



Determination of Resistance of Winter Wheat Varieties Against Root and Crown Rot *Fusarium culmorum* Under the Artificial Drought Conditions

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ABSTRACT

This study was conducted to investigate the relationship between the recent increase in the frequency of drought conditions and Root-Crown Rot (*Fusarium pseudograminearum*, *Fusarium culmorum*) in rainfed wheat growing areas of Central Anatolia. In 2018, the experiment was established in the greenhouse of Konya Bahri Dağdaş International Agricultural Research Institute using 25 registered wheat varieties in a randomised block split-plot experimental design with 4 replications. Irrigation levels (100% field capacity and 50% field capacity) were designed as main plots, inoculation (+ and -) as subplots and varieties as sub-subplots. In the study, the response of the cultivars to inoculation under artificial drought conditions was evaluated by measuring Crown Score (CR), Lesion Length (LL), Number of Diseased Leaves from outside to inside (NDL) and Plant Height (PH) from five plants in each pot. Statistically, the differences between inoculation, irrigation, NDL and LL were found to be significant at $P < 0.0001$ level, while the differences between varieties were found to be significant at $P < 0.001$ level for the CR parameter. On the other hand, when the interactions were evaluated for the NDL parameter, the differences were found to be significant at $P < 0.0001$ level for all three interactions of cultivar*inoculation, cultivar*irrigation and cultivar*inoculation*irrigation. When the effect of reduced water application intended to be used in resistance breeding was evaluated for 25 different varieties under *Fusarium culmorum* inoculation, there was an increase in CR from 41,7% (Gerek-79) to 487,5% (Altay-2000), NDL from 7,14% (Kirgiz-95) to 200% (Alpu-2001), LL from -36,84% (Karahana-99) to 283,33% (Altay-2000) and in PH reduction from 12,41% (Seval) to 32,22% (Kirgiz-95). The results showed that drought-stressed plants were already weakened and therefore more easily and severely infected by pathogens. According to these results, it has been determined that it is very important for the region to obtain resistance to drought and crown rot diseases, which have such an obvious relationship, in breeding studies.

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Introduction

The rapid growth of the world's population has a direct impact on the world and our Türkiye's nutritional needs. As the second most consumed crop in the world after rice and the main food source for around a third of the world's population, wheat is a strategic crop and ranks first among cultivated crops, accounting for about 221 million hectares planted. World wheat production is 776 million tons and the yield is about 351 kg/da (USDA, 2022). Among the agricultural areas of our country, wheat has the largest area under cultivation with 6.7 million ha and approximately 22 million tons of product are produced annually from these areas (TÜİK, 2022), placing our country among the top 10 wheat-producing countries in the world.

The importance of wheat as a strategic crop was better recognised during the COVID-19 pandemic and subsequent international crises. Wheat is grown almost everywhere in Turkey, but there are many barriers to production. Nearly 70% of the wheat production area in

Turkey is rainfed. Therefore, it is inevitable that dry areas are extremely important for production and it is essential to develop drought-tolerant varieties. So, varieties developed by the Konya Bahri Dağdaş International Agricultural Research Institute, Türkiye's drought testing center, using successful breeding-physiology studies and new strategies, have entered the market in recent years and made a reputation.

Yields and economic losses due to diseases and pests in wheat production are also major issues. Rust diseases (*Puccinia* spp.) and soil-borne pathogens are an important group of pathogens in wheat production areas and are responsible for significant yield losses in wheat (Kınaç, 1992; Wildermuth and McNamara, 1994). Other important diseases affecting wheat production include root rot and root crown disease (*Fusarium pseudograminearum*, *Fusarium culmorum*), a major problem in the rainfed wheat production areas of central Anatolia. Studies show that

soilborne pathogens reduce wheat yields by 42-45% (Nicol, 2008; Arici et al., 2013).

While rust diseases are important because they can rapidly cause epidemics, Fusarium is one of the most important yield-losing diseases in drylands worldwide and in Türkiye (Cook, 1992; Burgess et al., 2001; Backhouse et al., 2004, Nicol et al., 2008). Given that this disease can cause more severe infections in dry conditions, studying these diseases is particularly needed in Central Anatolia, where wheat is grown most intensively. This pathogen can infect the roots, crown and internodes upwards, particularly when the plant is under water stress and has low water potential (Papendick and Cook, 1974). Moist conditions immediately after planting and dry conditions between flowering and hard ripening favour the development of the disease (Paulitz et al. 2002; Smiley et al. 1996). White head formation is a clear symptom of the disease. Effective water transport through the primordial root system is prevented by the fungus attacking the root and crown internodes. Also, the fungus is more effective when there is insufficient surface moisture and lateral roots are underdeveloped, especially in drought conditions towards the end of the season. This situation also prevents grain filling and development (Burgess et al., 2001).

