ISSN 1479-2621

Plant Genetic Resources: Characterization and Utilization (2021) **19**(2); 167–173
doi:10.1017/S1479262121000216

An advanced lentil backcross population developed from a cross between *Lens culinaris* × *L. ervoides* for future disease resistance and genomic studies

Tadesse S. Gela*, Stanley Adobor, Hamid Khazaei and Albert Vandenberg*

Department of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan, S7N 5A8, Canada

Received 14 January 2021, revised 24 February 2021; Accepted 21 March 2021 – First published online 21 April 2021

Abstract

Genetically accessible variation to some of the abiotic and biotic stresses are limited in the cultivated lentil (*Lens culinaris* Medik.) germplasm. Introgression of novel alleles from its wild relative species will be useful for enhancing the genetic improvement of the crop. *L. ervoides*, one of the wild relatives of lentil, is a proven source of disease resistance for the crop. Here we introduce a lentil advanced backcross (LABC-01) population developed in cultivar 'CDC Redberry' background, based on *L. ervoides* alleles derived from an interspecific recombinant inbred population, LR-59-81. Two-hundred and seventeen individuals of the LABC-01 population at $BC_2F_{3:4}$ generation were screened for the race 0 of anthracnose (*Colletotrichum lentis*) and stemphylium blight (*Stemphylium botryosum*) under controlled conditions. The population showed significant variations for both diseases and the transfer of resistance alleles into the elite cultivar was evident. It also segregated for other traits such as days to flowering, seed coat colour, seed coat pattern and flower colour. Overall, we showed that LABC-01 population can be used in breeding programmes worldwide to improve disease resistance and will be available as a valuable genetic resource for future genetic analysis of desired loci introgressed from *L. ervoides*.

Keywords: biotic stresses, crop wild relatives, *Lens* spp, mapping population, pre-breeding

Introduction

Cultivated lentil (*Lens culinaris* Medik.) is an economically important cool-season grain legume with genome size of ~4.3 Gbp in the haploid complement (Bett *et al.*, 2016). The crop is cultivated in more than 70 countries worldwide and the production from Canada (40%), India (19%) and Australia (9%) provides most of the world's supply. Average global annual production was around 6.3 Mt (2015–2019 average, FAOSTAT, 2021). A genetic bottleneck limiting genetic variability exists in cultivated lentil germplasm (Erskine *et al.*, 1989, 1994; Gupta and Sharma, 2006; Khazaei *et al.*, 2016). Broadening the genetic base of breeding programmes by introducing new genetic resources is required for the development of improved lentil germplasm. Crop wild relatives have been used to improve the resistance and resilience of elite cultivars against various biotic and abiotic stresses in many crops (Hajjar and Hodgkin, 2007; Maxted and Kell, 2009; Maxted *et al.*, 2012) including grain legumes (Pratap *et al.*, 2021).

The *Lens* genus has seven closely related taxa with the same number of chromosomes ($2n = 14$) and have similar karyotypes (Ladizinsky *et al.*, 1984; Van Oss *et al.*, 1997). Presently, the *Lens* species are grouped into four gene pools; *L. culinaris*, *L. orientalis* (Boiss.) Hand.-Maz., *L. tomentosus* Ladiz. (primary gene pool); *L. odemensis* Ladiz., *L. lamottei* Czefr. (secondary gene pool); *Lens ervoides* (Brign.) Grand. (tertiary gene pool); and *L. nigricans*

