14	Susceptible
KSU 118	0.00	1.14	Resistant
KSU 110	3.25	2.53	Susceptible
L11-8	3.00	2.38	Resistant
L11-17	4.00	2.84	Susceptible
L11-21	2.75	0.66	Resistant
KSU 114	1.75	1.40	Resistant
KSU 119	2.25	0.92	Resistant
Yecora Rojo	17.00	35.11	Susceptible
LSD 5%	4.30	16.54	

Table 7. Screening wheat genotypes for *Cre* genes.

Genotype NO	Name	*Cre Y*	*Cre8*	*Cre5*	*Cre1*	*Cre 3*
1	KSU 102	0	1	1	1	0
2	KSU 118	1	1	1	1	1
3	KSU 110	1	1	1	0	1
4	L11-8	1	1	1	1	1
5	L11-17	1	1	1	1	1
6	L11-21	1	1	1	1	1
7	KSU114	1	0	1	1	1
8	KSU 119	1	1	0	1	0
9	Yecora Rojo	0	1	1	0	0

Table 8. General Linear Model of markers linked to CCN resistance in wheat genotypes.

Cre gene	Marker	p-value		R^{2a}	
		2007/08	2008/09	2007/08	2008/09
Cre1	Crecon	0.114	0.0244	0.03	0.12
Cre3	Xgwm301-2D	0.004	0.0014	0.45	0.56
Cre5	Xgwm140	0.8771	0.7726	0.0012	0.0022
Cre8	Xgwm147-6B	0.632	0.5221	0.009	0.012
CreX	Xgwm636-2A	0.5321	0.4272	0.013	0.017
CreY	OPY16	0.4221	0.3961	0.014	0.019

[a]The amount of genetic variance which would be explained by a marker at this gene, as a percentage.

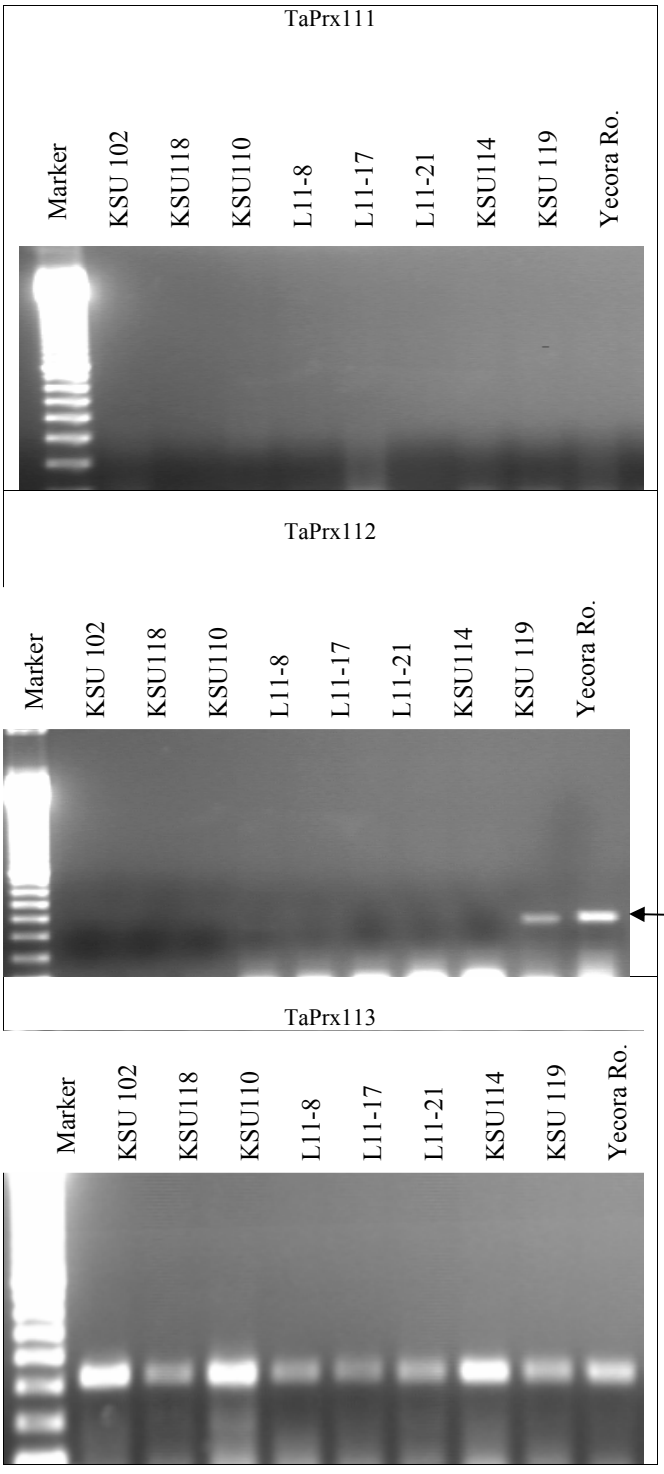

Fig1. Electrophortic analysis of PCR products obtained with specific primers for genes: *TaPrx111-A, TaPrx112-D, and TaPrx113-F* using genomic DNA from wheat genotypes. Specific primers for geneTaPrx111 did not amplify with wheat genotypes. Arrow shows the one polymorphic band for *TaPrx112-D* peroxidase gene in cultivar Yecora Rojo and line KSU 119. The PCR product of the *TaPrx113-F* peroxidase gene was present in all wheat genotypes.

it was shown that cultivar Yecora Rojo and advanced breeding line KSU 119 had the *TaPrx112-D* peroxidase gene. But, the advanced line KSU 119 was resistant while cultivar Yecora Rojo was susceptible to CCN. The possible explanation of this result may be that the advanced wheat line KSU 119 contained *Cre1*(Cereal root eelworm—locus 1) (*Triticum aestivum* 2B) (Williams *et al*. 1994), which was absent in cultivar Yecora Rojo. The *TaPrx112-D* peroxidase gene is located on the short arm of chromosome 2B (Simonetti *et al.*, 2009). The defense mechanism triggered by *Cre1* might have revealed a specific induction of the peroxidase (Delibes *et al*., 2008). Simonetti *et al.* (2009) suggested that wheat apoplastic peroxidases, because of their different expression in quantity and timing, play different roles in the plant response to nematode infection. Also, the work presented in this paper illustrated that wheat lines KSU118, L11-8, L11-21, and KSU 114 contained *Cre3* and were CCN resistant. Therefore, the presence of peroxidase genes alone did not explain differences among wheat genotypes for CCN resistance.

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