IMAM KHOMEINI INTERNATIONAL UNIVERSITY
IJGPB
Iranian Journal of Genetics and Plant Breeding
Print ISSN : 2251-9610
Online ISSN : 2676-346X

Screening lentil germplasm for resistance against *Ascochyta lentis* under field and controlled conditions

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ABSTRACT INFO

Research Paper

Received: 23 Jan 2023

Accepted: 08 May 2023

ABSTRACT

Lentil (*Lens culinaris*) is the third most important grain legume in the world after chickpeas and peas. Ascochyta blight (AB) caused by *Ascochyta lentis*, is one of the most damaging diseases of this crop worldwide. In this study response of 169 lentil genotypes against AB was appraised in the greenhouse and field conditions based on randomized complete block design (RCBD). Field screening was carried out in the growing season of 2019-2020 at Shirvan-Chardavol Agricultural Research Station, Ilam province, Iran. Conidial suspension with concentration of 1×10^6 spore per mL was used for artificial infection in the greenhouse condition. Analysis of variance discovered significant differences among studied genotypes in response to the pathogen. There were no detected resistant genotypes among the studied lentil germplasm against AB in either environments. In the field condition, the majority of genotypes were identified as resistant while in the greenhouse condition most of the genotypes retained susceptible responses. Regarding AUDPC and disease severity characteristics, genotypes “G121”, “G132”, and “G139” were recognized as resistant in both field and greenhouse coditions. Classification of lentil genotypes by using Ward algorithm in both conditions resulted in 4 heterotic groups. The identified resistant genotypes can be useful in the breeding program of interested susceptible lentil genotypes by the backcross approach. Also, the identified heterotic groups can consider in parental selection for mapping QTLs related to resistance to the disease.

Key words: Ascochyta blight, Genetic variability, *Lens culinaris*, Resistant genotype, Susceptible genotype.

How to cite this article:
Modareskia M., Shiran B., Hatami Maleki H., and Pouralibaba H. R. (2021). Screening lentil germplasm for resistance against *Ascochyta lentis* under field and controlled conditions. *Iranian Journal of Genetics and Plant Breeding,* 10(2): 25-36.
DOI: 10.30479/IJGPB.2023.18245.1334

INTRODUCTION

Legumes by having high amounts of protein are known as the second source of human food and in combination with grains can provide a valuable diet. Lentil (*Lens culinaris* L.), is a diploid plant with 2n=2x=14 chromosome, self-pollinate and annual plant. Regarding its large protein content (28%), micronutrients, and vitamins it has been considered as one of the most important legumes and widely used in the diets of people, worldwide (Iqbal *et al.,* 2006). Blight disease caused by *Ascochyta lentis* is one of the most important lentil foliar diseases globally and has been reported in many lentil leading countries comprising Argentina, Australia, Canada, Ethiopia, India, New Zealand, Pakistan, and the Russian Federation (Sheikh *et al.,* 2010). This disease has significant effects on both the quality and yield of the plant and has the ability to significantly diminish yield even up to 40% (Gossen and Morall, 1984). Regarding increasing of world population and the lack of food, it is necessary to provide agronomic and cross-breeding solutions to increase the resistance of this crop against this disease.

The causal agent of the disease, *Ascochyta lentis*, is similar to *Ascochyta fabae* in terms of morphological characteristics, with the difference that, unlike *Ascochyta fabae*, it specifically infects wild and cultivated lentil species (Tullu *et al.,* 2010). Populations of *A. lentis* are very diverse in terms of pathogenicity, and the reason for this variation can originate from the movement and spread of infected lentil seed germplasms around the world. Despite many studies in the field of agricultural and chemical control of Ascochyta blight (AB), no effective method has been introduced so far, and in the meantime, the use of disease-resistant cultivars is an eco-friendly solution that can also be used by the majority of farmers. Previous studies showed that the genetics of resistance to AB in lentils is controlled by genetic loci with large effects, although genes with small effects also play an important role in its control. In studies carried out by Tay *et al.* (1989) and Sakr (1994), it were shown that in lentils, resistance to AB is controlled by a dominant and a recessive gene. In another study (Ahmad *et al.,* 1997), by crossing the cultivated lentils with a wild lentil species (*L. ervoides*) showed that two dominant genes are involved in disease control. Parh (1998) using the F3 generation resulting from the crossing of genotypes Titore and W63261, reported thet only one recessive gene is present for the genetic control of resistance to AB in lentils. Later on, the existence of a dominant gene (*AbR*1) for the genetic control of resistance to AB was proven (Ford *et al.,* 1999). Other studies in the field of genetics of resistance to AB are related to the research studies of Ye *et al.* (2000 and 2001), they identified two genes associated with disease resistance by analyzing various generations of lentil plants such as F1, F2, BC1, F3, and BC2.

Limited information is available regarding the assessment of how lentil germplasm responds to disease. In a study by Hussain *et al.* (2008), the response of lentil germplasm including 590 genotypes was inspected simultaneously against three types of plant diseases comprising blight, rust, and viral diseases and they observed significant variations in the response of studied lentil genotypes against the mentioned pathogens. Hussain *et al.* (2008) further reported that most lentil genotypes were susceptible to rust disease whereas many genotypes showed resistance to viral and blight diseases. In another research conducted by Dadu *et al.* (2017), the response of 30 lentil genotypes belonging to five different species (*L. orientalis, L. odomensis, L. ervoides, L. nigricans, L. lamottei*) was evaluated against two Australian *Ascochyta lentis* isolates. Two *L. orientalis* accessions were found as a resistant source. Also, Zewdie and Gemachu (2020) studied the response of 148 lentil genotypes against AB in field conditions and identified 22 resistant genotypes, 58 semi-resistant genotypes and the rest as susceptible. The present study aimed to evaluate the genetic variability among large-scale genotypes of lentils to establish *Ascochyta* blight-resistant and susceptible subsets which can speed up lentils' future breeding programs.

MATERIALS AND METHODS

Plant material

A total of 169 lentil genotypes obtained from Iran's national lentil breeding program, as well as ICARDA lentil germplasm listed in Table 1, were tested for their resistance to AB.

Fungal pathogen

In May 2018, lentil stubble naturally-infested with *Ascochyta lentis* was collected from a field located in Shirvan-Chardavol (46° 34' 46" E 33° 47' 97" N), Ilam province. The stubbles were placed into large paper bags (20 cm in depth) and transferred into the lab. Infested leaves, stem and pod segments (1×1 cm) were produced, then surface sterilized in 0.5% NaClO for 10 min, dried on sterile paper, cultured on lentil seed meal-dextrose agar (LDA: 2% lentil seed flour, 2% dextrose, 2% agar in 1L deionized water) amended with 1 ml/L chloramphenicol and incubated

at 20±2 °C, 12 h light/12 h dark with fluorescent lights (100 µE m-2 s-1) supplemented with near UV radiation to stimulate sporulation. After one week, a dark grey colony appeared and it was purified using the single spore method (Khiang, 1999). The fungus was identified through colony morphology and spore dimension. For long-term storage, the purified culture was maintained on autoclaved stem pieces of lentils (Kaiser *et al.,* 1997). The plants were inoculated with the same isolate in both greenhouse and field trials.