*Corresponding authors. E-mail: tadesse.gela@usask.ca; bert.vandenbarg@usask.ca

(M.Bieb.) Grand. (quaternary gene pool, *see* Wong *et al.*, 2015). Among them, *L. ervoides* has been identified as a potential source of desirable genes for resistance to major lentil diseases such as anthracnose (*Colletotrichum lentis* Damm) (Tullu *et al.*, 2006; Vail *et al.*, 2012), ascochyta blight (*Ascochyta lentis* Vassilievsky) (Tullu *et al.*, 2010), stemphylium blight (*Stemphylium botryosum* Wallr.) (Podder *et al.*, 2013), and fusarium wilt (*Fusarium oxysporum* f. sp. *lentis*) (Singh *et al.*, 2017) along with yield and its components (Gupta and Sharma, 2006; Tullu *et al.*, 2011, 2013; Chen, 2018), and abiotic stresses (Gorim and Vandenberg, 2017; Yuan *et al.*, 2017). Introgression of the desirable alleles from *L. ervoides* to *L. culinaris* elite germplasm were facilitated by embryo/ovule rescue techniques that overcome the interspecific reproductive barriers (Fiala *et al.*, 2009; Tullu *et al.*, 2013). However, the introgressed gene from a distant wild relative into elite cultivars may result in disruption of the long-accumulated agronomic and quality traits due to linkage drag and/or epistatic interactions of deleterious genes of undesired wild traits (Tanksley *et al.*, 1989; Tanksley and Nelson, 1996). In many cases, these undesired traits are dominant and polygenic, making it difficult to select against and impeding the interspecific hybrid progeny from direct use in the breeding programmes. In lentil, Tullu *et al.* (2013) and Chen (2018) reported the presence of undesired traits such as seed dormancy, poor emergence, extremely small seed size and pod dehiscence in *L. ervoides* interspecific lines. Moreover, *L. culinaris* and *L. ervoides* genome are differed by a reciprocal translocation between chromosomes 1 and 5 (Gujaria-Verma *et al.*, 2014; Bhadauria *et al.*, 2017) that attributed to the postzygotic reproductive barrier (Tadmor *et al.*, 1987). Thus, the development of an advanced backcross (AB) population is critical to explore the genetic architecture and utilize the alleles from *L. ervoides*.

The AB populations are developed through multiple backcrossing (BC_2 or BC_3) followed by multiple rounds of selfing, and they may contain single or multiple, fixed or non-fixed segments of the introgressed genome of the wild species (Fulton *et al.*, 1997). The AB lines are useful genetic materials for the development of introgression lines (ILs), which consist of fixed lines that are carrying a single or a few genomic segments associated with desired traits (Frischa *et al.*, 1999; Dempewolf *et al.*, 2017; Prohens *et al.*, 2017). To make the introgression process more efficient and applicable, Tanksley and Nelson (1996) proposed an advanced backcross-quantitative trait loci (AB-QTL) mapping approach as a tool to minimize the undesirable segments of the wild genome through repeated backcrossing to the elite cultivar and simultaneous mapping of QTL underlying the trait of interest. The AB-QTL strategy has been used in many crop species for identifying introgression QTL for many traits of interest (*reviewed by* Bhanu *et al.*, 2017) including disease resistance (Yun *et al.*, 2006; Schmalenbach *et al.*, 2008; Taguchi-Shiobara *et al.*, 2013).

The main aim of the current paper is to introduce a lentil advanced backcross (LABC-01) population derived from a cross between the CDC Redberry and an interspecific line, LR-59-81 developed from *L. ervoides*. This population offers an opportunity to utilize beneficial traits introgressed from lentil wild relatives. As a showcase, the responses of the LABC-01 population at $BC_2F_{3:4}$ generation to anthracnose race 0 and stemphylium blight under climate-controlled conditions are presented.

Materials and methods

Parental lines selection

An LABC-01 population was developed from two founder lines, CDC Redberry and LR-59-81 (Fig. 1(a)). The recurrent parent, CDC Redberry, was a red lentil cultivar released by the Crop Development Centre (CDC), University of Saskatchewan (USask) for its high yield and partial resistance to anthracnose race 1 and ascochyta blight (Vandenberg *et al.*, 2006). CDC Redberry was used to develop a lentil reference genome (Bett *et al.*, 2016). The line LR-59-81 was selected from the LR-59 interspecific recombinant inbred line (RIL) population (Fiala *et al.*, 2009), which was developed from a cross between *L. culinaris* cv. Eston × *L. ervoides* accession L-01-827A (Fig. 1(b)). Embryo rescue techniques were used to obtain the F_1 seeds (Fiala *et al.*, 2009). Line LR-59-81 was originally selected for its high level of resistance to both races of anthracnose (races 0 and 1) under indoor and field evaluations (Fiala *et al.*, 2009; Vail *et al.*, 2012). The line was also evaluated for resistance to ascochyta blight and stemphylium blight (Table 1) and has been commonly used as a source of resistance for both races of anthracnose (Banniza *et al.*, 2018; Gela *et al.*, 2020).

