DIFFERENCES IN VIRULENCE OF *RHYNCHOSPORIUM COMMUNE* ISOLATES FROM CENTRAL ANATOLIA ON BARLEY CULTIVARS

Hakan Hekimhan[1], Aydan Araz[2], Mehmet Aydoğdu[3]

[1]Aegean Agricultural Research Institute, Menemen/İzmir, Turkey
[2]Central Plant Protection Institute, Yenimahalle/Ankara, Turkey
[3]West Mediterranean Agricultural Research Institute, Antalya, Turkey

Corresponding author: hakanhekimhan@hotmail.com

Abstract
Rhynchosporium commune, the causal agent of leaf disease and also known as leaf blotch or scald, affects plant growth and accordingly yield in barley worldwide. The aim of the study was to determine difference in virulence of isolates of *R*. *commune*(Rc) from main barley growing areas of Turkey. In the study, a total of 37 isolates were obtained from Central Anatolia (Ankara, Konya, Eskişehir, Kırşehir and Çorum provinces) in 2013 and 2014. To reveal difference in virulence of the isolates, susceptible cultivar Aydanhanımandcvs. Çetin 2000 and Tokak 157/37 were used as host plants. The study, set up a factorial design in randomized plots with three replications, was conducted under controlled conditions in Ankara. Cultivars at the seedling stage were inoculated by spraying inoculum on them. Using 0 to 4 scale, disease ratings were done. In the cluster analysis, the isolates were separated into four different groups. Mean disease severity (MDS) of the isolates on the cultivars was 1.92, while they were 2.59, 2.05 and 1.14 in cv. Tokak, Aydanhanım and Çetin 2000, respectively. Disease severity (DS) varied from 0.01 to 3,5 among the cultivars. With 2.83 and 2.66 DS rates, isolate 37 and 31 were the most virulent, respectively. However, the least virulent isolates were 10(1.17) and 11(1,0), 12(1,0), 13(1,0), 25(1,0). Differences determined among isolates and cultivars and isolate x cultivar interactions were significant statistically ($P<0.01$, R2 0,94). The study showed that virulences of the isolates of Rcwere significantly different on the hosts tested.

Keywords: *Rhynchosporium commune*, isolate, virulence, barley, seedling stage.

Introductıon

With a nearly 2,7 million ha cultivated area and 7.1 million tonnes production, barley (*Hordeum vulgare* L.) is ranked as the second cerealafter wheat in Turkey (Anonymous, 2015). It is mainly used in animal feeding and malt industry and usually grown in Central Anatolia under dry conditions in Turkey (Kün, 1988). Leaf blotch disease (LBD) of barley, caused by the fungus *R*. *commune* (Zaffarano, Mcdonald&Linde), is one of the most important diesease of barley in Turkey as well as the World (Zaffarano et al. 2011).LBD is generally leads to reduce yield by decreasing tillering and grain weight. Depending on the onset of the disease on the host, yield losses can reach up to 30-40% (Aktaş, 2001). However, yield losses ranging from 10 to 70% due to LBD was reported by Mathre (1982). Ensuing infection of *R*. *Commune* (Rc) on the second and third leaves of susceptible cultivars, leaf lesions covering leaf blade appear and then general chlorosis on the leaves occurs. On the other hand, on the leaves of resistant cultivars no lesions form or small, brownish-grey spots on the border and tips of the leaves emerge (Xue et al. 1991). Damage and intensity of LBD have tended to increase all around the world since 1980s. Growing barley in vast areas as a monoculture, using reduced tillage system, remaining infected crop residues in the soil, shifting climatic conditions and highly evolving genetic structure of the fungus, all of these factors could be responsible for those damage from LBD (Ellen, 2002). Thus, as the damage of LBD increases, fungicide usage against the disease goes up in the World. However, the fungicides used to control the disease causes soaring cost for production and adverse effects on the environment (Polley and King1993). In this regard, the best way to manage with the disease is to develop resistant barley cultivars and use them in barley

cultivation. There are many pathotypes of the fungus (Salamati and Tronsmo 1997, Lebedeva et al. 2006, Meles et al. 2008, Araz and Hekimhan 2017). Hence, determining reactions of existing barley cultivars against virulent pathotypes and monitoring genetic changes of the fungus are very important to manage with the disease (Avrova and Knogge 2012). The objective of the study was to determine differences in virulence of Rc isolates obtained from barley growing areas of Central Anatolia on widely grown cultivars.

