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RESEARCH ARTICLE

New sources of resistance to *Colletotrichum truncatum* race Ct0 and Ct1 in *Lens culinaris* Medikus subsp. *culinaris* obtained by single plant selection in germplasm accessions

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Abstract Anthracnose caused by the fungal pathogen *Colletotrichum truncatum* is a severe disease of lentil (*Lens culinaris* Medikus subsp. *culinaris*) causing premature defoliation and deep penetrating lesions on the stems leading to wilting and plant death. A total of 579 accessions from 20 countries were obtained from four germplasm collections in Russia, Poland, Bulgaria and Hungary. The accessions were collected between 1923 and 1988 and comprised mostly landraces. Consequently, many of the resistant entries contained susceptible plants which necessitated one or two cycles of selection of individual resistant plants for selfing and re-testing with the pathogen. Under controlled environmental conditions, plants of each accession were inoculated at early flower with *C. truncatum* race Ct0 (isolate 95A8) and race Ct1 (isolate 95B36), separately. Scoring of symptoms included number of lesions on the main stem, lesion penetration into the stem and amount of wilting. Resistance was obtained by single plant selection in 23 lentil accessions (4.0 %). Fifteen lines were generated with resistance to race Ct1 (2.6 %), seven with resistance to race Ct0 (1.2 %), and one line with resistance to both races. This is the first report on resistance in *L. culinaris* to *C. truncatum* race Ct0 as well as to the two races combined. Seed of homozygous resistant lines can be requested from the corresponding author, and are labeled with their original accession number with the prefix either -Ct0, -Ct1 or -Ct0Ct1 indicating resistance to one or both races of *C. truncatum*.

Keywords Lentil · *Lens culinaris* Medikus subsp. *culinaris* · Anthracnose · *Colletotrichum truncatum*

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Introduction

Anthracnose, caused by *Colletotrichum truncatum* (Schwein.) Andrus et W.D. Moore, is a damaging fungal pathogen of lentil (*Lens culinaris* Medikus subsp. *culinaris*) in North America. In Canada, the disease was first reported in the province of Manitoba in 1987 (Morrall 1988). A few years later it was found in both Saskatchewan (Morrall and Pedersen 1991) and North Dakota, United States (Venette et al. 1994). Anthracnose of lentil has also been reported from Bulgaria, Morocco, Ethiopia, Syria, Pakistan, Bangladesh and Brazil (Bellar and Kebabeh 1983; Baldanzi et al. 1988; Morrall 1997; Kaiser et al. 1998).

Colletotrichum truncatum survives in the form of microsclerotia in infested lentil debris for up to 4 years under western Canadian conditions (Buchwaldt et al. 1996). The first disease symptoms develop at early flowering when tan, necrotic lesions form on the lower leaflets leading to premature defoliation. Sunken tan colored lesions with dark margins, which are typical for anthracnose, start to develop at the stem base and spread to the upper part of the main stem and side branches throughout the growing season.

In Canada, resistance to anthracnose was initially identified in lentil cultivar 'Indianhead' and accession PI 320937 (U. S. Department of Agriculture—Agriculture Research Service, USDA-ARS; Bernier et al. 1992). 'Indianhead' which has a black seed coat was crossed into several different breeding lines with seed types suitable for human consumption (Buchwaldt et al. 1995) resulting in development of cultivars such as 'CDC Robin' and 'CDC Redberry' with improved level of anthracnose resistance under field conditions (Vandenberg et al. 2002, 2006). Fewer and smaller stem lesions combined with longer incubation and latent periods was the basis of resistance in 'Indianhead' and PI320937 significantly reducing the rate of disease development (Chongo and Bernier 1999). About one third of Canadian cultivars have some tolerance to anthracnose in indoor tests (Saskatchewan Ministry of Agriculture 2012). However, these cultivars become infected under field conditions primarily due to the lack of resistance to race Ct0. Before co-dominant molecular markers became available in lentil, RAPD (Randomly Amplified Polymorphic DNA) markers were used to map the location of a locus conferring anthracnose resistance in PI320937 (Tullu et al. 2003). It was later shown to be located close to a RAPD marker locus for resistance to *Ascochyta lentis* Vassilievsky, the cause of ascochyta blight (Tullu et al. 2006). These markers were used to combine anthracnose and ascochyta resistance from two parental lines and demonstrated the level of efficiency for each set of markers (Tar'an et al. 2003).

