

Assessment of resistance gene analog, peroxidase, and WRKY gene polymorphisms in the genus *Lens* Miller

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Abstract: Resistance gene analogs (RGAs), peroxidases (POXs), and WRKY transcription factors are gene families related to stress-response pathways that regulate enhancement of stress tolerance in crop plants. In this study, the polymorphisms of these gene families were determined in the genus *Lens* Miller and *Vicia montbretii* (Fish. & Mey) Davis and Plitmann. A total of 32 genotypes of 7 lentil taxa, including 1 accession of cultivated lentil (*Lens culinaris* Medik. subsp. *culinaris*), 11 accessions of *L. culinaris* subsp. *orientalis* (Boiss.) Ponert, 3 accessions of *L. culinaris* subsp. *tomentosus* Ladiz., 4 accessions of *L. culinaris* subsp. *odemensis* Ladiz., 3 accessions of *L. ervoides* (Brign.) Grande, 4 accessions of *L. lamottei* Czefer., 4 accessions of *L. nigricans* (M.Bieb.) Godr., and 2 accessions of *V. montbretii*, were used. Sixty-eight RGA, 22 POX, and 13 WRKY primers were used for molecular analysis. After using these degenerate primers in polymerase chain reaction, the highest polymorphism rate (90%) was observed in WRKY, followed by POX (89%) and RGA (79%). *Lens culinaris* Medik. subsp. *culinaris* had the lowest number of RGA and POX bands. Most RGA markers were obtained from *L. culinaris* subsp. *tomentosus*, *L. culinaris* subsp. *orientalis*, *L. culinaris* subsp. *odemensis*, *L. ervoides*, and *L. nigricans*. Similarly, the number of WRKY markers was the highest in *L. nigricans* and *L. culinaris* subsp. *orientalis*. *Lens nigricans* had the highest number of POX markers among lentils. The highest numbers of RGA, POX, and WRKY markers in *L. nigricans* and *L. culinaris* subsp. *orientalis* were in agreement with resistance for biotic and abiotic stresses, as these two taxa in the genus *Lens* Miller were resistant to many stresses. These results indicate that primers related to RGA, POX, and WRKY gene families may be considered potential markers for developing stress-related resistance genotypes.

Key words: Biotic and abiotic stress, disease resistance, lentil, molecular markers, polymorphism

1. Introduction

Many biotic and abiotic stress factors, such as cold, drought, salinity, diseases, and insects, adversely affect plant growth and productivity worldwide (Ujagir et al., 2009). Under these stress conditions, plants activate their defense mechanisms to provide resistance (Sharma and Muehlbauer, 2007). These mechanisms are often initiated by the presence of resistance (R) genes in the plants (Mutlu et al., 2006). Resistance gene analogs (RGAs), which are associated with R genes, are tightly linked to plant-pathogen interactions (Mutlu et al., 2006). Other gene families, including peroxidases and WRKY transcription factors, are also related to stress-response pathways that regulate enhancement of stress tolerance in crop plants (Century et al., 2008).

Many R genes have been determined in plants to identify resistance against viruses, bacteria, and nematodes. These genes have been sequenced, and results of these sequences indicate that the R genes are represented largely by domains of nucleotide-binding sites

(NBSs) and leucine-rich repeats (LRRs) (Martin et al., 2003). Although R genes exist in varied taxonomic groups, they share conserved domains and structural similarities. NBS-LRR, coiled-coil, extracellular transmembrane, and cytoplasmic serine/threonine kinase catalytic domains are parts of plant R genes (Ellis et al., 2000; Schulze-Lefert and Panstruga, 2011).

Plant peroxidases are heme-containing glycoproteins (Yoshida et al., 2003). Most peroxidase isoenzymes are encoded by multigene families in higher plants. Peroxidases have been characterized as having several physiological functions, such as oxidation of toxic reductants, biosynthesis and degradation of lignin in cell walls (Mader and Fussl, 1982; Lagrimini, 1991), auxin catabolism (Gazaryan and Lagrimini, 1996), and responses to pathogens or insects (Dowd and Lagrimini, 1997). The distal heme-binding domain, the central domain, and the proximal heme-binding domain are highly conserved motifs in plant peroxidases (Hiraga et al., 2001).

