

Evaluation of the Existence of a New Race of *Bremia lactucae* on Lettuce

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Abstract

Lettuce is one of the most consumed leafy vegetables in Turkey. The production of lettuce has been to get difficult due to infestation of cultivation areas with *Bremia lactucae*. This pathogen is genetically very variable. Pathogenic variation of *B. lactucae* has not been studied yet in Turkey. The objective of this study was to monitor the races of *B. lactucae* in the two geographic regions of Turkey. During the lettuce growing season in May and October 2018, totally 72 diseased leaf samples containing *B. lactucae* sporangia were collected from the fields of Bartın and Ankara provinces. From these samples 6 isolates were obtained. After a multiplication procedure, in which the susceptible lettuce cultivar 'Green Towers' was used, the *B. lactucae* sporangia were inoculated in differentiating lettuce cultivars. The response of the lettuce seedlings to pathogen was evaluated on the 7, 11 and 14 days after inoculation. The qualitative method was used for the assessment of infected seedlings. Sextet codes of Ankara and Bartın isolates were found as 44-00-01 and 13-03-04 respectively. The sextet codes of Turkish isolates did not match any of the present 37 races of *B. lactucae*. This result suggests that our isolates may be new races or pathotypes.

1. Introduction

Lettuce (*Lactuca sativa*), is the most widely used vegetable in Turkey for its availability all year-round. In Turkey, different types of lettuce are being cultivated on the area of 218 208 ha with an annual production of 520 151 tons (TUIK, 2020)

Many factors restrict the production of lettuce. Among these factors, downy mildew caused by *Bremia lactucae*, ranks first (Crute, 1992; Lebeda and Schwinn, 1994; Lebeda and Petzelová, 2004). *B. lactucae* is can infect any lettuce growth stage from seedling to mature plant and produce symptoms of pale-yellow angular leaf spots on the upper leaf surface and whitish fluffy sporulation on the lower side of the leaves (Crute and Harrison, 1988; Lebeda and Petzelová, 2010). Many factors affect the onset of the disease. Among these, humid and cool environments are of paramount

importance following solar radiation and wind which influence spore production and dispersal. The severity of damage caused by *Bremia* can vary depending on environmental conditions. High humidity and cool temperatures are optimal for infection and spread of the disease (Fletcher, 1976; Scherm and van Bruggen, 1994; Wu et al., 2002; Su et al., 2004). The pathogen may spread quickly over large areas during cool (5-17°C) and wet (100%) conditions, and may cause considerable damage, and economic losses (Lebeda and Petzelová, 2010).

There are various alternative for the farmers to control disease. Although growing resistant varieties together with fungicide application seems as the most effective way, it is not applicable everywhere having many pathogenic variation and fungicide resistance. *Bremia lactucae*, which has been reported to have two mating types (B1 and B2)

(Michelmore and Ingram, 1980) shows great genetic variability. New races overcoming the resistance of the varieties occur very often (De Vries, 1997; Lebeda et al., 2009). Because of this, breeding new resistant varieties is a necessity in the control of the disease. For successful resistance breeding, the regional variation of virulence in the *B. lactucae* population has to be known (Lebeda and Zinkernagel, 2003). From the years 1998 to 2021, 37 races of *B. lactucae* were determined in the European region (Ettেকoven and van der Arend, 1999; van der Arend et al., 2006; IBEB, 2021). Recently, The International Bremia Evaluation Board, European Union (IBEB-EU) reported that the most widely spread races are denominated BI: 34EU, BI: 35EU and BI: 36EU. These three races appear in 20% of the samples. More than 60% of the isolates, had new virulence patterns.

According to the IBEB (2021), the most recently found race BI:37EU (sextet code: 46-15-14) is widely spread in France but is also present in Spain, Portugal and Italy. Turkey is not in the scope of IBEB-EU. Therefore, resistance claims for lettuce varieties may be valid in Europe but not in Turkey. It is important to strengthen the scientific basis of the deployment of resistance in the Turkish market, given the importance of lettuce production for the Turkish economy and consumers. Therefore, identification of the races of *B. lactucae* in lettuce producing areas of Central Anatolia and West Black Sea regions of Turkey was taken into consideration in this study.

2. Materials and Methods

2.1. Collection and storage of the plant sample

Isolates of *Bremia lactucae* were obtained from lettuce fields in Bartın and Ankara (Beypazarı) provinces, during the lettuce growing season in May and October 2018. Leaves with typical symptoms of downy mildew (infected varieties: Cartagenas and Yedikule) were collected from the fields, placed in the cold plastic boxes with moistened filter paper, and transported to the laboratory as soon as

possible. Part of the samples were used freshly in the experiments and the rest of the samples were stored at -20°C.