The first systematic screening of cultivated lentil (*L. culinaris* Medikus subsp. *culinaris*) for resistance to *C. truncatum* was conducted by Buchwaldt et al. (2004). It encompassed 1567 germplasm accessions obtained from the USDA-ARS in Pullman, WA, and 204 accessions from the Institute für Pflanzengenetik und Kulturflanzenforschung (IPK) in Gatersleben, Germany. The lentil germplasm originated from 51 countries with 44 % of the entries collected in Turkey, Iran, Iraq, and Syria, which is the centre of origin of *Lens* as described in Barulina (1930) and reviewed by Cubero et al. (2009). However, only 16 accessions (1 %) were identified with resistance to *C. truncatum*. These accessions originated from Czechoslovakia, the former Soviet Union, Bulgaria, Germany, Greece and Brazil. Screening of resistant accessions with 50 isolates revealed the presence of two different races of the pathogen designated Ct0 and Ct1. The accessions were resistant to race Ct1, but were all susceptible to race Ct0 (Buchwaldt et al. 2004).

Future improvement of anthracnose resistance in lentil relies on identification of germplasm with resistance to both race Ct1 and Ct0 of *C. truncatum*. Since resistance to anthracnose in the previous study was identified among accessions from countries in Eastern Europe (Buchwaldt et al. 2004), the objective of the present study was focused on screening of more accessions from this region. Here we report on development of a set of lentil lines homozygous resistant to either one or both races of *C. truncatum* obtained by single plant selection in germplasm accessions.

Materials and methods

Plant material

A total of 579 *L. culinaris* subsp. *culinaris* accessions were obtained from four germplasm collections: the Vavilov Institute of Plant Industry, St. Petersburg, Russia (528 lines); Institute for Wheat and Sunflower, Bulgaria (14 lines); National Centre for Plant Genetic Resources, Radzikow, Poland (14 lines); and Institute for Agrobotany, Tapioszele, Hungary (12 lines). Some of the lines originally collected by the Vavilov Institute were received via ICARDA (International Centre for Agricultural Research in the Dry Areas), Aleppo, Syria. The number of lentil accessions by country of origin is shown in Table 1. Four seed morphology traits were recorded for each resistant lentil line developed. These included seed size (small 3.5–5.0 mm, medium 5.0–7.0 mm, and large 7.0–10.0 mm), cotyledon color (yellow or red), seed coat ground color (green, tan or black) and seed coat pattern (absent or dotted).

Table 1 *Lens culinaris* accessions resistant to one or both races of *Colletotrichum truncatum* the cause of anthracnose

Country of origin	Number of accessions	Number of accessions with resistance to *Colletotrichum truncatum*		
		Race Ct0 and Ct1	Race Ct0	Race Ct1
Afghanistan	63			
Armenia	29			
Azerbaijan	54			
Bulgaria	90			1
Czechoslovakia	22		4	7
France	1			
Georgia	13	1		
Germany	20		1	1
Hungary	51		1	2
Kazakhstan	4			
Libya	2			
Mexico	5			
Poland	14			
Romania	1			
Russia	116			3
Tajikistan	47			
Turkmenistan	11			
Ukraine	9			
Uzbekistan	15			
Yugoslavia	1			
Unknown	11		1	1
Total	579	1	7	15

Entries obtained from the Vavilov Institute of Plant Industry, St. Petersburg, Russia (528 accessions); Institute for Agrobotany, Tapioszele, Hungary (17 accessions); National Centre for Genetic Resources, Radzikow, Poland (14 accessions); Institute for Wheat and Sunflower, General Toshevo, Bulgaria (14 accessions)

Fungal isolates

All lentil accessions were inoculated with two isolates of *C. truncatum*, separately. Isolate 95A8 was representative of race Ct0 and isolate 95B36 of race Ct1 and were both part of a previous study (Buchwaldt et al. 2004). Stock cultures of the isolates were stored at −80 °C in a cryo-freezer solution (10 % skim milk and 40 % glycerol) until needed for inoculation. Aggressiveness was maintained by inoculating and re-isolating the pathogen from the susceptible cultivar 'Eston'. Inoculum for inoculation of lentil accessions was prepared by plating 30–50 µl of a stock culture on half strength oat meal agar (Difco) in 9 cm Petri dishes. The Petri dishes were inverted and incubated at 20/16 ± 1 °C in a 16 h photoperiod. Inoculum was prepared by flooding 10 day old cultures with sterile, distilled water. To remove mycelium, microsclerotia and nutrient media the suspension was filtered through one layer of Miracloth (Calbiochem-Behring Corp., La Jolla, CA). The final concentration of the inoculum was adjusted to 10^5 conidia per ml using a hemocytometer as previously described by Buchwaldt et al. (2004).