Table 1. Code and pedigree of the studied genotypes.

Code	Name/pedigree	Code	Name/pedigree	Code	Name/pedigree
G01	?	G58	09S 82109-04-X2006S147-ILL1005 x ILL5883	G115	08540124-08
G02	FLIP 2007-16L (ILL 2126×ILL 4659)	G59	09S 82109-01-X2006S147-ILL1005 x ILL5883	G116	FLIP2003-2L
G03	FLIP 2010-8L (ILL 2126×ILL 6199)	G60	ILL 5988-	G117	FLIP2011-45L
G04	FLIP 2011-1L (ILL 6443×ILL 1005)	G61	09S 82109-05-X2006S147-ILL1005 x ILL5883	G118	FLIP2007-55L
G05	FLIP 2011-5L (ILL 6434×ILL 6972)	G62	ILL 6536-	G119	FLIP2010-79L
G06	FLIP 2011 6L (ILL 6434×ILL 6972)	G63	ILL 6538-	G120	FLIP2007-95L
G07	FLIP 1996-15L(IBLA 1) (ILL 6209×ILL 5671)	G64	ILL 4605-	G121	8068
G08	ILL 4605×ADDA (2006-03-0GA-0GA-0GA-11)	G65	ILL 6183-	G122	FLIP2007-56L
G09	ILL 6434×ILL 8008 (2006-030G-0GA-0GA-11)	G66	ILL 590-	G123	×2009-5160K3
G10	ILL 4605×ADDA (2006-06-0GA-0GA-0GA-11)	G67	ILL7685-	G124	×2009-5160K1
G11	ILL 4605× ILL 6002 (2006-02-0G-0GA-0GA-11)	G68	09S 96510-21-X2007S69-ILL8072 x ILL7162	G125	×2009-5212K2
G12	ILL 7547×ILL 6211 (2006-02-0G-0GA-0GA-11)	G69	ILL7978-	G126	×2009-51146K3
G13	ILL 7547×ILL 6211 (2006-03-0G-0GA-0GA-11)	G70	2009S 96505-2-X2007S67-ILL6434 x ILL8199	G127	×2009-5237K1
G14	ILL 6211×ILL 6002 (2006-07-0G-0GA-0GA-11)	G71	2009S 96518-1-X2007S107-ILL7940 x ILL5883	G128	×2009-5210K1
G15	FLIP 2005-32 L	G72	2009S 96501-1-X2007S61-ILL5883 x ILL6458	G129	×2009-5223K4
G16	FLIP 2005- 53L	G73	09S 83193-02-X2006S148-ILL9977 x ILL5883	G130	ILLB6554
G17	KIMIA	G74	2009S 96101-4-X2007S61-ILL5883xILL6458	G131	ILL 5582
G18	Gachsaran	G75	ILL 5883-	G132	ILL 7947
G19	PRECOZ-4605	G76	09S 83253-04-X2006S280-ILL8009xILL7711	G133	09S-96S10-12
G20	FLIP84-51L-5722L 883/ILL470	G77	08S 41226-02-X2005S206-X2004S99xX2004S27	G134	2009S-96575-17
G21	FLIP96-46L-7978	G78	2009S 96501-5-X2007S61-ILL5883xILL6458	G135	ILL 5883-
G22	FLIP2010-81L-10811 (ILL 7620/ILL8113)	G79	ILL 4605-	G136	09S-96506-08
G23	FLIP2010-88L-10818 (AKM282/ILL662)	G80	09S 83194-04-X2006S149-ILL6434 x ILL5883	G137	08S-41226-02
G24	FLIP2011-17L-10897 (ILL8114/ILL590)	G81	08S 41205-13-X2005S140-ILL5883 x ILL7620	G138	ILL 5605
G25	FLIP2010-40L-10770 (ILL8119/ILL7686)	G82	08S 41193-16-X2005S124-ILL590 x ILL5883	G139	08S-41205-13
G26	FLIP2010-50L-10780 (ILL5883/ILL8188)	G83	2009S 96101-4-	G140	08S-41193-16
G27	FLIP2010-70L-10880 (ILL6199/ILL5769)	G84	ILL 6002-	G141	09S-83184-01
G28	FLIP2011-13L-10893 (ILL358/ILL590)	G85	X2012S-154-X2012S-154-ILL6002xILWL118	G142	ILL 6002

Table 1 (Continued). Code and pedigree of the studied genotypes.