2.2. Isolation, inoculation and incubation

Firstly, infected leaves were examined under a stereo microscope and light microscope to observe the sporangia and sporangiophore of the fungus on the lower surface of the leaves containing mycelial growth (Figure 1).

The inoculum was prepared by washing off spores from infected lettuce leaves by shaking the sporulating lesions in centrifuge tubes with tap water in a Vortex mixer. The suspension was filtered through a single layer cheesecloth. The concentration of suspension was determined by counting the number of sporangia with haemocytometer and adjusted to 8×10^4 sporangia ml⁻¹.

This initial inoculum was used to infect the susceptible lettuce variety 'Green Towers' on which the isolate was propagated (Figure 2). To determine of races of *Bremia*, seeds of the differential cultivars of IBEB in EU-C set (IBEB, 2019) were placed on moist filter paper in transparent plastic boxes. Then the seedlings with fully expanded cotyledons were sprayed to runoff with the sporangia suspension with small hand sprayer. The seedlings of the differentials set were kept in darkness for 24 hours (h) and then with a 12 h photoperiod (light intensity of 10,000 lux) in the climate chamber at night and day temperature of 15-17°C, respectively. The experiment was conducted with three repetitions, each repetition consisting of a box containing fifteen seedlings in cotyledon stage.

2.3. Disease assessment

Isolates of *B. lactucae* were tested on a series of 16 differential cultivars of IBEB in EU-C set as mentioned above. Each experiment was conducted at least three times. The response of the lettuce seedlings was evaluated on the 7, 11 and 14 days after inoculation. The qualitative method was used for the assessment of infected seedlings (Lebeda



Figure 1. Infected lettuce leaf (a) with *Bremia lactucae*, sporangia (b).



Figure 2. Inoculated fully expanded cotyledons of cv 'Green Towers' lettuce.

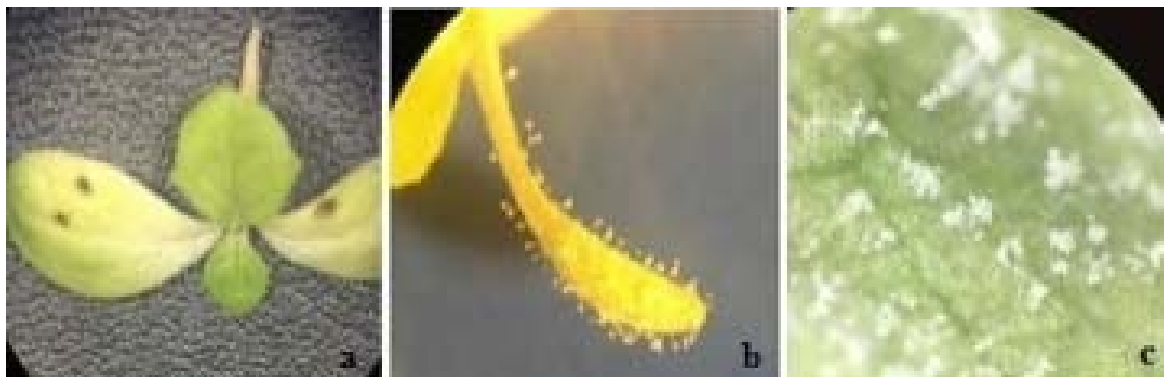


Figure 3. *Bremia lactucae* symptoms with necrosis and chlorosis (a), sporulation (b, c).

and Petrželová, 2010). Symptoms were observed with or without necrosis, chlorosis and sporulation (Figure 3).

3. Results and Discussion

In this study, quite a lot of lettuce leaf samples infected with *B. lactucae* containing sporangia were collected from the fields of Bartın and especially from the high lettuce cultivation areas in the Bey pazarı district of Ankara province. Sampling was carried out during the lettuce growing season in May and October 2018. However, it was seen that most of the isolates obtained in the spore viability tests lost their viability, possibly due to fungicide applications a few days before sampling. It has been observed that viable spores are associated with successful reproduction and non-viable spores with unsuccessful reproduction.

After a propagation procedure, in which the susceptible lettuce cultivar 'Green Towers' was used, the *B. lactucae* sporangia were inoculated in differentiating lettuce cultivars. The response of the lettuce seedlings was evaluated on the 7, 11 and 14 days after inoculation and the qualitative method was used for the assessment of infected seedlings.