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The WRKY genes encode important transcription factors for plants that regulate physiological responses to stress factors, senescence, seed development, and production of secondary metabolites (Kim et al., 2000; Yoda et al., 2002). These genes contain sequences of 60 amino acids, referring to WRKY domain. In this domain, a conserved WRKYGQK sequence is followed by a C₂H₂- or C₂HC-type of zinc-finger motif. The WRKY domain binds to a DNA sequence designated as the W-box in the promoter of the target gene to regulate gene expression (Eulgem et al., 2000).

Lentil (*Lens culinaris* Medik. subsp. *culinaris*) is a self-pollinating, diploid (2n = 14), annual, cool-season food legume crop (Zohary, 1972; Erskine, 1997). It is one of the important protein sources for human nutrition (Laserna-Ruiz et al., 2012). As in many crops, several abiotic stresses, especially drought, heat, salinity susceptibility, and iron deficiency, affect lentil and play a large role in reducing its seed yield (Saxena, 1993; Bansal et al., 2014). Various biotic stress factors, such as *Ascochyta* blight, anthracnose, *Fusarium* wilt, *Sclerotinia* white mold, rust, and various aphid-transmitted viruses, cause adverse effects during plant growth (Muehlbauer et al., 2006). Breeding for resistant varieties requires screening methodologies and selection for resistant plants, which are often laborious and require extensive knowledge of plant-pathogen interactions (Jayashree et al., 2010). Specific molecular markers related to the genes conferring resistance have great advantages to combat biotic and abiotic stress factors. The conserved motifs in all these domains have allowed, via polymerase chain reaction (PCR) using degenerate primers, for amplification of RGA, peroxidase (POX), and WRKY gene families from many plant species. The RGA (Mutlu et al., 2006; Satya et al., 2014; Wang et al., 2014), POX (Gulsen et al., 2007, 2010; Ceylan et al., 2014; Nemli et al., 2014; Satya et al., 2014), and WRKY (Borrone et al., 2004; Kim et al., 2008; Lee et al., 2011; Li et al., 2013) polymorphisms have been used in genetic diversity studies in different plants.

The goal of this study, therefore, was to identify RGA, POX, and WRKY gene families to enhance breeding and resistance studies in lentil. This is the first comprehensive report for which three different gene families were used among *Lens* species.

2. Materials and methods

2.1. Plant material

Thirty accessions of *Lens* and two accessions of *Vicia* were used as genetic material in this study (Table 1). Out of *L. culinaris* Medik. subsp. *culinaris*, 29 accessions of wild species were used, comprising 3 of *L. ervoides* (Brign.) Grande, 4 *L. lamottei* Czefr., 4 *L. nigricans* (M.Bieb.) Godr., 4 *L. culinaris* subsp. *odemensis* Ladiz., 11 *L. culinaris* subsp. *orientalis*

(Boiss.) Ponert, and 3 *L. culinaris* subsp. *tomentosus* Ladiz. Two accessions of *V. montbretii* (Fisch. and Mey.) Davis and Plitmann were used for confirmation, since *Vicia montbretii* was systematically classified as a member of *Lens* Miller before 1982 (Ladizinsky and Sakar, 1982).

2.2. DNA extraction

Total DNA was extracted from fresh leaves with some modifications of the CTAB method (Doyle and Doyle, 1990). Modifications consisted of extra chloroform and isoamyl addition and also ethanol washing in order to obtain higher purity. The concentration and the quality of DNA were tested using 1% agarose gel with a DNA standard. The DNA extracts were suspended in Milli-Q PCR water and stored at -20 °C until use.

2.3. Degenerate primers

Sixty-eight RGA degenerate primers were used in this study. Out of 68, 20 primers were designed by Mutlu et al. (2006) from the highly conserved motifs of Kinase-1a (K) and the hydrophobic domain (HD) of NBS-LRR-type resistance genes. In the PCR reaction, K and HD primers acted as forward and reverse primers, respectively. Other RGA degenerate primers were acquired from the work of Rajesh et al. (2002), using 48 of the available RGA primers and their 24 different combinations.