Stubble burning, ploughing, crop rotation and chemical control are the methods used to control the disease, but they are insufficient. So, the use of resistant varieties to reduce the harmful effects of the disease is an alternative to these methods. Breeding studies are very timeconsuming and expensive. The basic principle of breeding is that to produce a good variety, the objective should be welldefined, the appropriate parents should be selected and the experimental technique should be well determined

(suitable location and good observation). To this end, success in combination breeding is realised through the existence of the necessary variation resources and the efficient use of these resources. Knowing the heritability of the traits of the parents determined according to the purpose eliminates unnecessary combinations and gives an idea of the generation in which selection should start (Toklu and Yağbasanlar, 2005).

Taking into account the above studies and reasons, the fact that these factors are more frequent in our region, especially in areas severely affected by drought, and that diseases can cause more severe infections under stress factors, the idea that disease resistance studies should be carried out under artificial drought conditions and that drought and disease resistance traits should be found together in wheat varieties to be grown in production areas has come to the forefront.

Characterising the parents used in drought resistance breeding for their responses to disease will make it easier to develop varieties with more traits along with the right crosses, shortening this long-term process. Therefore, the study has been carried out under controlled conditions with 25 registered varieties currently in use and will be extended to more material according to the results obtained.

Material and Methods

Material

In this study, 25 bread wheat varieties developed by the TAGEM Institutes from national and international (IWWIP) programs were used as material (Table 1).

Table 1. Varieties used in the study and their characteristics.

No	Cultivars	Winter-Summer	Drought Resistance Status	Fusarium Resistance Status
1	Alpu-2001	Winter Bread Wheat	Susceptible	Susceptible
2	Altay-2000	Winter Bread Wheat	Moderate resistance (C)	Moderate resistance (C)
3	Çetinel-2000	Winter Bread Wheat	Susceptible	Susceptible
4	Bezostaya-1	Winter Bread Wheat	Susceptible	Susceptible
5	Gerek-79	Winter Bread Wheat	moderate resistance	Susceptible
6	İzgi-2001	Winter Bread Wheat	Susceptible	Susceptible
7	Kıraç-66	Winter Bread Wheat	Moderate resistance	Susceptible
8	Kırgız-95	Winter Bread Wheat	Susceptible	Susceptible
9	Kutluk-94	Winter Bread Wheat	Susceptible	Susceptible
10	Müfitbey	Winter Bread Wheat	Susceptible	Susceptible
11	Ahmetağa	Winter Bread Wheat	Susceptible	Susceptible
12	Dağdaş-94	Winter Bread Wheat	Moderate resistance (C)	Moderate resistance (C)
13	Ekiz	Winter Bread Wheat	Susceptible	Susceptible
14	Göksu-99	Winter Bread Wheat	Susceptible	Susceptible
15	Karahan-99	Winter Bread Wheat	Moderate resistance	Susceptible
16	Kınacı-97	Winter Bread Wheat	Susceptible	Susceptible
17	Konya-2002	Winter Bread Wheat	Susceptible	Susceptible
18	Bayraktar-2000	Winter Bread Wheat	moderate resistance	Susceptible
19	Demir 2000	Winter Bread Wheat	Susceptible	Susceptible
20	Gün-91	Winter Bread Wheat	Susceptible	Susceptible
21	İkizce-96	Winter Bread Wheat	Susceptible	Susceptible
22	Seval	Winter Bread Wheat	Susceptible	Susceptible
23	Tosunbey	Winter Bread Wheat	Susceptible	Susceptible
24	Uzunyayla	Winter Bread Wheat	Susceptible	Susceptible
	Yakar-99	Winter Bread Wheat	Susceptible	Susceptible

Methods

The experiments were conducted as pot trials in the fully controlled greenhouses in the Bahri Dağdaş International Agricultural Research Institute (BDUTAE) using 25 registered wheat varieties in a randomised block split-plot experimental design with 4 replications. Irrigation levels (100% field capacity and 50% field capacity) were designed as main plots, inoculation (+ and -) as subplots and varieties as sub-subplots.

First, wheat seeds were surface sterilized and germinated in an incubator. The seeds were first soaked in 98% ethyl alcohol for 5 minutes, then in 4.5% sodium hypochlorite solution for 1 minute, and then washed 3 times with sterile distilled water. To germinate the seeds, filter paper moistened with sterile water was placed on 9 cm diameter Petri dishes, and seeds were placed on top and incubated at 23 °C for 4 days. The 5kg pots used in the experiment were filled with soil (20% sand, and 80% soil). Pre-germinated seeds were placed in the pots with pliers, with 10 plants in each pot, then covered with the soil mixture. After the growing of the plants, the number of plants in each pot was reduced to five.