Material and methods

Barley Cultivars

2-row Tokak 157/37 and Aydanhanım, 6-row Çetin-2000.

Isolates

A total of 37 isolates were obtained from diseased leaves during surveys conducted in barley growing areas of Central Anatolia (Konya, Ankara, Eskişehir, Kırşehir and Çorum Provinces) in 2013 and 2014.

Isolation

Infected leaf tissues were cut as pieces with a size of 5 mm^2 and exposed to 70% ethyl alcohol for 10 seconds and then 5% Sodium Hypochlorite (NaoCl) for 90 seconds. These pieces were soaked into sterile distilled water for 30 seconds and later transferred to blotter medium at 20 °C for 24 hour to ensure sporulation. Afterwards, the leaf pieces were dipped into sterile distilled water and rinsed and then a drop from this solution was taken and spreaded onto 1% water agar medium and incubated at 20 °C for 24 hour (Fowler and Owen 1971, Döken, 1979). Conidia germinating on the medium were taken single by single through a sterile needle under a microscope and transferred to PDA (Potato Dextrose Agar). Petri dishes were put in an incubator by adjusting 17 °C for 15 days and in this way colony of the fungus appeared (Figure 1). By subculturing these colonies, pure isolates of the fungus was attained. Single spore isolates obtained were transferred into glass tubes (Microbank) and stored at -18 °C (Lange and Boyd 1968, Xue et al. 1991).

Figure 1. Colonies of *R. commune* on PDA

Greenhouse tests and evaluations of host responses

Stored isolates were transferred on PDA and incubated at 17 to 20 ° for 14 to 20 days. Later, 5 to 10 ml sterile water per petri was poured on colonies developing on PDA and the colonies were taken using a sterile fine brush and filtered via a sterile cheese cloth into a sterile beaker. Conidia concentration of the inoculum was adjusted as $5x10^5$ spores/ml through a haemocytometer (Xue et al. 1991). Since barley is a long-day plant, in the greenhouse experiments, durarion of lighting was adjusted as 16 hour light and 8 hour darkness (Vardar, 1983). During lightning and dark periods, temperatures of the ambient were arranged as 18±2°C and 16±2°C, respectively. (Xue et al. 1991). 10 seeds of each cultivars were sown per pot and plants were watered twice a week. At the seedling stage of plants(11^{st} growth stage of Zadoks) (Zadoks et al. 1974), plants were inoculated by spraying adjusted inoculum. However, sterile water was sprayed on cv. Aydanhanım (susceptible check cultivar). Experiments were set up a factorial design in randomized plots with three replications. Ensuing inoculation, plants were covered with polyethylene bags and kept at 18±2 °C under 90 to 100% relative humidity and 48 hour dark period conditions (Mayfield and Clare 1991). Nearly 18 to

20 days later inoculation, disease ratings were done according to 0 to 4 scale in Table1 (El-Ahmed, 1981). In the scale; values of 0,1 and 2 were evaluated as resistant, as for 3 and 4 were rated as susceptible.

Table 1. Scale, 0 to 4, used for disease evaluations (El-Ahmed, 1981)

0	No disease symptom on plant
1	Small, brownish-grey spots on tip and/or borders of leaves
2	Small, brownish-grey lesions scattered on leaves
3	Large lesions on an area, over 50% of leaves
4	Large, coalesced lesions and general chlorosis and leaf death