Twenty-two combinations of 14 forward and 16 reverse peroxidase primers (Gulsen et al., 2007) were handled to target the amplification of peroxidase sequences from genomic DNA for genotypes.

Thirteen WRKY degenerate primers were used, and 8 primers were studied by Kim et al. (2007). One of the primers (WS primer) was designed from nucleotides encoding WRKYG (the WRKY core sequence), and the other primer (G primer) was designed from conserved sequences of each group. The other 5 primers were obtained from the study of Borrone et al. (2004). Two of the 5 primers (WRKY 1 FP and WRKY 2 RP) were selected from the generated list. Three additional primers (WRKY 2 FP, WRKY 3 FP, and WRKY 3 RP) were selected based on the study of Borrone et al. (2004).

2.4. PCR amplification conditions

The PCR analyses were conducted with templates for the PCR reactions set up for 15 µL as follows: 1.5 µL of 10X PCR buffer, 200 µM of each dNTP, 2 mM MgCl₂, 10 pM µL⁻¹ of the primer pairs, 1 U of Taq DNA polymerase (Fermentas Life Sciences, Burlington, Canada), 1 µL of genomic DNA template, and Milli-Q water to a final volume of 15 µL. Amplification was performed in a programmable thermocycler (MyGenie, Bioneer) under the following conditions: 94 °C for 2 min; 34 cycles of 94 °C for 1 min, annealing temperature of 48-57 °C for 1 min, and 72 °C for 90 s; and a final extension of 10 min at 72 °C. The PCR products were separated in 2%–3% agarose gels in

Table 1. Plant identification numbers and origins of *Lens* and *Vicia* taxa.

Number	PI number	Species	Status	Origin
1	-	<i>Lens culinaris</i> subsp. <i>culinaris</i>	Cultivated	Turkey
2	PI 72558	<i>Lens culinaris</i> subsp. <i>odemensis</i>	Wild	Turkey
3	PI 72686	<i>Lens culinaris</i> subsp. <i>odemensis</i>	Wild	Syria
4	PI 572360	<i>Lens culinaris</i> subsp. <i>odemensis</i>	Wild	Israel
5	PI 572361	<i>Lens culinaris</i> subsp. <i>odemensis</i>	Wild	Israel
6	PI 72524	<i>Lens culinaris</i> subsp. <i>orientalis</i>	Wild	Cyprus
7	PI 72534	<i>Lens culinaris</i> subsp. <i>orientalis</i>	Wild	Syria
8	PI 72592	<i>Lens culinaris</i> subsp. <i>orientalis</i>	Wild	Uzbekistan
9	PI 72593	<i>Lens culinaris</i> subsp. <i>orientalis</i>	Wild	Iran
10	PI 72599	<i>Lens culinaris</i> subsp. <i>orientalis</i>	Wild	Palestine
11	PI 72847	<i>Lens culinaris</i> subsp. <i>orientalis</i>	Wild	Jordan
12	PI 72900	<i>Lens culinaris</i> subsp. <i>orientalis</i>	Wild	Uzbekistan
13	PI 72901	<i>Lens culinaris</i> subsp. <i>orientalis</i>	Wild	Turkmenistan
14	PI 72925	<i>Lens culinaris</i> subsp. <i>orientalis</i>	Wild	Lebanon
15	PI 572386	<i>Lens culinaris</i> subsp. <i>orientalis</i>	Wild	Turkey
16	PI 612249	<i>Lens culinaris</i> subsp. <i>orientalis</i>	Wild	Turkey
17	PI 72613	<i>Lens culinaris</i> subsp. <i>tomentosus</i>	Wild	Turkey
18	PI 72616	<i>Lens culinaris</i> subsp. <i>tomentosus</i>	Wild	Turkey
19	PI 72672	<i>Lens culinaris</i> subsp. <i>tomentosus</i>	Wild	Syria
20	PI 572335	<i>Lens ervoides</i>	Wild	Turkey
21	PI 572338	<i>Lens ervoides</i>	Wild	Turkey
22	PI 72565	<i>Lens ervoides</i>	Wild	Italy
23	PI 72552	<i>Lens lamottei</i>	Wild	Spain
24	PI 110813	<i>Lens lamottei</i>	Wild	Spain
25	PI 116006	<i>Lens lamottei</i>	Wild	Turkey
26	PI 136637	<i>Lens lamottei</i>	Wild	France
27	PI 72546	<i>Lens nigricans</i>	Wild	Italy
28	PI 72547	<i>Lens nigricans</i>	Wild	Croatia
29	PI 572344	<i>Lens nigricans</i>	Wild	Spain
30	PI 615676	<i>Lens nigricans</i>	Wild	Turkey
31	PI 515984	<i>Vicia montbretii</i>	Wild	Turkey
32	PI 632670	<i>Vicia montbretii</i>	Wild	Turkey