The inoculum was prepared as described by Nicol et al. 2008, and the isolate was grown in Petri dishes on PDA for 2 weeks. The isolates were then placed in moistened and autoclaved propylene bags filled ¼ full with wheat and left to develop conidia for 2-3 weeks at 23°C. Distilled water was added to the bags and the spore suspension was filtered through cheesecloth for particle removal. The resulting dense spore suspension was used for the inoculation of seeds using a thoma slide and diluted to a density of 1×10^6 . The prepared inoculum was applied to the root collar and soil using a micropipette at 100 µl 1 week after the seeds germinated (Mitter et al., 2006; Erginbaş et al., 2008; Özdemir, 2014).

To create artificial drought conditions, the field capacity of the experimental plants (pots) was first measured to obtain different water contents (100% field capacity and 50% field capacity) and the watering time was specified for all pots to determine the disease effect under dry conditions. The pots were then weighed at 5-day intervals throughout the study and the water deficit pots were replenished to the determined field capacity (for 100% irrigation) or half (for 50% irrigation).

Greenhouse conditions were set at $25 \pm 2^\circ\text{C}$ with 16 hours of light and 8 hours of darkness, and plants were uprooted after 8 weeks. Crown score (CS), lesion length (LL), number of diseased leaves from outside to inside (NDL) and plant height (PH) were measured from five plants in each pot at uprooting to assess the response of the cultivars to inoculation under artificial drought conditions. CS, after washing the root and crown parts in water, was scored on a scale of 0 to 10 at 10 cm discoloration of the crown (0= No discoloration, 1= 1-4%; 2=5-9%; 3=10-19%; 4=20-29%; 5=30-39%; 6=40-49%; 7=50-59%; 8=60-69%; 9=70-79%; 10=80 and more). To evaluate the inoculation, re-isolation studies were performed. Classical phytopathological methods were used to isolate and identify the fungi from samples taken from the medium.

Results and Discussion

Statistically, the differences between inoculation, irrigation, NDL and LL were found to be significant at $P < 0.0001$ level, while the differences between varieties were found to be significant at $P < 0.001$ level for the CS parameter. On the other hand, when the interactions were evaluated for the NDL parameter, the differences were found to be significant at $P < 0.0001$ level for all three interactions of cultivar*inoculation, cultivar*irrigation and cultivar*inoculation*irrigation.

In the study to determine the disease resistance of varieties under drought stress induced by reduced watering, different results were obtained depending on the parameters. However, compared to 100% water treatment, CS and NDL increased in all cultivars, LL increased in all cultivars except Izgi-2001, Kutluk-95, Kirgiz-94 and Karahan-99, and PH decreased in all cultivars in 50% water treatment (Figure 1,2,3,4, 5, 6, 7, 8).

When Figure 1 examined with these statistical analysis data, it is noticeable that the plants in the 50% water deficit block were divided into two sub-subjects inoculated and uninoculated, and in the inoculated sub-group plant development was weaker and yellowing was highly evident. This can be explained by the fact that deficit irrigation conditions create a stress situation in favour of the pathogen, allowing the pathogen to act more severely and symptoms to become more pronounced. These results are very similar to Papendick and Cook (1973), Cariddi and Catalano (1990) and Alahmad et al., (2018). However, Figure 2 shows that although the full irrigation block was divided into two sub-blocks with and without inoculum, the effect of the disease was not as marked as in the 50% water restriction block, and because there was no water restriction, the pathogen caused fewer symptoms in the plants due to the absence of drought stress conditions. These visual assessments are described in detail with the parameters CS, LL, PH and NDL taken at harvest.

Figures 3 and 4 indicate that, depending on the amount of irrigation and the inoculation conditions, there are apparent differences in plant height, volume, colour and development in plants with different genetic characteristics (developed for dryland and irrigated condition). In Figure 3, it is clear that the Bezostaya-1 variety, developed for relatively irrigated areas, is very strongly affected by water deficit (100%-50% comparison), and the difference becomes even more distinct when the effect of the disease is combined with the conditions (inoculated-uninoculated comparison under 100% irrigation or inoculated-uninoculated comparison under 50% irrigation). In Figure 4, Karahan-99, one of the most drought-tolerant cultivars, could tolerate water restriction (100% water inoculated vs. 50% water inoculated), but could not tolerate inoculation under the same irrigation conditions because it was not resistant to the disease (inoculated vs. uninoculated under 100% irrigation and inoculated vs. uninoculated under 50% irrigation).