Results and discussion

All cultivars tested showed resistant response to the isolates according to mean scale values. Differences in the isolates and cultivars and isolate x cultivar interactions were significant statistically ($P<0.01$, R^2 0,94). Mean disease severity (MDS) of the cultivars was 1.93. However, MDS of Tokak 157/37, Aydanhanım and Çetin-2000 were 2.59, 2.05 and 1.14, respectively (Table 2). Disease severity (DS) of the cultivars ranged from 0.01 to 3.5. Of the isolates, with 2.83 and 2.66 MDS values, isolate 37 and 31 were the most virulent ones, respectively. Whereas, with 1.17 MDS, the weakest virulent was isolate 10. Isolate 17 and 37obtained from Ankara and isolate 31 from Çorum constituted the highest DS on cv. Tokak 157/37. Isolate 37 also created the highest DS on cv. Çetin 2000. Besides, isolate 8 from Kırşehir and isolates 29 and 33 from Konya constituted the highest DS values on cv. Aydanhanım (Table 2). In the cluster analysis, isolates were separated into 4 different groups according to their virulence status. The isolates 10, 11, 12, 13, 25 and 31, 37 were grouped individually as the least and the most virulent, respectively (Figure 2). In the study, isolates obtained from Ankara, Konya and Eskişehir Provinces constituted both high and low DS on the hosts tested, indicating virulence difference in them. As it is known that responses of the cultivars to different isolates from different locations may vary. For example, cv. Çetin-2000, known as resistant to Rc, showed 2.50 DS against isolate 37, which near the value of 3.0 DS displayed by susceptible cv. Aydanhanım. In the study, the most virulent isolate was 37, obtained from Ankara. However, in a study conducted by Araz and Maden (2006), it was reported that the most virulent pathotypes of Rc in Central Anatolia were found on the samples from Eskişehir, Kayseri and Yozgat Provinces. In this regard, our finding revealed that isolates in one location could shift their genetic structure and accordingly, with time, new virulent isolates may appear. Likewise, although sexual stage of Rc has not been know, it has been stated that Rc has a high degree of genetic variation and evolving potential (Jackson and Webster 1976, Zhang et al. 1992,Forgan et al. 2007, Zhan et al. 2012). In the present study, DS values changed according to isolate and host. For instance, the isolates, e.g. 33, 34 and 35, constituted high DS on cv. Aydanhanım whereas, they displayed low DS on cv. Çetin-2000. In addition, the isolates, e.g. 2 and 3, showed higher DS on cv. Tokak 157/37 than cv. Aydanhanım. As for, Azamparsa et al. (2015) stated that 3 Rc isolates from Gaziantep, Eskişehir and Manisa Provinces created high DS on Tokak 157/37 under greenhouse conditions. However, Düşünceli et al. (2008) emphasised that Of 36 barley cultivars, cv. Çetin-2000, 6-row barley variety, was the most resistant one against Rc both under greenhouse conditions and in the field. This finding corroborated the ones of Barradas (1984). In that study, author reported that 6-row barley cultivars have more resistance sources. Additionally, virulence of isolates can change depending on the pathotype isolated from different location and hosts. Barradas (1984) stated that genetic and morphological structure of the hosts, cultural practices, climate could play an important role in Rc development on barley. Likewise, Bockelman (1984) tested 9 different isolates of Rc on 20 barley cultivars and reported that virulence of the isolates were different one another. In fungi, alterations in virulence may appear through gene flow, recombination, mutation and sexual production. As a result of these phenomenon, with time, cultivars, known as resistant, could become susceptible to those new emerging virulent fungi.

Thus, new emerging pathotypes of Rc could be more virulent than existing ones (Jorgensen and Smedegaard-Petersen 1995, Zencirci and Hayes 1990, Düşünceli et al. 2008).