1X TBE buffer and visualized under ultraviolet light after staining with ethidium bromide. The expected bands were determined visually and recorded.

2.5. Statistical analysis

Each band was scored as present (1) or absent (0), and data were analyzed with the NTSYS-pc version 2.1 software package (Exeter Software, Setauket, NY, USA) (Rohlf, 1993). A similarity matrix was constructed based on Dice's coefficient (Dice, 1945), which considers only one-to-one matches between genotypes for similarity.

3. Results

The RGA primers were tested with bulk DNA, and amplicons yielded were identified in all 32 accessions. After this process, polymorphic primer combinations were selected. These combinations are given in Table 2. A total of 103 bands for RGA primers were scored in *Lens* and *Vicia* taxa. Eighty-five (79%) bands were polymorphic. Band sizes ranged from 50-1500 bp (Table 3). Similarity of bands of RGAs ranged from 0.63 to 0.91, averaging 0.77. The LM 637/LM 638 primer combinations had the lowest number of bands with 5, while the S2-INV/AS3-INV combination produced the highest number of bands per amplification (Figure 1). The K03/HD01 primer combination had 7 polymorphic bands out of 10 that ranged from 50 to 1000 bp. Five polymorphic bands were obtained using the K04/HD01 combination in the 150-950-bp range. The K06/HD03 primer combination produced 6 bands in the 210-1400-bp range. One band of 210 bp was monomorphic in all genotypes. All other bands were polymorphic. All of the 12 polymorphic bands were observed with the S2INV/AS3INV primer combination.

The polymorphisms of the POX marker exhibited appropriate numbers of different fragments for 22 primer pairs. Primer sizes ranged from 13 (POX9F) to 24 (POX14R), averaging 18.4 bases. The highest number of bands was observed in the 7 primer combinations that were selected. A total of 66 DNA bands, amplified by 7 different oligonucleotide primers, were scored among each accession. Fifty-nine out of 66 (89%) bands were polymorphic (Table 3). The approximate sizes of the bands varied from approximately 150 to 1250 bp. Amplification with the POX 3 FP/RP and POX 5 FP/RP primer combinations yielded the highest number of bands with 11, while the POX 7 primer produced the lowest number of bands with 7 (Figure 1).

Of the 10 possible WRKY primer combinations, only the GI, GII, GIII, and WRKY 2 FP/WRKY 3 RP and WRKY 3 FP/WRKY 3 RP combinations consistently resulted in prominent PCR products. A total of 52 bands were obtained with the primer combinations. Band sizes ranged from 200 to 1450 bp. Forty-seven of the scored bands were polymorphic; the rate of polymorphism was 90% (Table 3). The WRKY 3 FP/WRKY 3 RP combination produced the fewest number of bands, while GI and GII produced the highest numbers of bands per amplification (Figure 1). All of the bands obtained using the GI and WRKY 3 FP/WRKY 3 RP primers were polymorphic.

The RGA, WRKY, and POX primers amplified different numbers of bands for each species (Figure 2). The cultivated species, *L. culinaris* subsp. *culinaris*, produced the least number of bands in agarose gel after RGA and POX analyses. *L. culinaris* subsp. *tomentosus*, *L. lamottei*, and *V. montbretti* produced the highest number of bands using RGA, WRKY, and POX primers, respectively.

Table 2. Polymorphic primers of RGA, POX, and WRKY primers of *Lens* and *Vicia* taxa.

