Arch. Biol. Sci., Belgrade, 63 (1), 49-53, 2011 DOI:10.2298/ABS1101049C

INDIRECT SELECTION OF *CRE1* GENE IN WINTER WHEAT POPULATIONS

M. ÇALIŞKAN[1], S. URANBEY[2], J. NICOL[3], T. AKAR[4], H. ELEKÇİOĞLU[5] and G. KAYA[6]

[1] *Central Research Institute for Field Crops,* 06100 Ankara, Turkey
[2] *Çankırı Karatekin University, Faculty of Arts and Sciences, Department of Biology, 18000* Çankırı, Turkey
[3] *CIMMYT Ankara Office, 06100 Ankara,* Turkey
[4] *Çukurova University, Department of Plant Protection, 01010 Adana,* Turkey

Abstract - The nematodes are important biotic constraint in rain-fed wheat production systems. In Turkey, they is found in 75.0% of soil samples in Central Anatolia with the dominant species being *Heterodera filipjevi*. Yield losses for winter wheat in rain-fed environments are documented between 27.0-46.0 %. A single dominant gene for resistance to *H. avenae*, designated as *Cre1*, was assessed in Turkey. It was also found to be effective to *Heterodera filipjevi*. In this research, a STS-based *Cre1* marker was applied in a number of segregating wheat populations from F_1 to F_4 to discriminate *Cre1*-positive lines among the wheat populations. Results clearly indicated that Marker Assisted Selection (MAS) is functioning effectively, with recovery of *Cre1* positive lines up to 88.0 % depending on the cross in early stage of breeding.

Key words: Bread wheat, nematode, *Cre1* resistance, STS-PCR, indirect selection.

UDC 595.132:591.5:633

INTRODUCTION

Turkey, with 8.95 million hectare acreage, 17.78 million tons production and 2.2 tons per hectare yield, is one of the largest wheat producers in the world (FAO, 2008). One third of this acreage is under rain-fed conditions and the main aims of wheat breeding in these conditions are to develop new wheat cultivars tolerant to biotic and abiotic stress factors. Nematodes, root rots and rusts are the common biotic factors causing important yield loses in the central plateau of Turkey.

Cereal cyst nematodes (CCN; *Heterodera* spp.) are a global problem and cause significant economic yield loses, especially under rain-fed cereal predominant systems of the world (Nicol et al., 2003). *H. avenae,* which is the most studied CCN species, has yield loses ranging from 15 to 20% of the wheat in Pakistan, 20% of barley and 23 to 50% of wheat in Australia (Nicol, 2002). Preliminary yield loss studies indicate that *H. filipjevi,* which is a common nematode species in Turkey, has resulted in up to 50% yield loss of Turkish common bread wheat cultivars (Nicol et al., 2005).

Chemical protection is not an economical and practical tool to prevent the yield loss caused by nematodes in cereals. However, rotations, different tillage systems and the use of wheat cultivars tolerant to nematodes are the most common methods. Among these, tolerant cultivars are the most effective and economical way to prevent nematode damage (Trudgill et al., 1998; Elekçioğlu et al., 2004).

In the last decade, many resistant sources against different nematode species have been determined. The *Cre1* gene, located in the 2B chromosome of wheat, has been found effective against many European nematode races and Anatolia pathotypes,

while the *Cre3* gene located at the 2D chromosome of wheat has been found to be highly effective against Australian pathotypes of nematodes. The resistance was generally incorporated to cultivated wheat varieties from the wild relatives of wheat (Dosba and Rivoal, 1982; Eastwood et al., 1991; Dhaliwal et al., 1993; Delibes et al., 1993; Bekal et al., 1998; Jahier et al., 1998; Romero et al., 1998; Jahier et al., 2001; Zaharieva et al., 2001).

In Turkey, several studies have determined genetic resistance to nematodes. Öztürk et al. (1999) tested some wheat cultivars against nematodes under field conditions and they could not determine any resistant cultivars in the rain-fed area of the Konya province. However, Akar et al. (2009) screened limited numbers of wild and cultivated wheat varieties and landraces of bread and durum wheat varieties by using *Cre1* and *Cre3* markers, and they determined a new *Cre1* resistant bread wheat landrace.

Marker Assisted Selection (MAS) or indirect selection is a very effective method to distinguish positive lines in crossing the *Cre1* resistant gene against *H. avenae* or *H. filipjevi.* Many studies have been conducted in Australia in order to determine tightly-linked PCR-based markers and the use of these markers in screening some wheat germplasms (Eagles et al., 2001; Ogbonnaya et al., 2001). The International Maize and Wheat Research Center (CIMMYT) also routinely use this strategy to select *Cre1* resistant bread wheat lines to distribute to different research centers around the world.