Inoculation and incubation

Eight to twelve seeds of each lentil accession were sown in Sunshine Germination Mix 3 (Sun Gro Horticulture Canada Ltd., Vancouver, BC) in two 10 cm square pots. As a control, two seeds of the susceptible lentil cultivar 'Eston' were placed in each pot to verify that adequate disease pressure had been achieved of the surrounding plants. Two weeks after germination, plants were fertilized weekly with 20-20-20 NPK. Each test consisted of around 30 accessions.

The pots were placed in a randomized complete block design in a growth chamber maintained at 21/16 ± 1 °C day/night temperature and 16 h day length. Lentil plants were inoculated at the early flowering stage when the main stem had 10–12 nodes. Each plant was inoculated with 1.5 ml conidial suspension using an air-brush sprayer set at 125–150 kPa. To support the plants, each pot was placed in a 30 cm tall plastic sleeve without disturbing the drops of inoculum. The plants were incubated for 24 h in a humidity chamber kept at 100 % RH and low light intensity. Plants were transferred to a greenhouse where disease symptoms developed over the following 2 weeks. The greenhouse was maintained between 20 and 24 °C day temperature and between 15 and 19 °C night temperature. Supplemental light was provided to maintain a 16 h day length.

Rating of disease symptoms

The anthracnose severity of each individual plant was rated 14 days after inoculation as described by Buchwaldt et al. (2004). The quantitative rating was based on the number of lesions on the main stem accurately counted between 0 and 30, but on stems with more lesions a >30 notation was used (Table 2). The qualitative rating was based on the depth of lesion penetration into the stem, location of lesions along the stem and amount of wilting using the notations in Table 2. Defoliation prevented an accurate rating of leaf damage which was therefore omitted. The susceptible control 'Eston' showed symptoms of wilting within 1 week of inoculation. In rare instances, when 'Eston' was not sufficiently infected, the surrounding plants were omitted and the accession was included in a later test date. There were 23 test dates for each of the two isolates.

Single plant selection and re-testing for resistance

The initial disease screening was performed on lentil plants grown from the original seed received from germplasm collections. Following rating of disease severity 14 days after inoculation, all susceptible plants were removed from the pots. These plants either had 30 or more stem lesions with shoot die back or were partly to completely wilted, often without showing distinct lesions on the stems. Putative resistant plants which had either no stem symptoms or less than 25 lesions on the main stem were kept to maturity. The S_1 seeds were harvested separately for each resistant plant. Plants grown from S_1 seed were re-tested for resistance to the appropriate race followed by another selfing to produce S_2 seed. In cases when S_1 plants yielded less than ten seeds, a seed increase was undertaken before re-testing. Only lines in which resistance to one or both races was successfully verified by re-testing are reported here.

Results

Symptoms on susceptible plants developed first on leaflets as tan, necrotic lesions followed by varying amount of defoliation. Inoculation of highly susceptible plants resulted in wilting of the entire plant without development of discrete lesions on the stems. This was particularly evident with race Ct0. Less susceptible plants developed typical anthracnose symptoms on the stems in which the lesions were necrotic, sunken and surrounded by a dark margin.

Putative resistant plants did not develop stem lesions or only superficial lesions in which just the epidermis of the stem was discolored brown. Occasionally, a mixture of superficial and deep lesion was

Table 2 Quantitative and qualitative notation used to rate anthracnose symptoms on individual lentil plants inoculated with *Colletotrichum truncatum*

Number of lesions	Depth of lesion penetration into the stem		Location of lesions on the main stem		Amount of plant wilt	
Accurately counted between 0 and 30 lesions. >30 if more than 30 lesions	spf	Superficial lesions affecting only the stem surface	sb	Stem base	sdb	Shoot die-back
	mix	A mixture of superficial and deep lesions	t	Top half of the stem	1/2w	Plant half wilted
	d	Deep lesions creating indents into the stem			w	Plant completely wilted

found on the lower half of the stem or a single lesion at the stem base, neither of which resulted in wilting of the plant. Resistant plants continued to grow vegetatively and S_1 seed were obtained at maturity.