Gene-specific primer	Combination	References
RGA primers	K03/HD01, K04/HD01, K05/HD01, K06/HD02, K06/HD03, K06/HD06, K07/HD03, K07/HD10, K08/HD03, K10/HD03, LM 637/LM 638, XLRR F/R, S2-INV/AS3-INV, Xa1-NBS F-R	Rajesh et al., 2002; Mutlu et al., 2006
POX primers	POX 1, POX 2, POX 3, POX 5, POX 7, POX 8c, POX 10	Gulsen et al., 2007
WRKY primers	G1, GII, GIII, WRKY 2/3, WRKY 3/3	Borrone et al., 2004; Kim et al., 2007

Table 3. Comparison of primers for their capacity to generate polymorphisms in *Lens* and *Vicia* taxa.

Primers	Band sizes (bp)	No. of resolvable bands	No. of polymorphic bands	Polymorphism (%)
RGA	50-1500	103	81	79
POX	150-1250	66	59	89
WRKY	200-1450	52	47	90

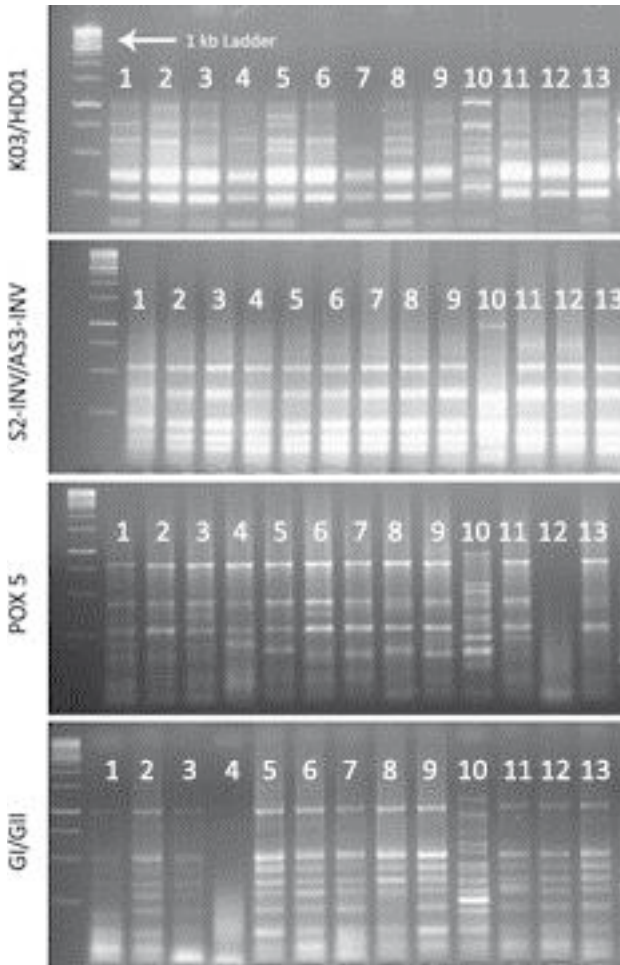


Figure 1. Agarose gel (2.5%) images of best primer pairs in 1: *L. culinaris* subsp. *culinaris*, 2–5: *L. culinaris* subsp. *odemensis*, 6–13: *L. culinaris* subsp. *orientalis*.