LABC-01 population development

The LABC-01 population was developed by crossing CDC Redberry × LR-59-81 to obtain the F_1 generation (Fig. 1(a)). All F_1 seeds were fertile and the hybridity of F_1 plants was confirmed by flower colour as a morphological marker. CDC Redberry had typical *L. culinaris* flower-type, white background with light blue veins and LR-59-81 had purple flowers (typical *L. ervoides*). All F_1 plants had purple flowers. Purple flower colour was dominant over white with blue veins and it is known to be inherited as a simple Mendelian fashion (Singh *et al.*, 2014). Two F_1 plants were backcrossed to CDC Redberry to obtain BC_1F_1 seeds. To avoid genetic drift, all efforts were made to achieve the maximum number of cross combinations. A total of 111 and 73 BC_1F_1 seeds were harvested from two F_1 plants, respectively. The segregation of the flower colours was checked for the BC_1F_1 population and fit a 1:1

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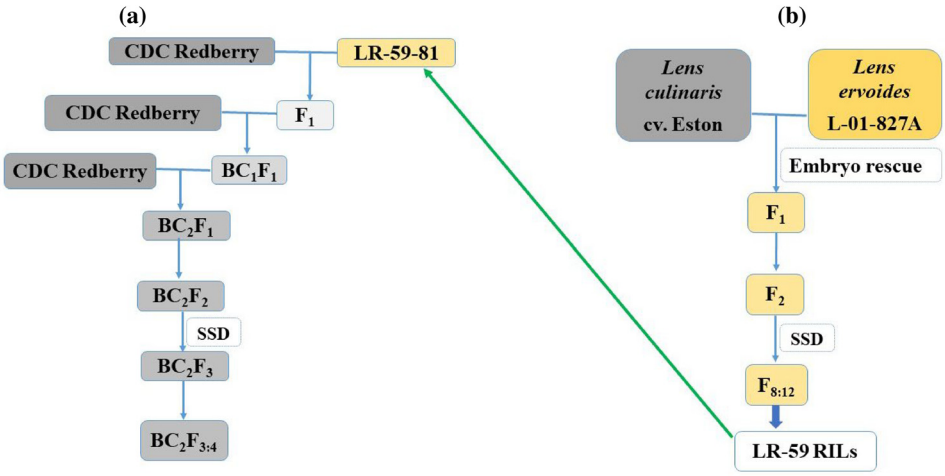

Fig. 1. Schematic diagram of lentil advanced backcross mapping (LABC-01) population development (a) and the pedigree of the LR-59-81 (b). SSD, single seed descent.

Table 1. Disease response and morphological characteristics of parental lines of the LABC-01 population

Characteristics	CDC Redberry	LR-59-81/L-01-827A	Reference
Disease resistance			
Anthracnose race 0	Susceptible	Resistant	Vail *et al.* (2012), Gela *et al.* (2020)
Anthracnose race 1	Partially Resistant	Resistant	Banniza *et al.* (2018)
Ascochyta blight	Resistant	Resistant	Tullu *et al.* (2010)
Stemphylium blight	Susceptible	Resistant	Podder *et al.* (2013)
Days to flower	Late	Early	This study
Days to maturity	Late	Early	Chen (2018)
Seed cotyledon colour	Red	Red	This study
Seed coat colour/pattern	Grey/unpatterned	Brown/black marble	This study
Seed weight	High	Low	Chen (2018)
Flower colour	White with light blue veins	Purple	This study
Plant height	Tall	Short/weak stem	Chen (2018)

ratio (88 white: 93 purple, chi-square $(\chi^2)_{(1:1)} = 0.138$, $P = 0.710$), indicating unbiased segregation of the BC_1F_1. The second backcross was made independently with all 184 BC_1F_1 plants to generate the BC_2 population, and one to two BC_2F_1 seeds were advanced to BC_2F_2 for each successful BC_1F_1 backcross. A total of 217 BC_2F_2 individuals was generated and one seed of each individual was arbitrarily selected and selfed to generate the BC_2F_3 generation and onward using a single seed descent approach.