After one or two cycles of single plant selection, 23 lentil lines were generated with resistance to one or both races of *C. truncatum* (Table 3). Fifteen lines were resistant to race Ct1, seven to Ct0 and one line, VIR 2633, was resistant to both races. This is the first report of resistance in lentil to the aggressive race Ct0 both alone and in combination with resistance to race Ct1. Eleven of the resistant accessions originated from Czechoslovakia, while one to three accessions were from Bulgaria, Georgia, Germany, Hungary and Russia. Two resistant accessions were of unknown origin. Accession VIR 2633 with resistance to both races came form Georgia. This accession had tan seed coat color and red cotyledon color (Table 3). Only one of the resistant accessions had large seed size, seven were medium, and fifteen lines had small seed size.

Selection for anthracnose resistance in a number of accessions is ongoing. Accession VIR 2687 contained plants resistant to race Ct1 and other plants resistant to Ct0, while about half of the plants were susceptible. This accession is identical to the cultivar 'Naslada' from Bulgaria showing promising levels of resistance to *C. truncatum* in the field (Mihov and Stoyanova 1998). Further single plant selection in VIR 2687 is in progress to obtain plants with resistance to both races combined (data not shown). Another line, VIR 916,

Table 3 *Lens culinaris* accessions resistant to one or both races of *Colletotrichum truncatum* obtained by single plant selection in gene bank accessions

Name of resistant line[a]	Country of origin	Year of inclusion	Other names	Seed size[b]	Seed coat colour	Cotyledon colour
VIR2633-Ct0Ct1	Georgia	1983		S	Tan	Red
VIR2058-Ct0	Czechoslovakia	1963	Moravska Krajova Drobnozrnna	S	Green	Yellow
VIR2068-Ct0	Czechoslovakia	1963	Ozima Ruzova	S	Green dotted	Yellow
VIR2076-Ct0	Czechoslovakia			S		
VIR2080-Ct0	Hungary	1964	Aproszemu Lense, Lens 111	S	Green	Yellow
VIR2086-Ct0	Germany	1964	Schwarze Linse, Lens 104	S	Black	Red
VIR2826-Ct0	Unknown			S	Green	Yellow
VIR2827-Ct0	Czechoslovakia			S	Green	Yellow
VIR61-Ct1	Russia			S	Grey dotted	Yellow
VIR81-Ct1	Russia			M	Green	Yellow
VIR306-Ct1	Russia	1923		S	Green	Yellow
VIR2051-Ct1	Czechoslovakia	1963	Slovenska Zemplinska	S	Green	Yellow
VIR2054-Ct1	Czechoslovakia	1963		S	Black	Yellow
VIR2056-Ct1	Czechoslovakia	1963	Branisovicka Alba	M	Green	Yellow
VIR2066-Ct1	Czechoslovakia	1963	Slovenska Krajova Zemplinska	S	Green	Yellow
VIR2069-Ct1	Czechoslovakia	1963	Slovenska Krajova Trebisovska	M	Green	Yellow
VIR2073-Ct1	Czechoslovakia		KM 2049	M	Green	Yellow
VIR2077-Ct1	Czechoslovakia	1963	Trebisovska Selena	M	Green	Yellow
VIR2106-Ct1	Hungary	1965	Spats Alpen linse	M	Green	Yellow
VIR2612-Ct1	Bulgaria	1988	Veseletc-3	M	Green	Yellow
PL85055-Ct1	Unknown			S	Black	Yellow
PL85064-Ct1	Unknown		Trebisovska	S	Green	Yellow
RCAT023809-Ct1	Hungary			L	Green	Yellow

[a] Original gene bank name with prefix -Ct0-Ct1, -Ct0 or -Ct1 indicating that single plant selection was successfully carried out in each accession for resistance to one or both races of *Colletotrichum truncatum*

[b] Small (S) 3.5–5.0 mm, medium (M) 5.0–7.0 mm, large (L) 7.0–10.0 mm

was a mixture of seed with yellow and red cotyledon color, respectively, each producing plants resistant to race Ct1. Generation of resistant lines of each seed type is in progress (data not shown). Some accessions were duplicated in different germplasm collections. One example was Lens 104 from IPK, which was duplicated at the Vavilov Institute as line VIR 2086. Lens 104 was resistant to race Ct1 in the previous study (Buchwaldt et al. 2004); while VIR 2086 was resistant to Ct0 in the current study and also contained plants with Ct1 resistance. Selection for resistance to both races in VIR 2086 is in progress (data not shown).