In this research, we also used *Cre1* markers, kindly provided by the CIMMYT, to test the efficiency of Marker Assisted Selection (MAS) in the different stages of segregation populations of bread wheat which were generated by the International Winter Wheat Improvement Program-TURKEY, CIMMYT, ICARDA (IWWIP).

MATERIALS AND METHODS

Plant material

Seven different winter bread wheat populations generated under the IWWIP were used as plant material in this study. The crossing stage of germplasm changed from F_1 to F_4 and the names of the populations and their pedigrees are summarized in the Table 1.

DNA isolation and PCR conditions

A hundred seeds from each population were labeled and then planted in the first week of October in 2009 in a greenhouse. In each population the fresh leaves were collected and stored in a deepfreeze from each line separately in order to isolate the DNA. DNA isolation was realized by using Fermentas (#K0512) Genomic DNA Purification Kit for each line within a population. The purification and quantification of the DNA was checked by spectrophotometry (Rogers and Bendish, 1988). In this study, we applied the PCR method described by Ogbonnaya *et al.* (2001). According to this method, genomic DNA was amplified in 20-µL reaction volumes consisting of 1x reaction buffer (50 mM Tris-HCI, pH 8.8, 250 mM KCI, and 0.05% gelatin), 1.5 mM $MgCI_2$, 200 µM of each deoxyribonucleotide of each primer, 1 unit of ampli-Taq, 50 pmol of each primer and 100 ng of genomic

Table 1. Plant material used in this study

Name of the populations	Pedigries of the populations
POP1/07F_1-TOP	BURBOT-6/3/SLVS//BAU/MILAN/4/SLVS//BAU/MILAN
POP2/07F_1-TOP	ALTAY 2000/3/SLVS//BAU/MILAN//SLVS/4/BAU/MILAN
POP3/07F_1-TOP	AUS4930 5.3/Spear DH#47//KATIA1/3/KATEA-1
POP4/07F_2-TCI	BURBOT-6/3/SLVS//BAU/MILAN
POP5/07F_2-TCI	JING411//PLK70/LIRA/3/GUN91/4/SLVS//BAU/MILAN
POP6/07F_4-TCI	SARDARI/FRAME//IZGI
POP7/07F_4-TCI	SARDARI/T-2004//GANSU-7

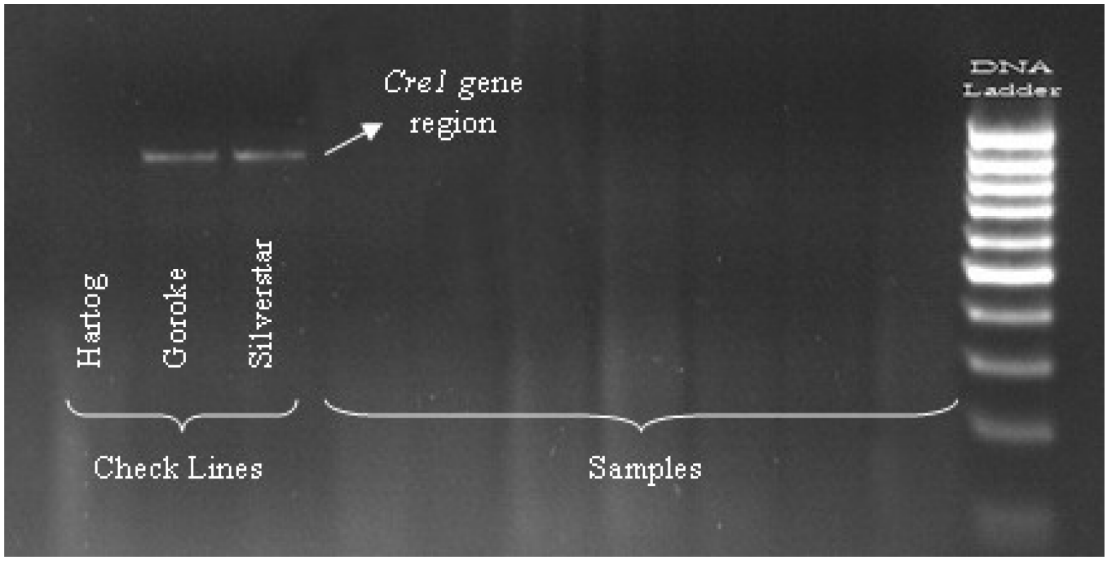

Fig. 1. Gene location of positive *Cre1* check lines.