4. Discussion

Generation of RGA, POX, and WRKY polymorphisms used a PCR-based approach with degenerate primer combinations that targeted the conserved domains in these gene families. The RGA, POX, and WRKY primers were evaluated in this study. They also were studied by Rajesh et al. (2002) in chickpea (*Cicer arietinum* L.), Borrone et al. (2004) in cacao (*Theobroma cacao*), Mutlu et al. (2006) in common bean (*Phaseolus vulgaris* L.), Gulsen et al. (2007) in buffalograss (*Buchloe dactyloides* [Nutt.] Engelm.), and Kim et al. (2007) in chili pepper (*Capsicum annuum* L.). Several primers yielded scorable bands that were used to understand resistance genes among the lentil genetic resources. Eighty-five out of the 103 RGA markers (79%) from *Lens* sp. and *Vicia* sp. were polymorphic. The polymorphism rate was 89% in POX markers and 90% in WRKY markers. Different results previously were reported using gene families in other plants. Only one RGA primer pair (Ptokin1: 5'GCATTGGAACAAGGTGAA-3'; Ptokin2: 5' AGGGGGACCACCACGTAG-3') revealed polymorphism in the *Cicer* genotypes studied by Rajesh et al. (2002). Such a low level of polymorphism in *Cicer* has also been observed using various other marker systems (Winter et al., 2000). In our study, we observed no scorable bands with this primer combination, and the polymorphism value was 79%. Toklu et al. (2009), using AFLP for 38 Turkish lentil landraces and 6 commercial lentil cultivars, observed 119 polymorphic bands with a polymorphism value of 56%. Thus, the level of RGA polymorphism was higher compared to AFLP polymorphism. The same POX primers were analyzed in apple collections by Gulsen et al. (2010). All primers used in their study yielded scorable bands, except for POX gene

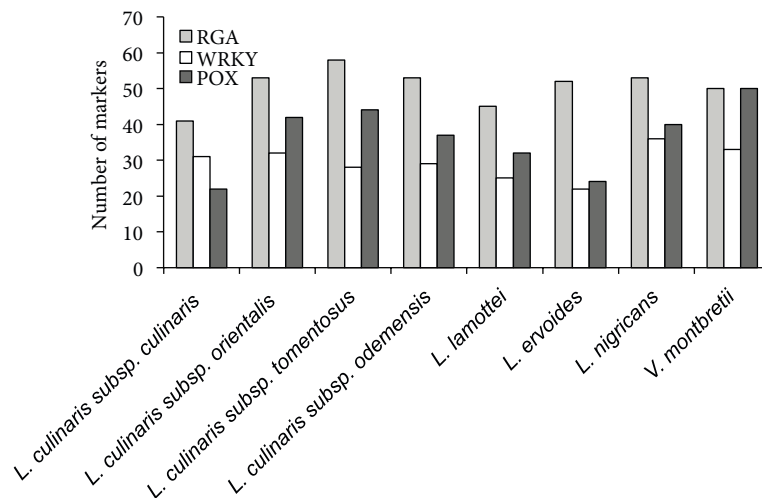


Figure 2. The distribution of the RGA, POX, and WRKY markers by species.

polymorphism (POGP) 9. Similarly, we also observed no scorable bands with this primer. Fifty-nine out of the 66 POGP markers (89%) from *Lens* sp. and *Vicia* sp. were polymorphic. Zhang et al. (2001) indicated that the POX gene family had the second-lowest sequence conservation among 25 multigene families. Degenerate PCR primer pairs designed to specifically amplify WRKY gene fragments directly from genomic DNA have been reported in cacao (Borrone et al., 2004) and chili pepper (Kim et al., 2007). A number of bands ranging from 250 bp to more than 1.5 kb were observed. These results were consistent with our results.

RGA polymorphism analysis indicated that similarity ranged from 0.63 to 0.91, averaging 0.77 among the 32 accessions. The results showed significant diversity among the wild and cultivated types of plants used in this study. The number of amplified fragments ranged from 5 to 12, with a mean of 11.2 bands per amplification. The

K07HD10 and LM 637/LM 638 primer combinations produced the lowest number of bands, while S2-INV/AS3-INV produced the highest number per amplification. In the POGP analysis, band size ranged from 150 to 1250 bp. The POGP bands exhibited a similarity range of 0.58-0.92, averaging 0.75. All of the amplified fragments were polymorphic using POX 7 and POX 10 primers. Results indicating POX markers appear to be useful for identifying polymorphism in lentil.

Turkey is within the diversity center for the genus *Lens* Miller. Although abundant variation exists among species of *Lens*, advances in breeding studies on lentil have not been achieved due to the lack of traits related to stress factors and low genetic diversity in cultivated lentils (Erskine et al., 1994; Erskine, 1997; Ferguson et al., 2000; Duràn and Pérez de la Vega, 2004). The wild species are good sources to combat biotic or abiotic stresses (Table 4) and for many other useful agronomic characters. As

Table 4. Sources of resistance to biotic and abiotic stresses in wild lentils (*Lens* sp.).