Growth conditions

In all experiments, the growth chamber (GR48, Conviron, Winnipeg, Canada) conditions were adjusted to 18 h light and 6 h dark, with the temperatures maintained at 21°C (day) and 18°C (night) and the photosynthesis photon flux density was set to 300 µmol m^{-2} s^{-1} during the light period at the crop canopy level. All experiments were carried out at the controlled-climate growth chambers at USask College of Agriculture and Bioresources phytotron facility, Saskatoon, Canada.

Disease phenotyping

Phenotyping for anthracnose resistance

A total of 217 $BC_2F_{3:4}$ individuals of the LABC-01 population and parental lines were evaluated for anthracnose race 0 under growth chamber conditions. Fungal inoculum production, inoculation and plant growth conditions were performed as described by Gela *et al.* (2020). Briefly, two

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plants of each line were grown in a set of 38-cell cone trays ($26.8 \times 53.5\ cm^2$) per replication filled with SUNSHINE Mix #4 plant growth medium (Sun Gro Horticulture, Seba Beach, AB, Canada) and perlite (Specialty Vermiculite Canada, Winnipeg, MB) in a 3:1 ratio (v/v). The susceptible control cv. Eston (Slinkard, 1981) and the parental lines were included in each tray. The experiment design was a randomized complete block (RCBD) with six replicates. Replicates were inoculated over time. Four-week-old seedlings were inoculated with a spore suspension (5×10^4 spores/ml) of *C. lentis* race 0 isolate CT-30 (Banniza *et al.*, 2018) at 3 ml per plant using an airbrush. Plants were placed in an incubation chamber (relative humidity 90–100%) for 48 h before being moved to misting benches (see Gela *et al.*, 2020). Individual plants were scored for anthracnose severity at 8–10 days post-inoculation (dpi) using a 0 to 10 rating scale with 10% increments. The mean disease severity score of the two plants per replicate was calculated and the data were converted to per cent disease severity using the class midpoints for statistical analysis.

Phenotyping for stemphylium blight resistance

Six seeds of each individual line of the LABC-01 population were sowed in 10-cm plastic pots filled with SUNSHINE Mix #4 plant growth medium and arranged in RCBD with three replicates. Two weeks post-emergence, plants were thinned to four plants per replicate and fertilized once every week using $3\ gl^{-1}$ of soluble N:P:K (20:20:20) PlantProd® fertilizer (Nu-Gro Inc., Brantford, ON, Canada). Cultivars Eston (Slinkard, 1981) and CDC Glamis (Vandenberg *et al.*, 2002) were used as resistant and susceptible checks, respectively.

A culture stock of the aggressive *S. botryosum* SB19 isolate collected from Southeast Saskatchewan was obtained from the Plant Pathology Laboratory, USask for mass spore production following a procedure described by Caudillo-Ruiz (2016). Plants were spray-inoculated at the pre-flowering stage with ~3 ml of conidial suspension per plant at a concentration of 1×10^5 conidia/ml using an airbrush (Badger Airbrush, model TC 20, USA). Two droplets of Tween® 20 (Sigma, Saint Louis, MO, USA) were added to every 1000 ml of suspension before inoculation to help reduce the surface tension of water and promote plant tissue contact. Plants were placed in an incubation chamber for 7 days. Two humidifiers (Vicks Fabrique Paz Canada, Inc., Milton, ON, Canada) were placed in the incubation chamber to ensure 90–100% relative humidity for infection and disease development. Blocks were inoculated over time.

Disease severity was assessed visually at 7 dpi using a semi-quantitative rating scale (0–10) where 0 – healthy plants; 1 – few tiny lesions; 2 – a few chlorotic lesions; 3 – expanding lesions on leaves, the onset of leaf drop; 4 – one-fifth of nodes affected by lesions and leaf drop; 5 – two-fifth of nodes affected; 6 – three-fifth of nodes affected; 7 – four-fifth of nodes affected; 8 – all leaves dried up; 9 – all leaves dried up but stem green; and 10 – plant completely dead. Disease severity was assessed on single plants within the experimental unit (pot). For each genotype, four single plants per replicate pot were assessed. Disease severity data was analyzed using the median disease severity score for each genotype.