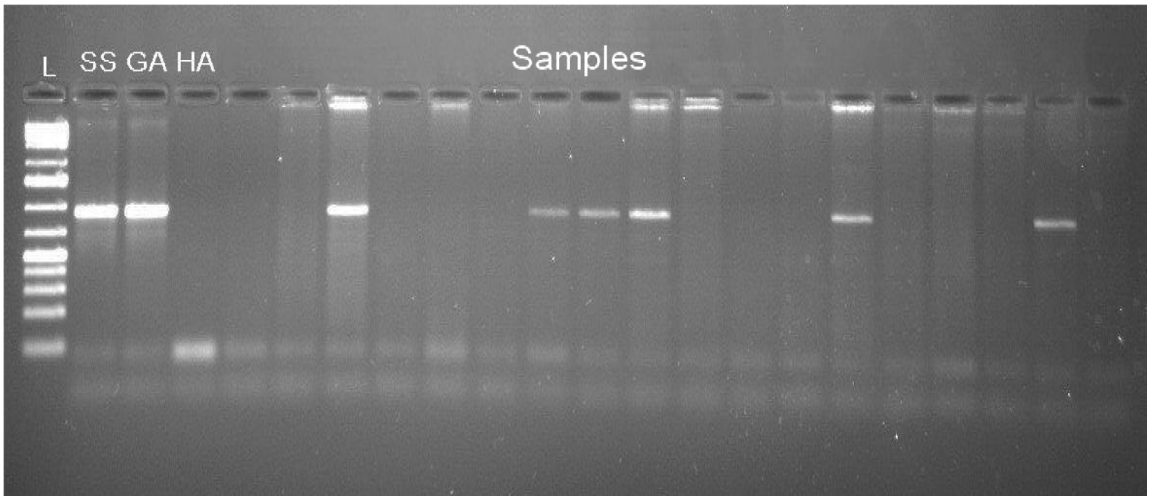

Fig. 2. *Cre1* variation in population 4.

DNA using a thermal cycler. PCR products were separated on 1.5% agarose gel in 1x TAE buffer, stained with ethidium bromide, visualized under UV light and then photographed. Prior to routine screening the wheat populations, gene location of two *Cre1* positive wheat cultivars "Goroke and Silverstar" together with a negative check "Hartog" were visualized (Figure 1).

RESULTS AND DISCUSSION

General evaluations of the wheat germplasms are summarized in Table 2. The highest percentage of the *Cre1*-positive lines was detected in population 2 with 87.64% and it was followed by population 1 with 72.41% (Table 2). These results were almost the same as those of Ogbonnaya et al. (2001). These two

Table 2. General evaluation of wheat germplasm

Name of The Populations	*Number of Screened Lines*	*Number of Cre1 Positive Lines*	Percentage of *Cre1* Positive Lines
POP1/07F_1-TOP	87	63	72.41
POP2/07F_1-TOP	89	78	87.64
POP3/07F_1-TOP	93	-	0.00
POP4/07F_2-TCI	93	16	17.20
POP5/07F_2-TCI	94	1	1.06
POP6/07F_4-TCI	91	-	0.00
POP7/07F_4-TCI	95	-	0.00
Total	**642**	**158**	

populations were at the F_1 breeding stages but the third population at the F_1 stage didn't have any *Cre1* positive lines. This situation can be explained by the use of different *Cre1* sources or the Spear DH#47/AUS4930 5.3 crosses not being realized in practice. For that reason Spear DH#47 could be self-pollinated.

In the F_2 stages, there were two populations, but the *Cre1* positive percentage was much lower when compared to populations in the F_1 stages. The clear variation among the different breeding lines is shown in Fig. 2. This can be explained by segregation in the F_2 stages and also in the effective crossing, especially for population 5. In this study there were also two populations at F_4 stages but resistance sources were completely different from the others. Although many attempts were made to determine *Cre1*-positive lines, we couldn't find any *Cre1*-positive lines among the two different populations. This situation can be attributed to the *Cre1* genetic sources, Sardari, or segregation and selection pressure could be the main reasons. This result cannot overlap the output of Akar et al. (2009). More research, especially in the early stage of crossing, is required with the Sardari landraces.

Our study clearly shows that the MAS of the *Cre1* gene is both time and labor saving and a more effective tool when compared to the physical testing of nematodes. Moreover, in order to obtain concrete data, more attention should be given to the self-pollination purity of *Cre1* parents, and MAS should be resumed at the early stage of crossing. This first study on MAS of nematode-resistant winter bread wheat lines demonstrated that the technique can be easily integrated into programs in order to increase the wheat breeding. Additionally, doubled-haploid production of these nematode resistant lines can also accelerate wheat breeding cycles.

Acknowledgments - We gratefully acknowledge the contribution of the Scientific and Technological Council of Turkey (TUBITAK) under 106 G 095 coded project.

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