Table 2. Origin of Rc isolates and disease severity(DS)of the cultivars

No	Origin of isolates	DS of the Cultivars			Mean DS
		Tokak 157/37	Çetin-2000	Aydan hanım	
1	Konya, Altınekin, Koçyaka	2,00 ef	1,00 h	3,00 b	2,00 dh
2	Ankara, Temelli	2,33 ce	1,00 h	2,00 ef	1,78 hj
3	Ayaş, Bayram	2,33 ce	1,00 h	2,00 ef	1,78 hj
4	Ankara, Ş.koçhisar	2,67 bc	2,00 ef	2,50 cd	2,39 b
5	Konya, Sarayönü, Baş höyük	2,50 cd	2,00 ef	2,50 cd	2,33 bc
6	Çubuk, Saraycık	1,50 g	1,83 fg	1,50 g	1,61 j
7	Eskişehir Agr.Res.Inst.BVD 44	2,50 cd	2,00 ef	2,67 bc	2,39 b
8	Kırşehir, Çiçekdağı, Safalı	2,00 ef	1,00 h	3,50 a	2,17 be
9	Ankara, Gölbaşı, İkizce	2,00 ef	1,00 h	3,00 b	2,00 dh
10	Ankara, Çubuk, Yazır	1,00 h	0,01 ı	2,50 cd	1,17 k
11	Konya, Yunak, Böğrüdelik	1,00 h	0,01 ı	2,00 ef	1,00 k
12	Eskişehir, Mahmudiye, Şerefiye	1,00 h	0,01 ı	2,00 ef	1,00 k
13	Eskişehir, Çifteler, Zaferhamit	1,00 h	0,01 ı	2,00 ef	1,00 k
14	Ankara, Sincan	2,00 ef	2,00 ef	2,50 cd	2,17 be
15	Konya, Kulu, Zincirlikuyu	2,67 bc	1,00 h	3,00 b	2,22 bd
16	Konya, Çumra, Karapınar yolu	2,33 ce	1,00 h	2,50 cd	1,94 eh
17	Ş. Koçhisar, Demirciobası	3,00 b	1,00 h	3,00 b	2,33 bc
18	Sivrihisar, Yeniköy	2,67 bc	1,00 h	2,50 cd	2,06 dg
19	Ankara, Akyurt, Kalaba	2,00 ef	1,00 h	3,00 b	2,00 dh
20	Konya, Çeltik, Göktepe	2,50 cd	1,00 h	3,00 b	2,17 be
21	Ş. Koçhisar, Evren	1,00 h	1,00 h	3,00 b	1,67 ıj
22	Ankara, Akyurt, Bügdüz	2,00 ef	2,00 ef	3,00 b	2,33 bc
23	Bala, Merkez	2,00 ef	1,00 h	2,50 cd	1,83 gj
24	Konya, Cihanbeyli, Acıkuyu	2,67 bc	1,00 h	2,00 ef	1,89 fı
25	Ankara, Kızılcahamam,Akdoğan	2,00 ef	1,00 h	0,01 ı	1,00 k
26	Konya, Cihanbeyli, K. Beşkavak	2,17 df	1,00 h	3,00 b	2,06 dg
27	Ankara, Başayaş	2,00 ef	1,00 h	2,50 cd	1,83 gj
28	Ankara, Polatlı	2,00 ef	1,83 fg	2,50 cd	2,11 cf
29	Konya, Kulu, Yaraşlı	2,00 ef	1,00 h	3,50 a	2,17 be
30	Konya, Kaşören, Çeltik	1,00 h	1,00 h	3,00 b	1,67 ıj
31	Çorum, Sungurlu, Tuğcu	3,00 b	2,00 ef	3,00 b	2,67 a
32	Ankara, Haymana,Yeşilyurt	2,00 ef	1,00 h	2,50 cd	1,83 gj
33	Konya, Kulu, Bahadırlı mah.	2,00 ef	1,00 h	3,50 a	2,17 be
34	Konya, Cihanbeyli, Karatepe	2,00 ef	1,00 h	3,00 b	2,00 dh
35	Ankara, Bala, İsmetpaşa	2,00 ef	1,00 h	3,00 b	2,00 dh
36	Eskişehir Agr.Res.Inst. Parcel 57	2,00 ef	1,00 h	2,00 ef	1,67 ıj
37	Ankara, Susuz	3,00 b	2,50 cd	3,00 b	2,83 a
Mean		2,59 a	1,14 c	2,05 b	1,93

