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# Pathogenic Variation among Isolates of *Rhynchosporium secalis* from Cultivated Barley Growing in Central Anatolia, Turkey

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**Abstract:** Pathotypes of barley scald pathogen, *Rhynchosporium secalis* (Oudem.) J.J. Davis, were determined based on the reactions of 50 single spore isolates of the pathogen obtained from the diseased leave samples collected from 8 provinces in Central Anatolia and 2 provinces in Aegean Region on 10 differential cultivars in the controlled conditions. Forty one pathotypes were distinguished based on the susceptible reactions formed on the differentials. Thirty six pathotypes were represented by 1 isolate, 1 pathotype by 3 isolates, 1 pathotype by 4 isolates and 3 pathotypes by 2 isolates, respectively. Osiris was susceptible against only one pathotype (pathotype 41), Nigrinidum being against 2 and Steudelli against 12 pathotypes. Tokak 157/37 showed susceptible reaction against 92.7% of the pathotypes (38 pathotypes). The other cultivars showed susceptible reactions between 18 and 29 of the pathotypes. The pathotype which is pathogenic to all of the differential cultivars (pathotype 41) was represented by 2 isolates collected from Eskişehir province. The pathotype (pathotype 40) which caused susceptible reaction on 8 differential cultivars was also determined from the isolates obtained from this province and in addition to this 7 more pathotypes were determined in this province. Distribution of the pathotypes in the provinces varied according to the number of the isolates, in other words the number of the pathotypes increased parallel to the increase of the number of the isolates. The most virulent pathotypes were detected from the isolates of Eskişehir, Kayseri and Yozgat provinces.

**Key words:** Barley, *Rhynchosporium secalis*, pathogenic variation, central anatolia, Turkey

## INTRODUCTION

Barley is the second most grown crop after wheat, with the acreage of 3.6 million ha and production of 8 million tons in Turkey (Anonymous, 2000). Scald, incited by *Rhynchosporium secalis* (Oudem.) J.J. Davis (Mitosporic fungi: Hyphomycetes) is an important disease of barley (*Hordeum vulgare* L.) world-wide (Jenkins and Jemmett, 1967; Jenkyn *et al*., 1989; Hoffmann and Schmutterer, 1983). It is also a severe disease of winter sown barley in Anatolia (Karaca, 1974; Döken, 1979; Aktaş, 2001). Pathogenic variability of this pathogen is well known and this was shown in many countries by using various differential varieties (Xue *et al*., 1991; Tekauz, 1991). Most of these differential varieties comprise identical resistance genes and pathotypes are differentiated by the reactions of these varieties against the isolates of the pathogen. For this reason there is no standard universal race discrimination in the world. Tokak cultivar comprises more than 90% of the whole cultivars sown in this region (Akar, 2001). Though this cultivar has very good agronomical characteristics it is very susceptible against scald. Resistance to scald has been reported in barleys (Habgood and Hayes, 1971; Starling *et al*., 1971; Webster *et al*., 1980), but the effectiveness of this resistance against Central Anatolian isolates of the pathogen is unknown. The purpose of this research was to obtain Turkish isolates of *R. secalis* and find out the reactions of some differential cultivars against these isolates.

## MATERIALS AND METHODS

**Collection and isolation of the fungus:** Most of the 50 isolates of *R. secalis* used in this study were recovered from scald-infected fields and research plots in Central Anatolia. Isolates were obtained in proportion the acreage of barley and origins of the isolates are as following: Ankara, 11; Çorum, 1; Eskişehir, 10; Kayseri, 2; Kırıkkale, 1; Kırşehir, 2; Konya, 13; Kütahya, 1; Uşak, 2 and Yozgat, 6. Majority of the isolates originated from winter barleys of cultivar (cv.) Tokak of farm fields. One of the Ankara isolates was obtained from experimental plots of Central Anatolia Field Crops Research Institute, Ankara; One isolate from wild oat (*Secale montanum* Guss); One from cv. Tarm 92; One from a local cultivar (Çakır).

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To isolate the fungus, a 5 $mm^2$ segment cut from a scald lesion was surface sterilized in 70% alcohol for 10 sec, then in 0.5% aqueous NaOCl for 90 sec, rinsed in Sterile Distilled Water (SDW) and placed in a humid chamber at 20°C for 24 h to have sporulation of the pathogen (Fowler and Owen, 1971; Döken, 1979). After sufficient sporulation, leaves were agitated in SDW until having a slightly turbid spore suspension, a drop of suspension was spread on to 1% Water Agar (WA) in Petri dishes and the dishes were kept in an incubator set to 20°C (Fowler and Owen 1971, Döken, 1979). Single germinated conidia were transferred to Lima Bean Agar (LBA) and incubated at 17°C for 15 days to establish the single-spore cultures used to assess pathogenicity. Cultures were stored on porcelain beads in cryo vials of Micro-Bank at -18°C.