Code	Name/pedigree	Code	Name/pedigree	Code	Name/pedigree
G29	FLIP2010-90L-10820 (ILL7115/AKM27)	G86	X2012S-102-X2012S-102-(ILL7986 X ILWL 74)X(ILL4605 X ILL5677)	G143	010S-96122-3
G30	FLIP2011-13L-19893 (ILI358/ILL590)	G87	Gachsarn	G144	ILL 1323
G31	FLIP2007-133L (ILL7978/ILL98)	G88	Sepehr	G145	09S-26510-15
G32	FLIP2009-52L (ILL5883/91517)	G89	FLIP2012-2L(ILL10977)-ILL7985/ILL6037	G146	ILL 5883
G33	ILL 7547×ILL 9892 (2006-06-0G-0GA-0GA-11)	G90	PRECOZ(ILL4605)-ILL 5888 / ILL 5782	G147	08S-41102-11
G34	ILL 6434×ILL 8008 (2006-02-0G-0GA-0GA-11)	G91	FLIP2011-43L(ILL10947)-ILL 7537 X ILL 590	G148	07S-96811-5
G35	?	G92	FLIP2014-021L(ILL11431)-ILL9977 x ILL 1005	G149	08S-41137-02
G36	ILL 5782	G93	FLIP2014-032L(ILL11442)-ILL5883 x ILL6458	G150	ILL 79978
G37	ILL 10707	G94	FLIP2014-031L(ILL11441)-ILL5883 x ILL6458	G151	Bilesavar
G38	ILL 9896	G95	FLIP2014-029L(ILL11439)-ILL6037 x ILL7012	G152	FLIP2012-2L
G39	Qaz-89-90PR52	G96	FLIP2012-77L(ILL11052)-ILL6129XILL7980	G153	FLIP2012-8L
G40	Qaz-89-90PR73	G97	FLIP2012-240L(ILL11215)-ILL7711XILL8176	G154	FLIP2002-9L
G41	Qaz-89-90PR85	G98	FLIP2012-244L(ILL11219)-ILL7711XILL5480	G155	FLIP2002-2L
G42	Qaz-89-90PR108	G99	FLIP2014-103L(ILL11513)-ILL9892 x ILL7978 ICARDA 3 111 139 45 4.0 732 98 C	G156	FLIP2002-15L
G43	FLIP 2007-30L	G100	ILL8006	G157	FLIP2002-25L
G44	FLIP2003-2L	G101	FLIP2010-95L(ILL10825)-ILL 7620 X 91517	G158	FLIP2002-29L
G45	FLIP 2011-33L	G102	FLIP 86-16L(ILL6002)-ILL 4349 x ILL 4605	G159	FLIP2014-021L
G46	?	G103	Gachsarn	G160	FLIP2012-3L
G47	2009S 96575-10-	G104	Sepehr	G161	FLIP2012-196L
G48	2009S 96575-1-	G105	Yazd Local	G162	FLIP2012-207L
G49	010S 96105-1-X2007S76-ILL8108 x ILL7938	G106	Bilesavar	G163	FLIP2012-245L
G50	2009S 96101-4-	G107	FLIP97-10L	G164	FLIP2012-262L
G51	010S 96143-4-	G108	0854-124-8	G165	FLIP2013-13L
G52	010S 96130-2-X2007S116-ILL7947 x ILL1005	G109	0854124-08	G166	FLIP2013-24L
G53	010S 96131-2-	G110	FLIP2002-7L	G167	FLIP2014-45L
G54	08S 40111-01-	G111	FLIP2011-45L	G168	PRECOZ
G55	09S 96510-13-X2007S69-ILL8072 x ILL7162	G112	FLIP2011-42L	G169	FLIP96-59L
G56	010S 96129-3-X2007S119-ILL10005 x ILL1005	G113	FLIP97-6L		
G57	010S 96130-6-X2007S120-ILL9977 x ILL1005	G114	FLIP2007-45L		

Field assay

Field screening was carried out in the growing season of 2019-2020 at Shirvan-Chardavol Agricultural Research Station, Ilam province, Iran. The research station's climate had an average temperature of 15.6 °C and a long-term precipitation of 550 mm. The plots were plowed and leveled in the autumn. In February 2020, the seeds of each genotype (=treatment) were sown by hand in two one-meter rows with 25 cm space and 100 seeds per row (a total of 200 seeds per genotype per two rows). The distance between treatments was adjusted to 50 cm. Around the field and every 10 rows (=5 treatments), a local susceptible landrace was sown as a check and spreader of the disease agent. The experiment was established in the form of a randomized complete block design (RCBD) with three replications. In spring, in the early podding stage of the lentil plants, and at sunset time when air humidity was above 80%, the plots were inoculated twice at one-week intervals, with *A. lentis* spore suspension at a concentration of 500,000 spores per mL using a 20 L backpack sprayer. The inoculum harvested from young and fresh *A. lentis*

colonies had already been grown on LDA in the lab. The disease assessment was carried out after appearing first disease symptoms on the susceptible check (10–15 days after inoculation) on 10 randomly selected single plants from each plot, three times with a one-week interval using a 1-9 scoring system proposed by Nasir and Bretag (1998).

Greenhouse tests

Conidia of seven-day-old colonies of *A. lentis* were prepared on LDA. Petri dishes were flooded with sterile distilled water, the surface of the culture was gently scratched with a sterile needle and after macerating and squashing in a clay mortar, the harvested segments were transferred into an Erlenmeyer containing sterile water. The liquid was filtered throw a four-layer cheesecloth, transferred into Falcone tubes and centrifuged at 10,000 rpm for 10 min. The harvested conidial suspension was fixed at 1×10^6 spore per mL using a hemocytometer.

Lentil seeds were surface sterilized in 0.5% NaClO for 5 min, dried on paper and were sown in 8 cm wide pots (5×8×6 cm), two seeds per pot, filled with pasteurized clay loam soil (3:2:1 clay soil: river bed sand: compost). Three pots (=6 replication in total) were considered for each genotype. Plastic trays measuring 97×55×27 cm were used to hold the pots, which were filled with pasteurized river-bed sand to a depth of 5 cm at the bottom. The plants were watered twice a week through this layer of sand. The trays were kept at 20±2 °C, 12h light/12 h dark photoperiod at 200 µmol/m^2S. The grown plants at the 4-6-leaf stage were inoculated by the spore suspension containing surfactant Tween 20 (0.01%) with a hand sprayer. After inoculation, the pods were kept under a plastic bag for 48 h to maintain leaf wetness under the above-mentioned temperature/light conditions. The disease symptoms were evaluated using the nine-digit scale (Nasir and Bretag, 1998) after observing the first disease symptoms in the susceptible check, three times with one weak intervals.

Data analysis

Disease assessment was performed using procedure introduced by Nasir and Bretag (1998). A non-destructive whole-plant 1–9 scoring system was used which ranged from 1=no visible disease, to 9=stem girdling and/or plant death. Genotypes with a mean score of 3.0 or less to the every isolate tested, were considered resistant. Those with mean scores from 3.1 to 4.9 were considered to be moderately resistant and those with a mean score of 5.0 and above were considered susceptible. The values for the areas under disease progress curve were calculated in Microsoft Excel software, following this formula:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left(y_i - y_{i+1}/2\right)(t_{i+1} - t_i) \quad (1)$$

Where y_i is an assessment of the disease (percentage, proportion, ordinal score, etc.) at the i^{th} observation, t_i is time (in days, hours, etc.) at the i^{th} observation, and n is the total number of observations. Friedman test as nonparametric alternative to the randomized block design was performed for the analysis of variance. Then, calculation of mean and standard error was carried out by package "dplyr"in R. The function of "hclust from package "stat" was implemented to group the studied germplasm. For two dimentional graphicaly depiction of resistance or susceptible responses of the studied germplasm, package "ggplot2" was utilized and function of "abline" was used to marke the threshold level.

RESULTS

One-way ANOVA revealed significant differences among the studied lentil genotypes based on disease severity as well as AUDPC (Area Under Disease Progress Curve) characteristics in both field and greenhouse conditions. Means of disease severity accompanied by AUDPC correspondence to each lentil genotype in the field (Figure 1) and greenhouse (Figure 2) conditions rely on the existence of potential genetic variation among studied plant germplasm. The field experiment was conducted under natural infection conditions where the climatic conditions during the experiment were favourable for the development of the disease. In this condition, the resistance response of the studied lentil genotypes revealed that the majority of genotypes were resistant albeit some susceptible and moderately resistant genotypes were also detected (Table 2 and Figure 1). Considering disease severity scores in field conditions, 7 genotypes with S (susceptible) response, 7 genotypes with MR (moderate resistance) responses, and the rest of the genotypes with R (resistance) response against AB were detected (Table 2). The AUDPC as a measurement for quantifying host resistance ranged between 14 and 45.5 (Table 2 and Figure 1). Artificial infection of the studied genotypes in greenhouse conditions showed that 34 and 15 genotypes out of the studied lentil genotypes were MR and R, respectively. In the greenhouse condition, the majority of genotypes (120 genotypes) were assessed as S in response to the disease (Table 3 and Figure 2). The AUDPC values in the greenhouse were higher than that seen in the field and ranged between 21 to 175 (Table 3 and Figure 2).