Discussion

Considering the economic importance of lentil anthracnose in Canada and some European countries, there was an urgent need to identify new resistant sources for improvement of lentil cultivars. The main objective of this research was to generate lines homozygous resistant to both races of *C. truncatum* identified in western Canada (Buchwaldt et al. 2004). Of special interest was resistance to race Ct0, since the first screening of more than 1,771 lentil accessions from gene banks in the USA and Germany did not identify resistance to this race (Buchwaldt et al. 2004). Another priority was to identify resistant accessions from diverse parts of the world in the expectation that some of the resistance genes would be non-allelic. Combining non-allelic resistance genes in lentil cultivars would reduce the risk of resistance breaking down due to changes of pathogenicity in the fungal population.

After screening of 579 accessions in this study and 1,771 accessions in a previous study (Buchwaldt et al. 2004), a total of 39 accessions (1.6 %) was identified as sources of resistance to race Ct1, seven with resistance to race Ct0 (0.3 %) and one with resistance to both races. The frequency of *C. truncatum* resistance in the two studies was summarized for each country as follows: 15 resistant accessions from Czechoslovakia (47 %), 4 from Germany (13 %), one resistant accession from Brazil (8 %) and Georgia (7 %), and five resistant accessions from Russia (3 %). A total of 48 accessions were of unknown origin and seven of these were resistant to the pathogen. Resistance to *C. truncatum* was not identified among 244 accessions from the Middle East (Turkey, Lebanon, Syria and Jordan) or 776 accessions from Asia (Iran, India and Iraq).

Lentil is an ancient crop dating back to 9000 B.P. The centre of origin for *Lens* species and domestication of *Lens culinaris* was recently reviewed by Cubero et al. (2009). The spread of lentil cultivation can be traced to Greece 8000 B.P., Central Europe 5000–7000 B.P., Russia 5000 B.P., Afghanistan and India 3000–4000 B.P. and to France and Germany 3000–3500 B.P. The crop was introduced in the Americas about 500 years ago with the colonization by Europeans. Modern-day lentil has an East–West distribution from Morocco to Bangladesh and North–South distribution from Russia to Ethiopia. Lentil is also grown in North America and Australia where it is a relative new crop. Due to the wide distribution of lentil and very little information on the occurrence of the fungal pathogen *C. truncatum*, it was necessary to include accessions collected in as many countries as possible in the first round of disease resistance screening. The results showed that anthracnose resistant accessions originated mainly in Czechoslovakia, Russia, Greece, Germany and Brazil (Buchwaldt et al. 2004). Consequently, the second screening focused on screening of accessions from these and neighboring countries; however, additional accessions from South America could not be obtained. The frequency of resistant lines was less than one percent in the initial screening compared to 4.0 % in the more targeted screening.

Although the majority of resistant accessions originated in Czechoslovakia it has not been possible to find any reports on the occurrence of *C. truncatum* from here. Nevertheless, given that anthracnose resistance is a rare trait in most parts of the world, and that half of the Czechoslovakian accessions showed resistance, it is likely that the pathogen has been present in this area for many years, resulting in either intended or unintended selection of anthracnose resistance. *Colletotrichum truncatum* has been reported from Bulgaria where the cultivar 'Naslada' was the best source of resistance under field conditions (Mihov and Stoyanova 1998). Higher moisture levels during the growing season in Czechoslovakia and Bulgaria have likely favored the pathogen in this part of the world compared to dryer climates of the Middle East and Asia, where all accessions proved to be susceptible likely due to a lack of selection pressure. Screening of a around 500 of the same lentil accessions for resistance to *A. lentis* showed that the majority of ascochyta blight resistant accessions originated in Greece (Buchwaldt, unpublished). These

findings suggest that the geographical origin of disease resistant lentil is difficult to predict. Core collections are generally created based on geographical location, morphological and physiological traits and lately on genetic diversity determined by molecular marker polymorphisms (Furman et al. 2009). However, these criteria and the limited number of accessions in core collections will hamper detection of rare traits such as disease resistance. A focused identification of germplasm strategy (FIGS) has been described by Endersen et al. (2011) linking eco-geographical parameters at collection sites to specific plant traits. FIGS demonstrated that climatic factors could help identify regions more likely to sustain wheat accessions resistant to stem rust (*Puccinia graminis* Pers.) and of barley resistant to net blotch (*Pyrenophora teres* Drechs.) and would therefore be more efficient than random sampling. The strategy may be useful for there cereal pathogens which have evolved along with domestication of the host (Stukenbrock and McDonald 2008), but may not be suitable for lentil anthracnose. The occurrence of *C. truncatum* in widely separated areas in both temperate (Canada, USA and Bulgaria) and warmer regions (Morocco, Ethiopia, Syria, Pakistan, Bangladesh and Brazil) indicate a high degree of pathogen adaptability, and also raises the question whether a host-shift of *Colletotrichum* from legume species to lentil might have happened in some regions as described for several other host-pathogen relationships by Stukenbrock and McDonald (2008). A host-shift is further supported by the fact that *Colletotrichum* species on legumes have a board host range (Lenné 1992). In Canada, *C. truncatum* from lentil easily infect *Vicia* species such as faba bean (*V. faba* L.) and narrow vetch (*V. americana* Muhl. ex Willd. var. *minor* Hook.) and to a lesser extent pea (*Pisum sativum* L.) (Gossen et al. 2009).