**Test plants:** Each isolate of the fungus was tested against five plants of each of ten cultivars (Nigrinudum, Steudelli, Modoc, Osiris, Brier, Atlas, Turk, Kitchin and Wisconsin winter×Glabro (Ww×G) and Tokak (a local susceptible cultivar)) having various resistance genes (Goodwin *et al*., 1990) with two replication. Each barley was grown as groups of five plants in 7 cm-diameter plastic pots containing a mixture of field soil, sand and organic manure (50: 45: 5, w/w/w) in a growth room at 18±2°C day and 16±2 night with a photoperiod of 12 h and a light intensity of 13000 lux provided by fluorescent lamps. Pots were watered from the bottoms twice a week.

**Preparation of inoculum and inoculation:** Spore suspension used as inoculum was prepared from 10-14 days old single spore cultures of *R. secalis* grown on LBA in an incubator at 17°C with a photoperiod of 14 h having 4 fluorescent lamps of 20 Watt. Each well sporulated dish was submerged with 10 mL of SDW and spores dislodged by a hard brush. The resulting spore suspension was filtered through two layers of cheesecloth and adjusted to $5\times10^5$ spores per milliliter. A drop of Tween 20 was added to the spore suspension of 25 mL and it was shaken some time. At the three leaf stage (growth stage 11 according to Zadoks *et al*. (1974) scale), the group of differentials that consisted of 10 pots in a chamber were sprayed with a total of 25 mL of spore suspension with a sprayer. Two pots, each of five plants of cv. Tokak sprayed with distilled water were included with each group of differentials as a check. cv. Tokak was also included in the inoculated group. The pots then were transferred in to a humid chamber and kept there 48 h in dark. The tests were repeated twice. This study was carried out at a climatically controlled room at Central Plant Protection Institute in Ankara in 2002-2003.

**Disease estimation:** Disease severity was rated after two weeks on the second and third leaf according to the following scale: 0 = no visible symptoms, 1 = small brown or gray spots confined to the leaf margins and tips, 2 = small brown or gray spots scattered over the leaf surface, 3 = large lesions covering more than 50% of leaf area and 4 = large, coalescing lesions, leaf withering (Jackson and Webster 1976, Xue *et al*., 1991). The mean of ten leaves in two replications was accepted as the reaction of the differential. Disease scores of 0, 1 and 2 were classified as resistant responses and scores of 3 and 4 as susceptible responses.

## RESULTS

Forty-one pathotypes of *R. secalis* were identified using the barley set of ten cultivars (Table 1). These have been designated as pathotypes TR 1 to TR 41. The cv. Tokak known as susceptible showed resistant reaction against 8 of the pathotypes. All the test cultivars were susceptible to the pathotype TR 41. Distribution of

Table 1: Susceptible reactions of 10 barley differentials to Turkish (TR) pathotypes of *Rhynchosporium secalis**

| | Turkish pathotype No. | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cultivar | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| Nigrinudum | | | | | | | | | | | | | | | | | | | | |
| Steudelli | | | | S | | | | | | | | | S | | | | | | | |
| Modoc | | | | | | S | | | | | | | S | | S | S | | S | | S |
| Osiris | | | | | | | | | | | | | | | | | | | | |
| Brier | | | | | S | | | S | | S | S | S | S | S | S | S | | S | S | |
| Atlas | | S | | | | | | | S | | | S | | S | S | | S | | | |
| Turk | | | | | | S | | S | | S | | | | | | S | S | | S | |
| Kitchin | | | | | | | S | | S | | S | | | S | | | | S | | S |
| WwxG[1] | | | | | | | S | | | S | | S | | | | S | S | S | S | S |
| Tokak 157/37[2] | | | S | S | S | S | S | S | S | | S | S | | S | S | | S | | S | S |
| No. of isolates per pathotype | 4* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| % of total | 8 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |

Table 1: Continued

| | Turkish pathotype No. | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cultivar | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | + |
| Nigrinudum | | | | | | | | | | | | | | | | | | S | | | S | 2 |
| Steudelli | | | | | | S | S | S | | | | S | | S | | S | | S | S | S | S | 12 |
| Modoc | | S | S | S | | S | | | | S | S | S | | S | S | S | S | S | S | S | S | 21 |
| Osiris | | | | | | | | | | | | | | | | | | | | | S | 1 |
| Brier | | S | | S | S | S | S | | S | S | S | S | S | S | S | S | S | S | S | S | S | 29 |
| Atlas | S | S | S | | | | | S | S | | S | | S | S | | S | S | | S | S | S | 20 |
| Turk | S | | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | | S | S | 25 |
| Kitchin | S | | | | S | | S | | | S | | S | S | | S | S | S | S | S | S | S | 18 |
| WwxG[1] | S | S | S | S | S | | S | S | S | | S | | S | S | S | | S | | S | S | S | 24 |
| Tokak 157/37[2] | S | S | S | S | S | S | | S | S | S | S | S | S | | S | S | S | S | S | S | S | 33 |
| No. of isolates per pathotype | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 3 | 2 | |
| % of total | 8 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 6 | 4 | |

* Pathotypes were designated based on the susceptible reactions (scores of 3-4 in the 0-4 scale) of the differentials. + The column shows the total No. of the pathotypes the cultivars having susceptible reaction

Table 2: Distribution of the Turkish pathotypes of *Rhynchosporium secalis* in central Anatolian provinces

| Provinces | No. of isolates* | Turkish (TR) pathotype No. ** |
|---|---|---|
| Ankara | 11 | 1, 4, 6 ,12, 13, 21, 22, 23, 24, 26, 33 |
| Çorum | 1 | 19 |
| Eskişehir | 10 | 1, 2, 7, 16, 27, 31, 34, 40, 41 (2) |
| Kayseri | 3 | 11, 40 (2) |
| Kırıkkale | 1 | 36 |
| Kırşehir | 2 | 29, 30 |
| Konya | 13 | 1 (2), 5, 14, 17, 18, 24, 25, 28, 32, 35, 39 |
| Kütahya | 1 | 35 |
| Uşak | 2 | 10, 20 |
| Yozgat | 6 | 3, 8, 9, 15, 37, 38 |

* The number of the isolates was collected proportional to the acreage of the provinces. ** The numbers in brackets show the number of the isolates in the designated pathotype

the pathotypes in the provinces also showed a variation (Table 2). Mostly each isolate represented a different pathotype. Only Pathotype 1 was obtained from three different provinces and Pathotype 24 and 40 from two provinces. This shows a high rate of pathogenic variability of *R. secalis* in Turkey.

## DISCUSSION

The purpose of this study was to determine pathogenic variability in *R. secalis* from Central Anatolia, Turkey. Since there are no standard set of host differentials and no standard pathotype nomenclature for this pathogen the differential hosts were chosen among the varieties having different resistance genes described in other works (Goodwin *et al*., 1990). We chose 9 differential hosts (Nigrinudum, Steudelli, Modoc, Osiris, Brier, Atlas, Turk, Kitchin, and Wisconsin winter×Glabro (Ww×G) having various resistance genes and a cultivar Tokak, the most widely grown Turkish cultivar and known as susceptible since we have not been able to obtain a universally susceptible one.

The characterization of numerous pathotypes demonstrated that a high degree of pathogenic variability exists in the *R. secalis* population in Central Anatolia. We allocated isolates to pathotypes on the bases of the responses of the differentials and named them as TR since the nomenclature for pathotypes of *R. secalis* is done in the same way in the other countries (Schein, 1960; Kajiwara and Iwata, 1963; Williams and Owen, 1973; Jackson and Webster, 1976; Xue *et al*., 1991; Tekauz, 1991; Lyngs Jorgensen and Smedegaard-Petersen, 1995). The possibility of pathotypes identifiable by the 10 differentials is 1024 but since 50 isolates were used the expected number of the pathotypes is 50 and 41 were found. This variability (82% of the expected) was high compared to the other findings in the other countries (Kajiwara and Iwata, 1963; Williams and Owen, 1973; Jackson and Webster, 1976; Brown, 1990; Tekauz, 1991).

The wild oat isolate did not produce any symptoms on any of the differentials. There is either a host speciation of the pathogen or cv. Tokak is resistant against it.

The widely grown Turkish winter barley cultivar Tokak was found resistant against 8 of the pathotypes (19.51%) and this shows that it does have some resistance genes. This cultivar is preferred by most of the farmers because of its good agronomic features for this reason its resistance should be increased.

The main asset of the work is the presence of various pathotypes of *R. secalis* in central Anatolia and distribution of these pathotypes should be considered in the future breeding programs. Specially pathotype 41 should be handled with caution since it overcame all the resistance genes in the differentials and new sources of resistance should be searched. This pathotype was obtained from the region where barley scald is so widespread.