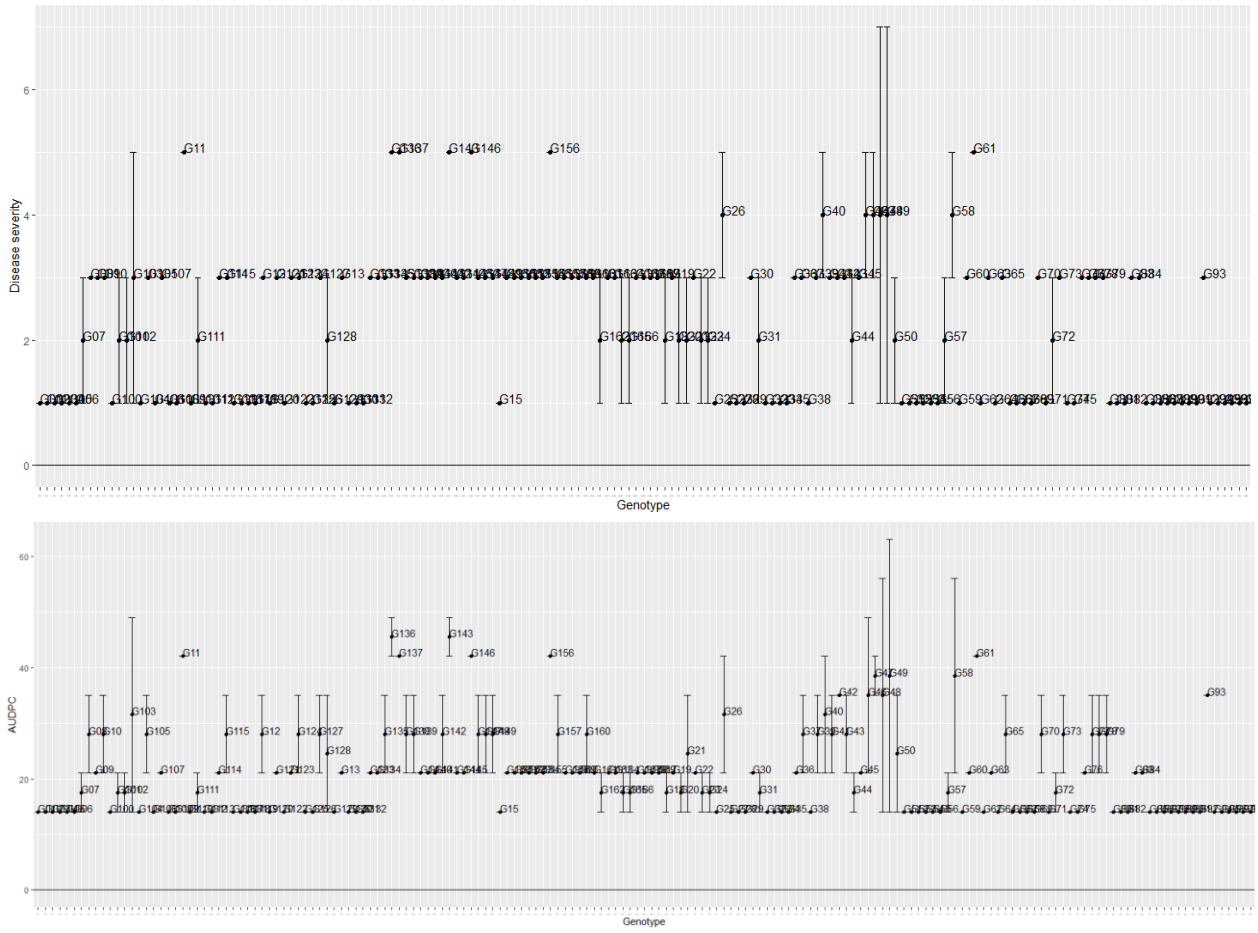

Figure 1. Mean and standard deviation of disease severity and AUDPC related to the studied genotypes in field condition.

Variables including disease severity and AUDPC were implemented by the Ward clustering algorithm to classify the germplasm in terms of their response to AB. Four groups were inferred from the germplasm in field conditions (Figure 3A) where resistant genotypes were discrete from the rest. Regarding Figure 1A, genotypes including "G11" "G61", "G136", "G137", "G143", "G146", and "G156" were located in distinguished groups (Group III). Some of the lentil genotypes with MR responses such as "G47", "G49", and "G58" were also located with the susceptible genotypes in the same cluster (Figure 3A). In the field conditions the majority of lentil genotypes were classified inside groups I, II, and IV (Figure 3A) and among the identified groups, group IV included the rest of MR lentil genotypes "G26", "G40", "G46", and "G48". Results of the classification of lentil genotypes in greenhouse conditions (Figure 3B) showed 4 separate groups. All of the resistant genotypes were classified inside group I while susceptible genotypes were located in groups II, III as well as IV (Figure 3B). Likewise, the majority of MR genotypes were located in group I (Figure 3B).

With the aim of screening and recognizing lentil genotypes harbouring suitable levels of disease severity and AUDPC, the two-dimensional scatter plot was applied (Figure 4). Since genotypes with a mean disease severity score of ≤3 were reported as resistant (Ford *et al.,* 1999), this value was applied as the disease severity threshold in the scatter plot graph. Considering the disease severity threshold as well as the minimum value of AUDPC, a bundle of lentil genotypes wins in the field trial (Figure 4A). Scatter plot illustrated that genotypes "G121", "G132", "G139", and "G143" possessed disease severity scores below 3 and AUDPC values lower than 30 in the greenhouse conditions (Figure 4B). Among the genotypes mentioned above; "G121", "G132", and "G139" were also resistant in the field conditions. There was no detected resistant genotype among the studied germplasm against AB (Figures 4A and 4B) in either conditions.

DISCUSSION

During the study, which involved screening 169 lentil genotypes for AB resistance in both field and

Table 2. Disease severity, resistance/susceptible response, and AUDPC values related to studied lentil genotypes in field condition.