%CV: 13,65, $LSD_{0,05}$Variety:0,07, Isolate:0,24, Variety x Isolate: 0,42

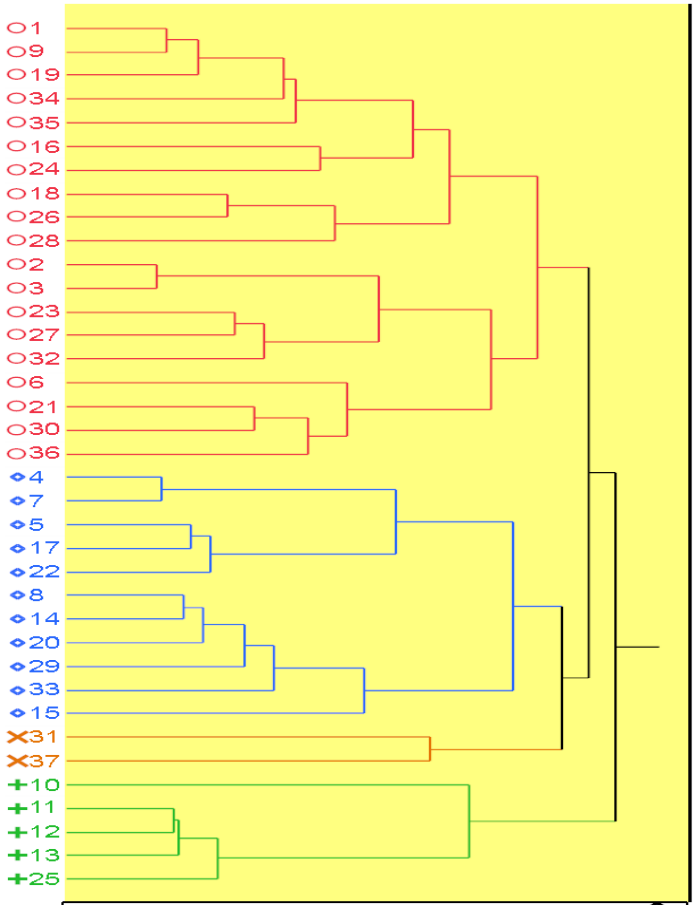

Figure 2. Dendogram of the virulence status of the isolates

Conclusions

Monitoring changes in virulence of Rc on the hosts is very crucial to manage with Rc. In this regard, in the present study, Rc isolates were obtained from main barley producing areas of Central Anatolia (Konya, Ankara, Eskişehir, Kırşehir and Çorum Provinces) in Turkey and difference in virulence of the isolates were determined. This study suggested that considerable changes in virulences of Rc isolates of Central Anatolia exist.

Acknowledgements

The study was conducted within the scope of the Project (Determining Pathotypes of Leaf Blotch Disease (*R. commune*) in Barley Growing Areas of Central Anatolia (Konya, Ankara, Eskişehir, Kırşehir and Çorum Provinces) and Investigating Management Practices of the Disease, Project No: TAGEM-BS-12/12-05/02-15) and funded by General Directorate of Agricultural Research and Policies.

References

1. Aktaş, H.(2001). Önemli Hububat Hastalıkları ve Survey Yöntemleri. Tarım ve Köyişleri Bakanlığı Tarımsal Araştırmalar Genel Müdürlüğü Bitki Sağlığı Araştırmaları Daire Başkanlığı, Bağdat cad. No: 208, PK78, 06171 Yenimahalle-Ankara (in Turkish).
2. Anonymous (2015). www.tuik.gov.tr
3. Araz, A. and Hekimhan, H. (2017). Determination of pathotypes of barley scald disease [*Rhynchosporium commune* (Zaffarano,McDonald&Linde)] in some provinces in central Anatolia. II. International Iğdır Symposium, 9-11 October 2017, Iğdır, Turkey.
4. Araz, A. and Maden, S. (2006). Pathogenic variation among isolates of Rhynchosporium secalis from cultivated barley growing in Central Anatolia, Turkey. Plant Pathology Journal, 5(2): 244-247.