This wide range of variability could be higher if this was determined by a higher numbers of isolates and this variability should be found out with short intervals since central Anatolia is a gene center for cereals. The most cumbersome stage of this work is the isolation of the pathogen and it is not so difficult. Storage of the isolates should also be handled carefully.

This is the first finding on the determination of pathotypes of *R. secalis* in Turkey and when the results are compared with the findings in other countries we can say that variability of this pathogen is more diverse in Anatolia.

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## REFERENCES

Akar, T., 2001. (Barley cultivars grown at state farms). Tigem., Year: 16 No: 80, pp: 26-29.

Aktaş, H., 2001. (Important cereal diseases and their survey methods). Ministry of Rural Affairs and Agriculture, General Directorate of Agricultural Research, Publication of head of the office of plant health, No: 208, pp: 44-47.

Anonymous, 2000: www.FAO.org.

Brown, J.S., 1990. Pathogenic variation among isolates of *Rhynchosporium secalis* from barley grass growing in south eastern Australia. Euphytica, 50: 81-89.

Döken, T., 1979. (Studies on the morphology, biology, damage and control methods of *R. secalis* (Oudem) J.J. Davis isolated from barley in Erzurum province). Atatürk Univ. Plant Protection Deparment, pp: 89.

Fowler, A.M. and H. Owen, 1971. Studies on leaf blotch of barley (*Rhynchosporium secalis*). Trans. Br. Mycol. Soc., 56: 137-152.

Goodwin, S.B., R.W. Allard and R.K. Webster, 1990. A nomenclature for *Rhynchosporium secalis* pathotypes. Phytopathology, 80: 1330-1336.

Habgood, R.M. and J.D. Hayes, 1971. The inheritance of resistance to *Rhynchosporium secalis* in barley. Heredity, 27: 25-37.

Hoffmann, G.M. and H. Schmutterer, 1983. Parasitic Diseases and Pests on Agricultural Culture Plants. Verlag Eugen Ulmer, Stutgard, pp: 488.

Jackson, L.F. and R.K. Webster, 1976. Pathotype differentiation, distribution and frequency of *Rhynchosporium secalis* in California. Phytopathology, 66: 719-725.

Jenkins, J.E.E. and J.L. Jemmett, 1967. Barley leaf blotch. N.A.A. S.Q. Rev., 75: 127-132.

Jenkyn, J.F., O.J. Stedman, G.V. Dyke and A.D. Todd ,1989. Effects of straw inoculum and fungicides on leaf blotch (*Rhynchosporium secalis*), growth and yield of winter barley. J. Agric. Sci. Cambridge, 112: 85-95.

Kajiwara, T. and Y. Iwata, 1963. Studies on the strains of the barley scald fungus *Rhynchosporium secalis* (Oud.) Davis. Bull. Nat. Inst. Agric. Sci., Ser. C., 15: 1-82.

Karaca, İ., 1974. (Systematic Plant Diseases, Deuteromycetes (Fungi-Imperfecti). Ege University Press, Bornova, pp: 272.

Lyngs Jorgensen, H.J.V. and Smedegaard-Petersen, 1995. Pathogenic variation of *R. secalis* in Denmark and sources of resistance in Barley. Plant Dis., 79: 297-301.

Schein, R.D., 1960. Physiology and pathogenic specialization of *Rhynchosporium secalis*. Penn. Agric. Exp. Stn., Bull., 644: 1-29.

Starling, T.M., C.W. Roane and K. Chi, 1971. Inheritance of reaction to *Rhynchosporium secalis* in winter barley cultivars. Proceedings of the 2nd Intl. Barley Genetics Symposium, 1969: 513-519.

Tekauz, A., 1991. Pathogenic variation in *Rhynchosporium secalis* on barley in Canada. Can. J. Plant Pathology, 13: 298-304.

Webster, R.K., L.F. Jackson and C.W. Schaller, 1980. Sources of resistance in barley to *Rhynchosporium secalis*. Plant Dis., 64: 88-90.

Williams, R.J. and H. Owen, 1973. Physiological pathotypes of *R. secalis* on barley in Britain. Trans. Br. Mycol. Soc., 60: 223-234.

Xue, B., R. Hall and D. Falk, 1991. Pathogenic variation in *Rhynchosporium secalis* from southern Ontario. Plant Dis., 75: 934-938.

Zadoks, J.C., T.T. Changi and C.F. Konzak, 1974. A decimal code for the growth stages of cereals. Weed Res., 14: 415-421.