Gen.	DS[a]	DR[b]	AUDPC[c]	Gen.	DS	DR	AUDPC	Gen.	DS	DR	AUDPC	Gen.	DS	DR	AUDPC
G01	1	R	14.0	G44	2	R	17.5	G87	1	R	14.0	G130	1	R	14.0
G02	1	R	14.0	G45	3	R	21.0	G88	1	R	14.0	G131	1	R	14.0
G03	1	R	14.0	G46	4	MR	35.0	G89	1	R	14.0	G132	1	R	14.0
G04	1	R	14.0	G47	4	MR	38.5	G90	1	R	14.0	G133	3	R	21.0
G05	1	R	14.0	G48	4	MR	35.0	G91	1	R	14.0	G134	3	R	21.0
G06	1	R	14.0	G49	4	MR	38.5	G92	1	R	14.0	G135	3	R	28.0
G07	2	R	17.5	G50	2	R	24.5	G93	3	R	35.0	G136	5	S	45.5
G08	3	R	28.0	G51	1	R	14.0	G94	1	R	14.0	G137	5	S	42.0
G09	3	R	21.0	G52	1	R	14.0	G95	1	R	14.0	G138	3	R	28.0
G10	3	R	28.0	G53	1	R	14.0	G96	1	R	14.0	G139	3	R	28.0
G11	5	S	42.0	G54	1	R	14.0	G97	1	R	14.0	G140	3	R	21.0
G12	3	R	28.0	G55	1	R	14.0	G98	1	R	14.0	G141	3	R	21.0
G13	3	R	21.0	G56	1	R	14.0	G99	1	R	14.0	G142	3	R	28.0
G14	3	R	21.0	G57	2	R	17.5	G100	1	R	14.0	G143	5	S	45.5
G15	1	R	14.0	G58	4	MR	38.5	G101	2	R	17.5	G144	3	R	21.0
G16	3	R	21.0	G59	1	R	14.0	G102	2	R	17.5	G145	3	R	21.0
G17	3	R	21.0	G60	3	R	21.0	G103	3	R	31.5	G146	5	S	42.0
G18	2	R	17.5	G61	5	S	42.0	G104	1	R	14.0	G147	3	R	28.0
G19	3	R	21.0	G62	1	R	14.0	G105	3	R	28.0	G148	3	R	28.0
G20	2	R	17.5	G63	3	R	21.0	G106	1	R	14.0	G149	3	R	28.0
G21	2	R	24.5	G64	1	R	14.0	G107	3	R	21.0	G150	3	R	21.0
G22	3	R	21.0	G65	3	R	28.0	G108	1	R	14.0	G151	3	R	21.0
G23	2	R	17.5	G66	1	R	14.0	G109	1	R	14.0	G152	3	R	21.0
G24	2	R	17.5	G67	1	R	14.0	G110	1	R	14.0	G153	3	R	21.0
G25	1	R	14.0	G68	1	R	14.0	G111	2	R	17.5	G154	3	R	21.0
G26	4	MR	31.5	G69	1	R	14.0	G112	1	R	14.0	G155	3	R	21.0
G27	1	R	14.0	G70	3	R	28.0	G113	1	R	14.0	G156	5	S	42.0
G28	1	R	14.0	G71	1	R	14.0	G114	3	R	21.0	G157	3	R	28.0
G29	1	R	14.0	G72	2	R	17.5	G115	3	R	28.0	G158	3	R	21.0
G30	3	R	21.0	G73	3	R	28.0	G116	1	R	14.0	G159	3	R	21.0
G31	2	R	17.5	G74	1	R	14.0	G117	1	R	14.0	G160	3	R	28.0
G32	1	R	14.0	G75	1	R	14.0	G118	1	R	14.0	G161	3	R	21.0
G33	1	R	14.0	G76	3	R	21.0	G119	1	R	14.0	G162	2	R	17.5
G34	1	R	14.0	G77	3	R	28.0	G120	1	R	14.0	G163	3	R	21.0
G35	1	R	14.0	G78	3	R	28.0	G121	3	R	21.0	G164	3	R	21.0
G36	3	R	21.0	G79	3	R	28.0	G122	1	R	14.0	G165	2	R	17.5
G37	3	R	28.0	G80	1	R	14.0	G123	3	R	21.0	G166	2	R	17.5
G38	1	R	14.0	G81	1	R	14.0	G124	3	R	28.0	G167	3	R	21.0
G39	3	R	28.0	G82	1	R	14.0	G125	1	R	14.0	G168	3	R	21.0
G40	4	MR	31.5	G83	3	R	21.0	G126	1	R	14.0	G169	3	R	21.0
G41	3	R	28.0	G84	3	R	21.0	G127	3	R	28.0				
G42	3	R	35.0	G85	1	R	14.0	G128	2	R	24.5				
G43	3	R	28.0	G86	1	R	14.0	G129	1	R	14.0				

a: Mean of disease severity score across replications, b: Disease response of plant, c: Area under disease progress curve.

greenhouse environments, no genotypes were found to be resistant, suggesting that non-host resistance was not present (Mysore and Ryu 2004) in this collection. Results from the screening of genotypes against AB also manifested a broad variability in disease severity among lentil genotypes which can be interpreted as the existence of a high genetic variability among the studied lentil genotypes (Vandenberg *et al.,* 2002). In field conditions, by challenging 169 lentil genotypes with *A. lenttis* isolates, approximately 4% of genotypes were identified as S, and 4% as MR, whereas the majority of genotypes were R. These results are in coincidence with the findings of Bayaa *et al.* (1994) and Tullu *et al.* (2010) who reported that the majority *L. ervoides* as wild species are resistant to the disease. Also, Zewdie and Gemachu (2020) reported that 80 Ethiopian lentil