Stress factors	Species	Accessions	References
Anthracnose	<i>L. ervoides</i>	ILWLs-123, 128, 131, 276, 280, 292, 461, 406, 413	Tullu et al., 2006
	<i>L. nigricans</i>	ILWLs-27, 28, 34, 190	Tullu et al., 2006
	<i>L. lamottei</i>	ILWLs-428, 429	Tullu et al., 2006
Ascochyta	<i>L. culinaris</i> subsp. <i>odemensis</i>	ILWLs-20, 83, 100, 170, 254, W6 3244, W6 3222	Ahmad et al., 1997; Tullu et al., 2010
	<i>L. culinaris</i> subsp. <i>orientalis</i>	ILWLs-99, 119, 306, 88, 69, 382, W6 3241, W6 3261, W6 3248	Bayaa et al., 1994; Ahmad et al., 1997; Tullu et al., 2010
	<i>L. ervoides</i>	ILWLs-123, 128, 131, 276, 292, W6 3173, W6 3176	Bayaa et al., 1994; Ahmad et al., 1997; Tullu et al., 2006; Tullu et al., 2010
Powdery mildew	<i>L. lamottei</i>	ILWL-432	Tullu et al., 2010
	<i>L. nigricans</i>	ILWLs-24, 30, 34, 37, 110, 190, 272, 452, W6 3208, W6 3210, W6 3218, W6 3221	Bayaa et al., 1994; Ahmad et al., 1997; Tullu et al., 2010
	<i>L. culinaris</i> subsp. <i>orientalis</i>	ILWL-200 ILWLs-40, 41, 42, 60, 61, 204, 207, 251, 271, 337 ILWLs-22, 26, 29, 37, 38	Gupta and Sharma, 2006 Gupta and Sharma, 2006 Gupta and Sharma, 2006
Rust	<i>L. culinaris</i> subsp. <i>odemensis</i>	ILWLs-21, 153, 165, 167, 174, 237, 238, 252, 403	Gupta and Sharma, 2006
	<i>L. culinaris</i> subsp. <i>orientalis</i>	ILWLs-70, 72, 74, 117, 189, 200, 226, 247	Gupta and Sharma, 2006
	<i>L. ervoides</i>	ILWLs-40, 41, 42, 50, 54, 61, 133, 159, 207, 251, 258, 259, 280, 299	Gupta and Sharma, 2006
	<i>L. nigricans</i>	ILWLs-22, 24, 25, 31, 37, 38, 191, 430	Gupta and Sharma, 2006
Sitona weevils	<i>L. culinaris</i> subsp. <i>odemensis</i>	ILWLs-166, 203, 207, 254	El-Bouhssini et al., 2008
	<i>L. culinaris</i> subsp. <i>orientalis</i>	ILWL-245	El-Bouhssini et al., 2008
	<i>L. ervoides</i>	ILWLs-136, 258	El-Bouhssini et al., 2008
	<i>L. nigricans</i>	ILWL-110	El-Bouhssini et al., 2008
Stemphylium blight	<i>L. culinaris</i> subsp. <i>odemensis</i>	ILWLs-20, 100, 222, 254, 436	Podder et al., 2013
	<i>L. culinaris</i> subsp. <i>orientalis</i>	ILWLs-99, 119, 88, 69, 384, 382, 421	Podder et al., 2013
	<i>L. ervoides</i>	ILWLs-123, 128, 131, 276, 292, 413	Podder et al., 2013
	<i>L. nigricans</i>	ILWLs-16, 24, 26, 27, 37, 110, 272, 446	Podder et al., 2013
Cold	<i>L. culinaris</i> subsp. <i>orientalis</i>	ILWLs-89, 91, 309	Hamdi et al., 1996
	<i>L. culinaris</i> subsp. <i>odemensis</i>	ILWLs-175, 167	Hamdi and Erskine, 1996
Drought	<i>L. ervoides</i>	ILWLs-311, 303	Hamdi and Erskine, 1996
	<i>L. nigricans</i>	ILWLs-266, 305	Hamdi and Erskine, 1996