Sextet codes of Ankara isolates (TR-1201 and BC-1) which were successful in propagation and inoculation were found as 44-00-01 and that of Bartın isolates (TB-1, TB-2, TB-3 and TB-4) were 13-03-04 (Table 1).

Bremia lactucae was prevalent in all major cultivation areas in Turkey before lettuce growers have started using fungicides and resistant varieties. However, in recent years, the losses caused by *Bremia* are relatively small due to the introduction of licensed fungicide against this disease in our country and the conscious preference of resistant varieties by the producer. Nevertheless, if the environmental conditions are favourable for the pathogen *B. lactucae* is still a major threat.

The pathogen is genetically very variable. Multiple isolates that differ in their ability to overcome resistance genes may be present even within one lettuce production field. Many isolates are of minor importance because they do not persist. Isolates with the same virulence that occur at several geographic locations, persist over multiple years, and have stable virulence are considered for nomination as a race. Between 2005 and 2010 in Australia, three distinct patterns of virulence in *B. lactucae* were identified and they were named AUS4, AUS5 and AUS6 (Nordskog et al., 2014). During 2008 and 2009 *B. lactucae* races occurring in lettuce producing areas of São Paulo state were identified. Two *B. lactucae* codes SPBI:05 and SPBI:06 were identified (Castoldi et al., 2012). Another study was monitoring the races of *B. lactucae* in the state of Minas Gerais, Brazil, in 2010. The data have identified the sextet codes in the state of Minas Gerais 63/63/51/00, 63/63/03/00, 63/63/19/00 and

Table 1. Reactions of six *Bremia lactucae* isolates to the IBEB C set of differentials.

Isolates	TR-1201	BC-1	TB-1	TB-2	TB-3	TB-4
Green Towers	+	+	+	+	+	+
Dandie	-	-	+	+	+	+
R4T57D	-	-	-	-	-	-
UC Dm14	+	+	+	+	+	+
NunDm15	+	+	+	+	+	+
CGDm116	-	-	-	-	-	-
Coloeado	+	+	-	-	-	-
FrRsal-1	-	-	+	+	+	+
Argeles	-	-	+	+	+	+
RYZ 2164	-	-	-	-	-	-
RYZ910457	-	-	-	-	-	-
Bedford	-	-	-	-	-	-
Balesta	-	-	-	-	-	-
Bartoli	+	+	-	-	-	-
Design	-	-	-	-	-	-
Kibrille	-	-	+	+	+	+
Sextet code	44-00-01	44-00-01	13-03-04	13-03-04	13-03-04	13-03-04

63/63/02/00 (Vargas, 2017). There are a lot of races or pathotypes in the population of *B. lactucae* in Europe and 37 races were found on lettuce from 1998 to 2021 (Ettkovén and van den Arend, 1999; van der Arend et al., 2006; IBEB, 2021). IBEB-EU evaluated 800 *B. lactucae* isolates for pathogenic variation, 300 collected in 2018 and 500 in 2017. Most isolates in 2018 belonged to local races. However, BI: 36EU already found in 2016 and 2017 was established in many places. In 2021 a new race of *B. lactucae*, BI: 37EU (sextet code: 46-15-14) identified and denominated in Europe (IBEB, 2021). So far, information about the pathogenic variability or races of *B. lactucae* in Turkey have not been recorded. But, 37 races of the pathogen (EU BI 1-37) have been identified in Europe and 9 (US BI 1-9) races in the United States (IBEB, 2021). The sextet codes of none of the Turkish isolates of *B. lactucae* matched the sextet codes of the races detected in Europe (EU BI 1-37) and the United States (US BI1-9). This situation reveals the existence of different pathotypes or land races in our country.

4. Conclusion

Bremia lactucae, the causal organism of downy mildew in lettuce, is a major threat to lettuce production. Farmers often need to use both fungicides and resistance genes to prevent heavy losses. Reliable information about resistances in relation to the local strains of *Bremia* is essential for a successful and durable disease control strategy.

In the present study, it is seen that there is a difference between isolates collected from lettuce cultivation areas in two different regions (Central Anatolia and West Black Sea). While the Ankara isolates (TR-1201, BC-1) formed a group, all of the Bartın isolates (TB1, TB2, TB3, TB4) formed a separate group. It is seen that the pathogenicity of the isolates is different in two different geographical

regions within the same country. The fact that the sextet codes obtained from the reactions of the six isolates tested on IBEB differentials did not match any of the present 37 races suggests a high pathogenic variability of the pathogen.

The future significance of the two local races that we describe here is hard to predict. Only continuous monitoring can further strengthen the scientific basis of resistance breeding.

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