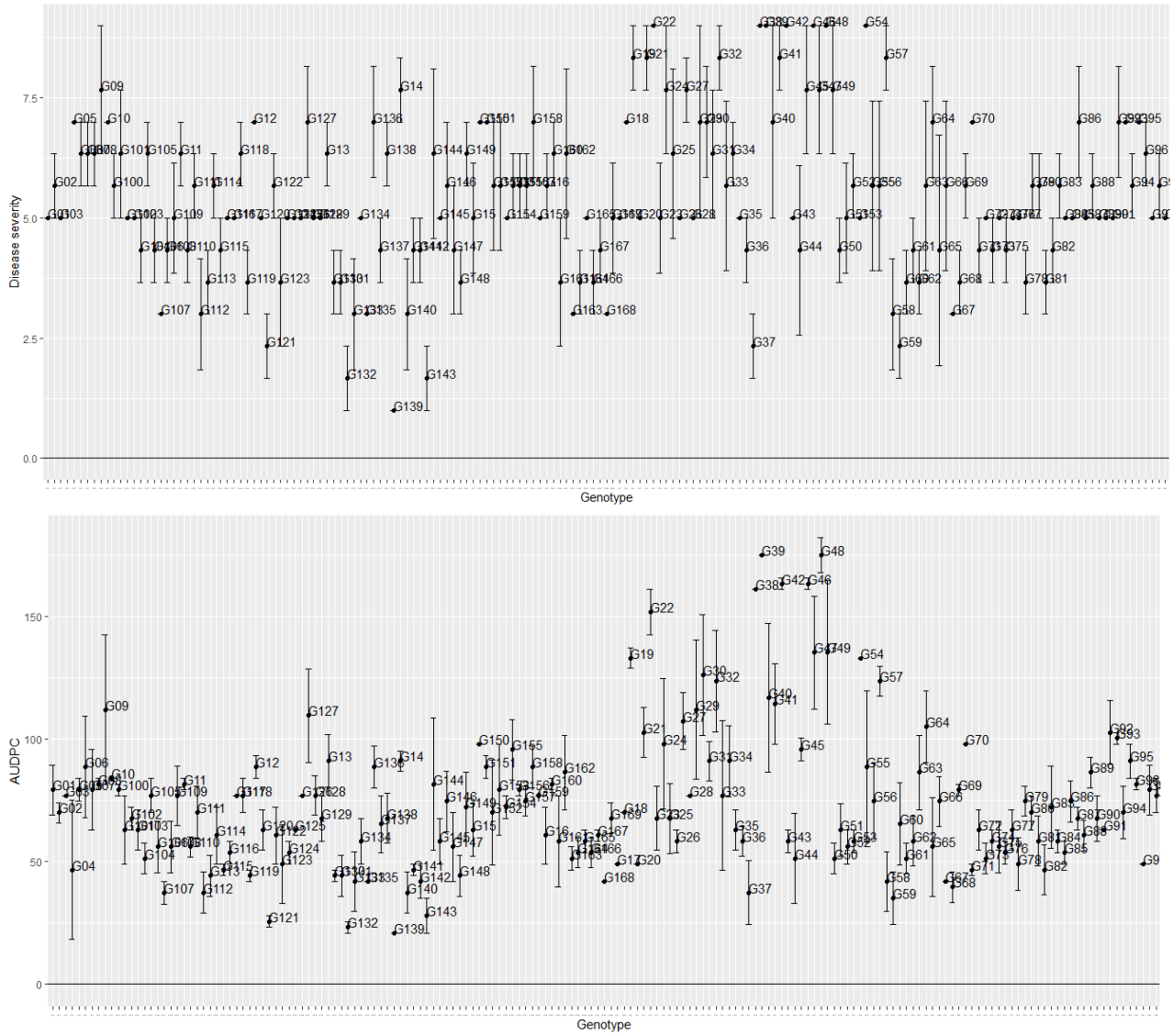

Figure 2. Mean and standard deviation of disease severity and AUDPC related to the studied genotypes in greenhouse condition.

accessions out of 148 undertaken accessions were R or MR. In another study, Bedasa (2021) evaluated a total of sixty five lentil entries at Alemtena and Minjar naturally hot spot field condition during the year 2018-19 and 2019-20. They reported high variations among the tested genotypes and 7 genotypes were resistant in Alem Tena location as well as one genotype being resistant in Minjar location. Albeit, field screening of disaease is reliable indicator but an important point to consider is that the disease does not develop naturally in the field-screening nurseries (Weaver *et al.,* 1988). Therefore, in this study, a greenhouse experiment was conducted, and unlike the field site, a wide range of responses to AB was observed among the germplasm under investigation. In this condition, 8% (15 genotypes) of genotypes identified with R response, out of which 13 were also resistant in field conditions (Table 2 and Table 3). The variation between the outcomes observed in the greenhouse and field conditions, as well as the more robust resistance observed in the field, could be attributed to the accumulation of resistance genes in a given genotype, which includes both seedling and mature plant resistance genes (McIntosh, 1988). As a result, genotypes that are susceptible during the early stages of growth, such as the seedling stage, may develop resistance at the mature plant stage. The literature review showed scarce research studies on screening lentil germplasm for AB resistance/ susceptibility by simultaneous trials in greenhouse and field conditions. Herein, the identified resistant lentil genotypes in both circumstances could be introduced for further evaluation such as multilocational yield trials

Table 3. Disease severity, resistance/susceptible response, and AUDPC values related to the studied genotypes in greenhouse condition.