Phenological measurement

The number of days to the onset of flowering was recorded.

Data analyses

Statistical analyses were conducted for both anthracnose and stemphylium blight severity using SAS software SAS 9.4, SAS Institute, Cary, North Carolina (SAS Institute, Inc., 2011). Normality and variance homogeneity of the residuals were tested using the Shapiro-Wilk normality test and Levene's test for homogeneity, respectively. The data did not fit the assumptions of a Gaussian distribution and were normalised using a lognormal distribution in the GLIMMIX procedure. Genotypes were treated as fixed effect and blocks as random effect and significance of variances were declared at a 5% significance level. Least square means were estimated for genotype using LSMEANS statements.

Results and discussion

In this study, we developed a LABC-01 population to explore the valuable genetic variation introgressed from lentil wild relative *L. ervoides* into adapted cultivar CDC Redberry. *L. ervoides* accession L-01-827A, the parent to the interspecific LR-59-81, has previously shown adaptation to drought (Gorim and Vandenberg, 2017) and resistance to diseases such as ascochyta blight (Tullu *et al.*, 2010), stemphylium blight (Podder *et al.*, 2013), anthracnose (Vail *et al.*, 2012; Gela *et al.*, 2020), and the parasitic weed broomrape (*Orobanche crenata* Forsk.) (Bucak *et al.*, 2014). Our results revealed variation for desirable traits in the LABC-01 population that were inherited from L-01-827A in the cultivated lentil background including disease resistance and phenological traits.

The LABC-01 population could possibly combine important key traits from *L. ervoides* for lentil genetic improvement as a pre-breeding genetic source and as a valuable resource on which to conduct further genetic studies. Our data showed that $BC_2F_{3:4}$ generation had a continuous distribution for days to flowering (online Supplemental

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Fig. S1) and also segregated for morphological traits such as flower colour (191 white: 26 purple), seed coat colour (190 grey: 27 tan), and seed coat pattern (185 absent: 32 marbled). Vail (2010) and Chen (2018) have reported segregation of several agronomic and phenotypic characteristics including plant vigour, yield and its components in the genetic populations derived from LR-59-81 or L-01-827A. A similar trend was also reported for seed iron concentration (Podder, 2018). Multi-location evaluation of LABC-01 population lines will be considered necessary for genetic analysis of these traits and selection of advanced lines for the lentil breeding programmes.

Significant variation for anthracnose race 0 resistance was observed among the 217 LABC-01 individuals (F-value = 3.98, $P < 0.0001$). The LR-59-81 had a resistant reaction with mean disease severity of 36%, whereas the recurrent parent CDC Redberry showed susceptible reactions with a mean of 85%. A large number of LABC-01 individuals showed to be susceptible to race 0. The disease severity ranged from 17 to 95% with a mean of 70.2%. Transgressive variation for race 0 resistance relative to that of the resistant LR-59-81 was observed (Fig. 2(a)) which is consistent with the findings (Fiala *et al.*, 2009; Tullu *et al.*, 2013), who reported the transgressive segregation and skewed distributions of lines toward the higher level of disease severity. Two pathogenic races of anthracnose (races 0 and 1) have been described (Buchwaldt *et al.*, 2004). Resistance to race 1 is abundant in cultivated lentil germplasm, however, resistance to the more virulent race 0 is mainly limited to *L. ervoides* (Tullu *et al.*, 2006; Barilli *et al.*, 2020; Gela *et al.*, 2020). Transfer of race 0 resistance alleles into the cultivated background could widen the lentil breeding genepool and provide benefits for cultivar development.

Significant variation in stemphylium blight severity was observed among the 217 LABC-01 individuals (F value = 1.81, $P < 0.0001$). The resistant line, LR-59-81, had significantly less disease severity (2.32) compared to the resistant check cv. Eston (4.16) and recipient parent CDC Redberry (5.13). The distribution of disease severity as a measure of stemphylium blight response for LABC-01 lines showed continuous variation ranging from 1.59 to 5.80 (Fig. 2(b)), suggesting polygenic regulation of stemphylium blight severity. None of the LABC-01 individuals had significantly less disease than the resistant parent LR-59-81 (online Supplementary Table S1). This observation is consistent with the findings of Adobor *et al.* (2020) who also reported the absence of resistant transgressive segregants in *L. ervoides* interspecific population screened for stemphylium blight resistance in the greenhouse, growth chamber and the field conditions. A high proportion of the LABC-01 individuals (144) had similar disease severity when compared to the resistant parental line LR-59-81, indicating that resistance genes were transferred from the resistant parent to LABC-01 individual lines (online Supplementary Table S1).