5. Avrova, A. and Knogge, W. (2012). *Rhynchosporium commune*: a persistent threat to barley cultivation. Molecular Plant Pathology, 13(9): 986-997.
6. Azamparsa, M. R., Karakaya,A., Mert, Z., Aydın, G., Peşkircioğlu, H., Seçer, E., Özmen, D., Tutluer, İ. and Sağel, Z.(2015). İki arpa çeşidinin ve Gamma ışınlanması ile elde edilmiş ileri kademe arpa hatlarının *R. commune*'ye fide dönemi tepkileri. Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi, 24 (1), 75-78 (in Turkish).
7. Barradas, M.T. (1984). Leaf Scald - A Major Barley Disease in Portugal. Barley diseases and Associated Breeding Methodology Workshop, p90-94, Rabat, Morocco.
8. Bockelman, H.E. (1984). Virulence Patterns of *Rhynchosporıum secalis* from Several Barley-Growing Regıons. Barley diseases and Associated Breeding Methodology Workshop, p88-89, Rabat, Morocco.
9. Döken, M.T. (1979). Erzurum'da arpadan izole edilen *Rhynchosporium secalis* (Oudem) J.J.Davis'in morfolojisi, biyolojisi, zarar durumu ve savaş yöntemleri üzerinde çalışmalar (Unpublished, in Turkish).
10. Düşünceli, F., Çetin, L., Albustan, S., Mert, Z., Akan, K. and Karakaya, A. (2008). Determination of the Reactions of Some Barley Cultivars and Genotypes to Scald Under Greenhouse and Field Conditions. Tarım Bilimleri Dergisi, 2008, 14 (1) 46-50, Ank. Üniv. Ziraat Fakültesi.
11. Ellen, O. (2002). Plant Protection in spring cereal production with reduced tillage III. Cereal Diseases. Crop Protection, 21: 195-201.
12. El-Ahmed, A.M.(1981). Seedling reaction of the 7th IBON to *R. secalis* in the greenhouse and source of resistance. Barley diseases and Associated Breeding Methodology Workshop, p95-105, Rabat, Morocco.
13. Forgan, A. H., Knogge, W. and Anderson, P. A. (2007). Asexual genetic exchange in the barley pathogen *Rhynchosporium secalis*. Phytopathology,97:650-654.
14. Fowler, A. M. and Owen, H. (1971). Studies on leaf blotch of barley (*R. secalis*). Trans. Br. Mycol. Soc., 56: 137-152.
15. Kün, E., (1988). Serin İklim Tahılları, Ankara Üniversitesi Ziraat Fakültesi Yayınları: 1032, Ders Kitabı:299, Ankara
16. Jackson, L.F. and Webster, R.K. (1976). The dynamics of a controlled population of *Rhynchosporium secalis*, changes in race composition and frequencies. Phytopathology, 66: 726–8.
17. Jorgensen, H.J. L. and Smedegaard-Petersen, V.(1995). Pathogenic variation of *Rhynchosporium secalis* in Denmark and sources of resistance in barley. Plant Disease, 79: 297-301
18. Lange, B.J. and Boyd, W.J.R. (1968). Preservation of fungal spores by drying on porcelain beads. Phytopathology, 58: 1711.
19. Lebedeva, L.V., Tvaruzek, L. and Konovalova, G.S. (2006). Virulence analysis of Russian and Czech barley scald populations causes by *Rhinchosporium secalis*. Mikologiya I, Fitopatologiya, 40(4): 337-343.
20. Mathre, D.E., (ed.) (1982). Compendium of Barley Diseases. APS Press., 78 pp.
21. Myfield, A., and Clare, B.G., (1991). Effects of *Rhynchosporium secalis* at specific growth stages on barley grown in a controlled environment. Plant Pathology, Volume 40, Issue 4, December 1991 , Pages 561–567
22. Meles, A. K., Yahyaoui, A., Abu-Blan, H. and Ahmed, M. (2008). Variation in virulence in ethiopian and Jordanian barley scald (*Rhinchosporium secalis*) field populations. Proceedings of the 10th International Barley Genetics Symposium, 5-10 April 2008, 391-392, Alexandria, Egypt.
23. Polley, R.W. and King, J.E. E. (1993). Surveys of diseases of spring barley in England and Wales, 1976-1980. Annals of Applied Biology, 123: 271-285.
24. Salamati, S. and Tronsmo, A.M. (1997). Pathogenicity of *Rhinchosporium secalis* isolates from Norway on 30 cultivars of barley. Plant Pathology, 46, 416-424.
25. Vardar, Y.(1983). Bitki Fizyolojisi Dersleri (Bitkilerin Büyüme ve Gelişme Olayları) . Ege Üniv. Yayınları No: 69-221 s(in Turkish).

26. Xue, B., Hall, R. and Falk, D. (1991). Pathogenic variation in *Rhynchosporium secalis* from southern Ontario. Plant Disease, 75: 934-938.
27. Zadoks, J.C., Chang, T.T. and Konzak, C. F. (1974). A Decimal Code For The Growth Stages Of Cereals. Weed Research, 14: 415-421.
28. Zhang, Q., Webster, R.K, Crandall, B.A., Jackson, L.F.and Saghai Maroof, M.A. (1992). Race composition and pathogenicity associations of *Rhynchosporium secalis* in California. Phytopathology, 82: 798–803.
29. Zhan, J., Yang, L., Zhu, W., Shang, L. and Newton, A. C. (2012). Pathogen populations evolve to greater race complexity in agricultural systems – evidence from analysis of *Rhynchosporium secalis* virulence data. PLoS ONE, 7(6): e38611.
30. Zaffarano, P. L., Mcdonald, B. A. and Linde, C. C. (2011). Two new species of *Rhynchosporium*. Mycologia, 103 (1): 195-202.
31. Zencirci, N. and Hayes,P. M. (1990). Effect of scald (*Rhynchosporium secalis*) on yield components of twelve winter barley genotypes. Journal of Turkish Phytopathology, 82: 798 –803.