Gen.	DS[a]	DR[b]	AUDPC[c]	Gen.	DS	DR	AUDPC	Gen.	DS	DR	AUDPC	Gen.	DS	DR	AUDPC
G01	5.5	S	79.3	G44	4.0	MR	51.3	G87	5.0	S	67.7	G130	4.0	MR	44.3
G02	5.5	S	70.0	G45	7.0	S	95.7	G88	5.5	S	60.7	G131	4.5	MR	44.3
G03	5.0	S	77.0	G46	9.0	S	163.3	G89	5.0	S	86.3	G132	1.5	R	23.3
G04	5.7	S	46.7	G47	8.0	S	135.3	G90	5.0	S	67.7	G133	3.0	R	42.0
G05	7.0	S	79.3	G48	9.0	S	175.0	G91	5.5	S	63.0	G134	4.5	MR	58.3
G06	6.5	S	88.7	G49	8.0	S	135.3	G92	6.5	S	102.7	G135	3.0	R	42.0
G07	6.5	S	79.3	G50	4.5	MR	51.3	G93	7.0	S	100.3	G136	7.0	S	88.7
G08	7.0	S	81.7	G51	4.5	MR	63.0	G94	6.0	S	70.0	G137	4.0	MR	65.3
G09	8.0	S	112.0	G52	6.0	S	56.0	G95	6.5	S	91.0	G138	6.0	S	67.7
G10	7.0	S	84.0	G53	5.0	S	58.3	G96	6.5	S	81.7	G139	2.5	R	21.0
G11	6.0	S	81.7	G54	9.0	S	133.0	G97	5.0	S	49.0	G140	4.0	MR	37.3
G12	6.5	S	88.7	G55	5.5	S	88.7	G98	6.0	S	79.3	G141	4.0	MR	46.7
G13	6.5	S	91.0	G56	5.5	S	74.7	G99	5.0	S	77.0	G142	4.5	MR	42.0
G14	7.5	S	91.0	G57	8.5	S	123.7	G100	5.5	S	79.3	G143	2.0	R	28.0
G15	4.5	MR	63.0	G58	3.0	R	42.0	G101	6.5	S	63.0	G144	6.5	S	81.7
G16	6.0	S	60.7	G59	2.5	R	35.0	G102	5.5	S	67.7	G145	5.5	S	58.3
G17	5.5	S	49.0	G60	4.0	MR	65.3	G103	5.0	S	63.0	G146	5.5	S	74.7
G18	7.0	S	70.0	G61	4.0	MR	51.3	G104	4.5	MR	51.3	G147	5.0	S	56.0
G19	8.5	S	133.0	G62	5.0	S	58.3	G105	6.5	S	77.0	G148	4.5	MR	44.3
G20	5.0	S	49.0	G63	5.0	S	86.3	G106	4.5	MR	56.0	G149	6.5	S	72.3
G21	8.5	S	102.7	G64	7.0	S	105.0	G107	3.0	R	37.3	G150	6.0	S	98.0
G22	7.5	S	151.7	G65	5.0	S	56.0	G108	4.5	MR	56.0	G151	7.0	S	88.7
G23	6.0	S	67.7	G66	6.5	S	74.7	G109	5.5	S	77.0	G152	6.0	S	70.0
G24	7.0	S	98.0	G67	2.5	R	42.0	G110	5.0	S	56.0	G153	6.5	S	79.3
G25	7.0	S	67.7	G68	4.0	MR	39.7	G111	5.0	S	70.0	G154	5.5	S	72.3
G26	5.0	S	58.3	G69	5.0	S	79.3	G112	3.5	MR	37.3	G155	6.0	S	95.7
G27	7.0	S	107.3	G70	7.0	S	98.0	G113	4.0	MR	44.3	G156	5.5	S	79.3
G28	5.0	S	77.0	G71	4.5	MR	46.7	G114	6.0	S	60.7	G157	5.5	S	74.7
G29	7.5	S	112.0	G72	5.5	S	63.0	G115	4.0	MR	46.7	G158	6.5	S	88.7
G30	7.5	S	126.0	G73	4.5	MR	51.3	G116	5.0	S	53.7	G159	5.0	S	77.0
G31	7.0	S	91.0	G74	5.0	S	58.3	G117	5.0	S	77.0	G160	6.0	S	81.7
G32	7.5	S	123.7	G75	4.5	MR	56.0	G118	6.0	S	77.0	G161	4.0	MR	58.3
G33	5.5	S	77.0	G76	4.5	MR	53.7	G119	3.0	R	44.3	G162	6.0	S	86.3
G34	6.0	S	91.0	G77	5.0	S	63.0	G120	5.0	S	63.0	G163	3.0	R	51.3
G35	5.0	S	63.0	G78	4.0	MR	49.0	G121	2.5	R	25.7	G164	3.5	MR	53.7
G36	4.0	MR	58.3	G79	5.5	S	74.7	G122	5.5	S	60.7	G165	5.0	S	58.3
G37	2.0	R	37.3	G80	5.5	S	70.0	G123	3.0	R	49.0	G166	4.0	MR	53.7
G38	9.0	S	161.0	G81	3.5	MR	58.3	G124	5.0	S	53.7	G167	4.5	MR	60.7
G39	9.0	S	175.0	G82	4.0	MR	46.7	G125	5.0	S	63.0	G168	3.0	R	42.0
G40	7.5	S	116.7	G83	6.5	S	72.3	G126	5.0	S	77.0	G169	4.5	MR	67.7
G41	8.5	S	114.3	G84	6.0	S	58.3	G127	7.5	S	109.7				
G42	9.0	S	163.3	G85	5.5	S	53.7	G128	5.0	S	77.0				
G43	5.0	S	58.3	G86	6.5	S	74.7	G129	5.0	S	67.7				

a: Mean of disease severity score across replicates, b: Disease response of plants, c: Area under disease progress curve.

in dryland cropping regions. Regarding Sandoval-Islas *et al.* (2007), AUDPC as a measurement at different times is a suitable criterion for screening resistant genotypes. Interestingly, some of the identified lentil genotypes ("G121", "G132", "G139", and "G143") which was resistant in any situations had also lower AUDPC values (Figure 4) and therefore, considered as resistance source for lentil. Since genetic control of resistance to AB is controlled by a few number of genes (Ye *et al.,* 2003), therefore; these genotypes can be used to improve target lentil genotypes with high yield and resistance to AB through the back cross method. From a breeding point of view, simultaneous selection could lead to a significant progress in the selection of genotypes with high-favorable traits (Baker, 1986). Therefore, a multivariate hierarchical