resistance sources, anthracnose resistance in *L. ervoides*, *L. lamottei*, and *L. nigricans* (Tullu et al., 2006); *Ascochyta* blight resistance in *L. culinaris* subsp. *odemensis* and subsp. *orientalis*, *L. ervoides*, and *L. nigricans* (Bayaa et al., 1994; Ahmad et al., 1997; Tullu et al., 2010); *Fusarium* wilt resistance in *L. culinaris* subsp. *orientalis* and *L. ervoides* (Bayaa et al., 1994; Gupta and Sharma, 2006); powdery mildew resistance in *L. culinaris* subsp. *orientalis* and *L. nigricans* (Gupta and Sharma, 2006); rust resistance in *L. culinaris* subsp. *odemensis* and subsp. *orientalis*, *L. ervoides*, and *L. nigricans* (Gupta and Sharma, 2006); *Stemphylium* blight resistance in *L. culinaris* subsp. *odemensis* and subsp. *orientalis*, *L. ervoides*, and *L. nigricans*; drought resistance in *L. culinaris* subsp. *odemensis*, *L. ervoides*, and *L. nigricans* (Hamdi and Erskine, 1996; Gupta and Sharma, 2006); cold tolerance in *L. culinaris* subsp. *orientalis* (Hamdi et al., 1996); and *Sitona* weevil resistance in *L. culinaris* subsp. *odemensis* and subsp. *orientalis*, *L. ervoides*, and *L. nigricans* (El-Bouhssini et al., 2008) have been identified. In the present study, a parallelism was found between the number of identified RGA, POX, and WRKY markers and resistance for biotic and abiotic stresses in wild species (Table 4), as wild lentils had more markers than cultivated lentil (Figure 2). Furthermore, *V. montbretii* had more markers than the cultivated lentil (Figure 2). Most RGA markers were obtained from *L. culinaris* subsp. *tomentosus*, followed by *L. culinaris* subsp. *orientalis*, *L. culinaris* subsp. *odemensis*, *L. ervoides*, and *L. nigricans* (Figure 2). These 5 taxa were reported as resistance sources (Table 4). Similarly, the number of WRKY markers was the most in *L. nigricans* and *L. culinaris* subsp. *orientalis* (Figure 2). *Lens nigricans* had the most number of POX markers among lentils and *V. montbretii* (Figure 2). As seen in Table 1, *L. nigricans* and *L. culinaris* subsp. *orientalis* had the

most desirable genes responsible for resistance to biotic and abiotic stresses (Bayaa et al., 1994; Ahmad et al., 1997; Gupta and Sharma, 2006; El-Bouhssini et al., 2008; Tullu et al., 2010). The cultivated lentil had fewer resistance markers for biotic and abiotic stresses compared to wild species (Figure 2), since the cultivated lentils, especially local types, lost most biotic and abiotic resistance genes during domestication. Erskine et al. (1994) reported that indigenous lentils were lacking desirable genes. Therefore, these markers should be evaluated for further crop breeding and selection. Introgression of desirable genes from wild *Lens* taxa to cultivated lentil might help the flow of useful genes into cultivated lentil. However, there has been limited success in transferring biotic and abiotic stress resistance from wild *Lens* to cultivated lentil, mainly because of postzygotic barriers (Muehlbauer et al., 2006). *L. culinaris* subsp. *orientalis* is readily crossable with the cultivated lentil (Ladizinsky et al., 1984), and *L. culinaris* subsp. *odemensis* and both other subspecies of *L. culinaris* crosses produce partially fertile hybrids (Abbo and Ladizinsky, 1991). However, cultivated lentil crossed with *L. ervoides* or *L. nigricans* causes pod abortion (Abbo and Ladizinsky, 1991). Embryo rescue techniques make possible the development of interspecific hybrids between the cultivated lentil and *L. ervoides* and *L. nigricans* (Ladizinsky et al., 1985).

Identification of resistance-related genes using wild species is important for developing new markers. The markers obtained in this study were considered to be related to abiotic or biotic stresses. These potential markers should also be taken into consideration to develop stress-related resistance genotypes via crosses between wild types and cultivars.

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