Understanding the genetic architecture of the favourable traits from the wild germplasm provides breeders information that can aid in the introgression of the traits while avoiding linkage drag of deleterious characteristics of the wild species (Tanksley and Nelson, 1996). The LABC-01 population can be used for preliminary QTL mapping and genetic characterization of agronomic traits and disease resistance that have been introgressed into CDC Redberry. Since no selection was carried out during population creation, some of the LABC-01 lines may be of interest as starting materials for the development of fixed

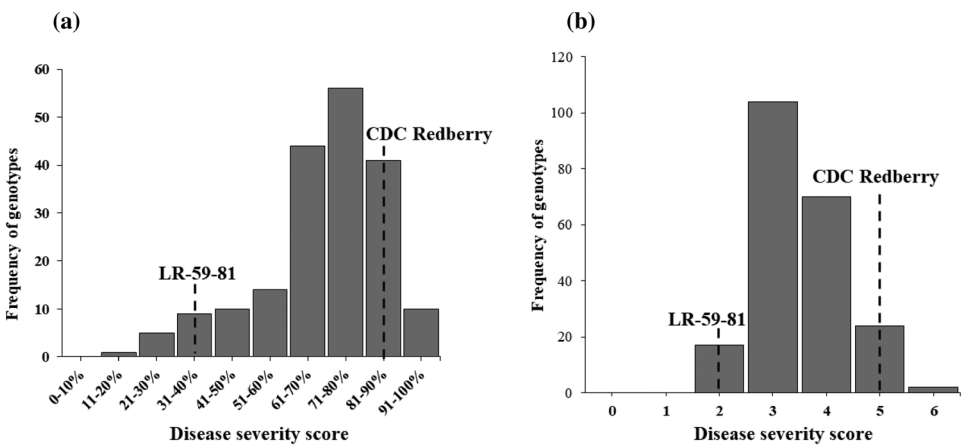

Fig. 2. Frequency distribution of mean anthracnose race 0 ($n = 217$, $P(W) = 0.001$) (a) and stemphylium blight severity ($n = 217$, $P(W) = 0.018$) (b) for lentil advanced backcross mapping (LABC-01) population at $BC_2F_{3:4}$ generation under growth chamber condition. The vertical lines indicate the average disease severity of the parents. $P(W)$, P value of the Shapiro–Wilk test for normality. Disease was rated using a 0–10 scale with 10% incremental increases in disease severity (a) and using a semi-quantitative rating scale (b).

https://doi.org/10.1017/S1479262121000216 Published online by Cambridge University Press

introgression populations for specific traits of interest (Prohens *et al.*, 2017). For instance, QTL analysis can be conducted with the anthracnose race 0 and stemphylium blight data and then the identified markers can be used to facilitate the development of ILs such as chromosome segment substitution lines (CSSLs) and/or near-isogenic lines (NILs) by means of marker-assisted selection. The ILs are important for fine QTL mapping studies and developing genetically characterized elite materials that can be directly incorporated into breeding programmes (Zamir, 2001; Eduardo *et al.*, 2005; Tian *et al.*, 2006). The present population is being genotyped using an exome capture array described by Ogutcen *et al.* (2018) which will provide a valuable genetic tool for collaborative lentil research. The plant materials are currently managed and stored at the Crop Development Centre, University of Saskatchewan, Saskatoon, Canada.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S1479262121000216

Acknowledgements

The authors gratefully acknowledge funding from the Natural Sciences and Engineering Research Council of Canada (NSERC) Industrial Research Chair Programme, the Saskatchewan Pulse Growers, and the University of Saskatchewan. We are also thankful for the technical assistance of the Pulse Pathology lab staff and Pulse Crop Breeding and Genetics group at the University of Saskatchewan.

Conflict of interest

None.

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