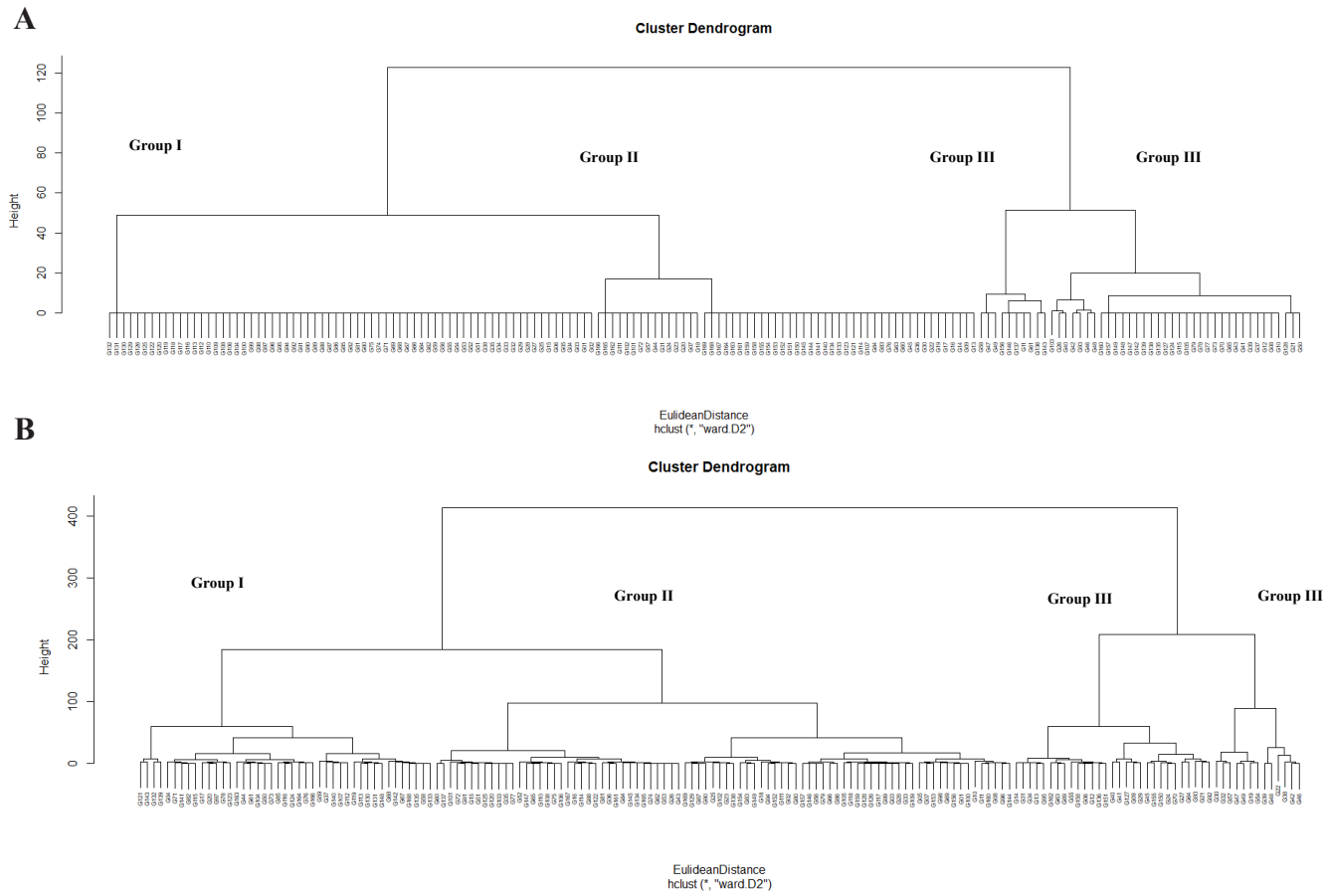

Figure 3. Classification of studied lentil germplasm according to their disease severity and AUDPC data using the Ward clustering algorithm and Euclidean distance in the **A:** field and **B:** greenhouse conditions.

clustering method was implemented to classify the studied lentil germplasm simultaneously based on disease severity and AUDPC data. Resulted cluster analysis was effectively used to screen the lentil germplasm in response to the disease. Resistant genotypes were separated from susceptible genotypes in both experimental conditions. The identified distant groups (Figure 3) named heterotic groups, could be considered in the parental selection for the construction of mapping populations and identification of genetic loci controlling resistance to AB. In addition, potential genetic variability among studied germplasm promoted its utilization for genome-wide association mapping approach.

CONCLUSION

AB is a significant threat to lentil production both in Iran and globally. The lentil germplasm that was evaluated demonstrated significant variability in its response to blight in both experimental conditions. The results obtained from this research indicate that both AUDPC and DS criteria were effective for screening the germplasm. This information could be useful for breeders to select parental lines in future lentil breeding programs, such as those focused on mapping resistance loci, using marker-assisted selection, and achieving multiple disease resistance. Our findings suggest that the genotypes G121, G132, and G139 exhibited resistance in both experimental conditions and could be suitable for future lentil programs that involve yield evaluation and regional trials.

ACKNOWLEDMENTS

The authors thank Dryland Agriculture Research Institute for their practical help in field experiment and Shahrekord University about lab facilities.

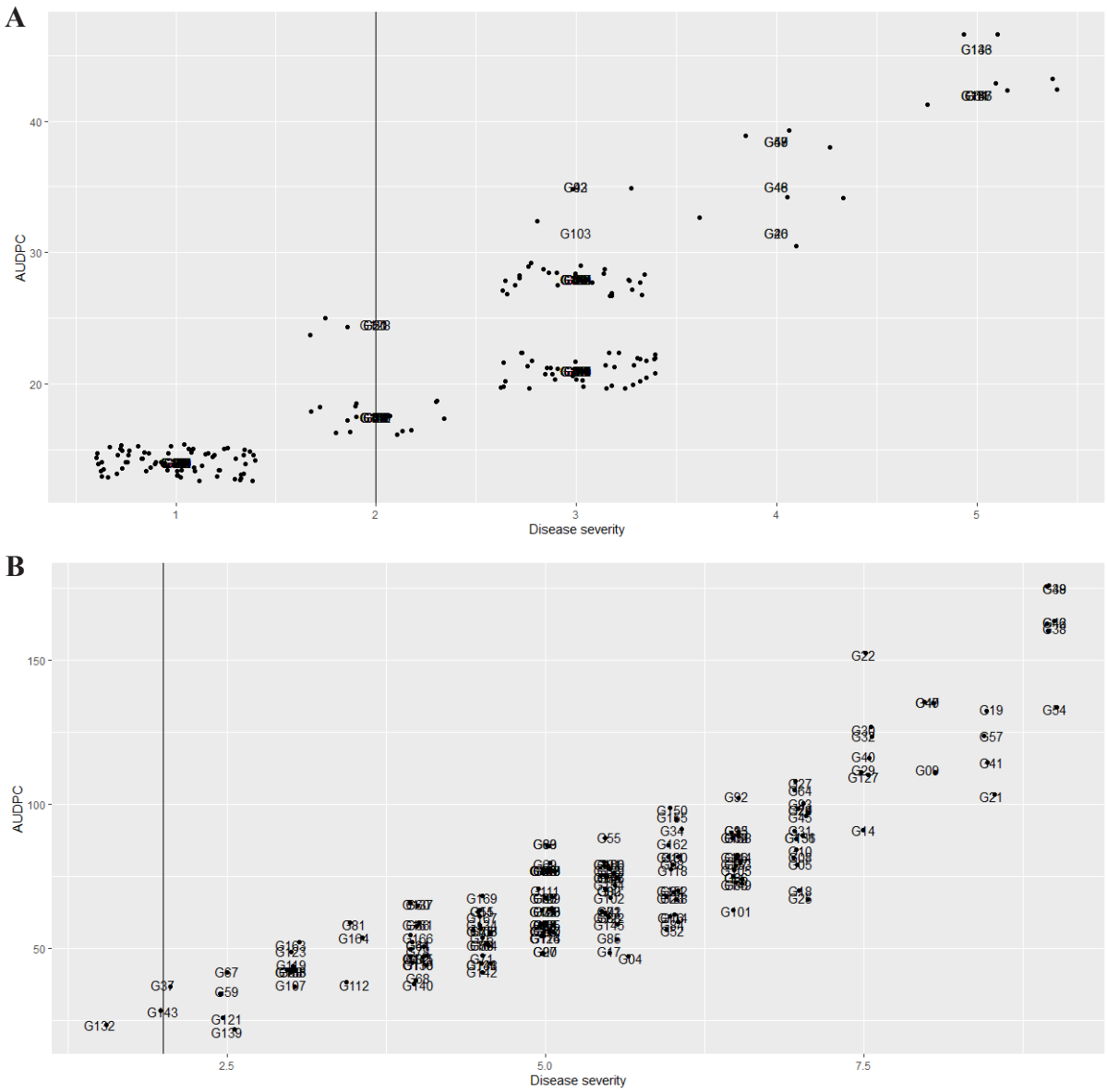

Figure 4. Screening lentil germplasm through two dimensional plot of disease severity and AUDPC in **A:** field and **B:** greenhouse conditions.

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