The previous lack of resistance to *C. truncatum* race Ct0 in the cultivated lentil prompted screening of wild *Lens* species. Resistance to race Ct0 was identified in three *L. ervoides* (Brign.) Grande accessions out of 167 tested and in one *L. lamottei* Czefr. accession out of nine (Tullu et al. 2006). Resistance to Ct0 was transferred to *L. culinaris* by crossing a resistant accession of *L. ervoides* with the susceptible cultivar 'Eston' followed by embryo rescue of an F_1 hybrid (Fiala et al. 2009). Since *L. ervoides* belongs to the secondary gene pool (Ladizinsky 1993) development of a population of recombinant inbred lines (RIL) was hampered by irregularities in chromosomal alignment during meiosis (Fiala et al. 2009). The conclusion that two recessive genes conferred anthracnose resistance in *L. ervoides* was therefore speculative as the ratio in the segregating population could be skewed. Furthermore, the spore concentration used for inoculation of the RIL was more than ten times lower than normally used for inoculation of *L. culinaris* in other studies (Buchwaldt et al. 2004; Gossen et al. 2009; Chongo and Bernier 1999). Nevertheless, a single resistant line was selected from the above study by Fiala et al. (2009) for crossing with cultivar 'CDC Redberry' resistant to race Ct1 and 'Eston' susceptible to both races. The resulting segregating ratios of progenies were unclear and further confounded by an effect of plant growth stage at the time of inoculation (Vail and Vandenberg 2011). Both studies illustrated the difficulties with transfer of resistance genes from outside the primary gene pool, which by definition is associated with crossing barriers and linkage drag as also seen in other crop species (Foolad et al. 2005). Despite initial problems, *L. culinaris* × *L. ervoides* hybrids were produced and anthracnose resistant progenies were back crossed to *L. culinaris* resulting in some F_4 lines with improved seed yield attributed to enhanced disease resistance in the field (Tullu et al. 2010). However, even after 20 or more years of traditional breeding, a single gene transferred from a wild species can be associated with enough linked chromosomal DNA to contain more than 100 other and potentially undesirable genes from that species (Tanksley and Nelson 1996).

Reproductive barriers and undesirable genes can be avoided by utilizing resistance in *L. culinaris*. Solh and Erskine (1981) described how natural and artificial selection over millennia have resulted in development of myriads of lentil land races adapted to specific locations. Consequently, genetic resources comprise these landraces and primitive cultivars. Due to the low level of natural cross-pollination in lentil, the landraces are largely composed of mixtures of homozygous genotypes, but some level of heterozygosity can not be ruled out. The available passport information for lentil accessions in this study showed they were primarily landraces. The majority of the resistant accessions were collected between 1923 and 1988 (Table 3) with most of the Czechoslovakian entries collected in 1963. Variation was therefore expected in phenotypic traits such as disease resistance. This was previously demonstrated for resistance to *C. truncatum* race Ct1 as

three resistant lines, Lens 104, Lens 102 and Lens 195 from the PIK collection, contained 2–10 % susceptible plants (Buchwaldt et al. 2004). This heterogeneity necessitated selection of single resistant plants for selfing and re-testing with the pathogen. Resistance to race Ct0 was identified in *L. culinaris* alone and in combination with resistance to race Ct1 in accession VIR 2633. The resistance to race Ct0 was not complete as some lesions develop on stems and leaves, but allow the plants to mature and produce seeds. Ct0 resistance was identified in small-seeded lines, which are easily crossed into adapted cultivars with appropriate seed characteristics. Research is now in progress to determine the number, allelism and dominance of these resistance genes. Seed of homozygous resistant lines can be requested from the corresponding author, and are labeled with their original accession number with the prefix either -Ct0, -Ct1 or -Ct0Ct1 indicating resistance to one or both races of *C. truncatum*.

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