In addition to the different responses of irrigated and drought-tolerant cultivars to the effect of reduced water application on CS, different reactions were observed in drought and disease tolerant cultivars.

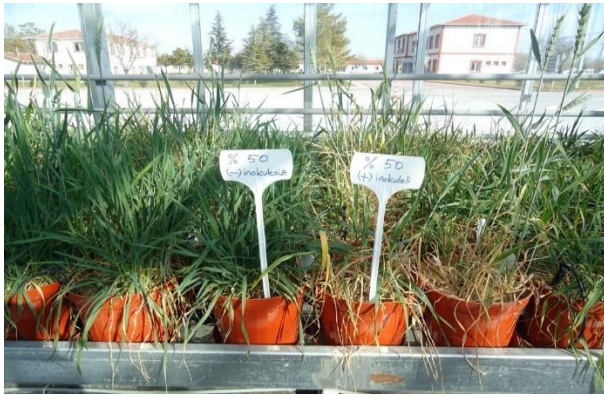


Figure 1. View of pots with and without inoculum in the reduced water block of the greenhouse study. Although the irrigation level (50% water restriction) was the same, it is easy to see the yellowing of the plants and the reduction in volume due to the effect of the disease.



Figure 2. View of pots with and without inoculum in the full field capacity treatment block in the greenhouse study. Although the watering level (100%) was the same, the yellowing of the plants and reduction in volume can be seen due to the effect of the disease.



Figure 3. Bezostaya-1 cultivar under full (100%)-limited irrigation (50%) and *Fusarium culmorum* inoculated-non-inoculated conditions.



Figure 4. Karahan-99 cultivar under full (100%)-limited irrigation (50%), *Fusarium culmorum* inoculated-non-inoculated conditions.

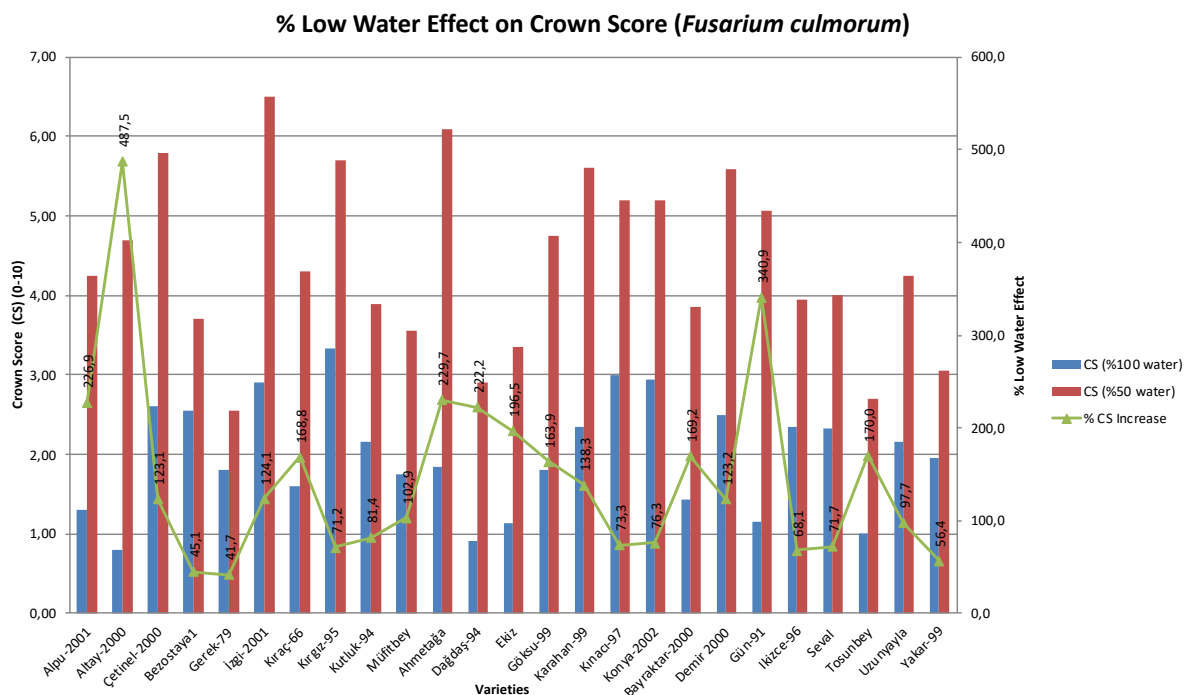


Figure 5. Evaluation of CS in wheat varieties at 50% and 100% irrigation and % change in CS according to irrigation difference

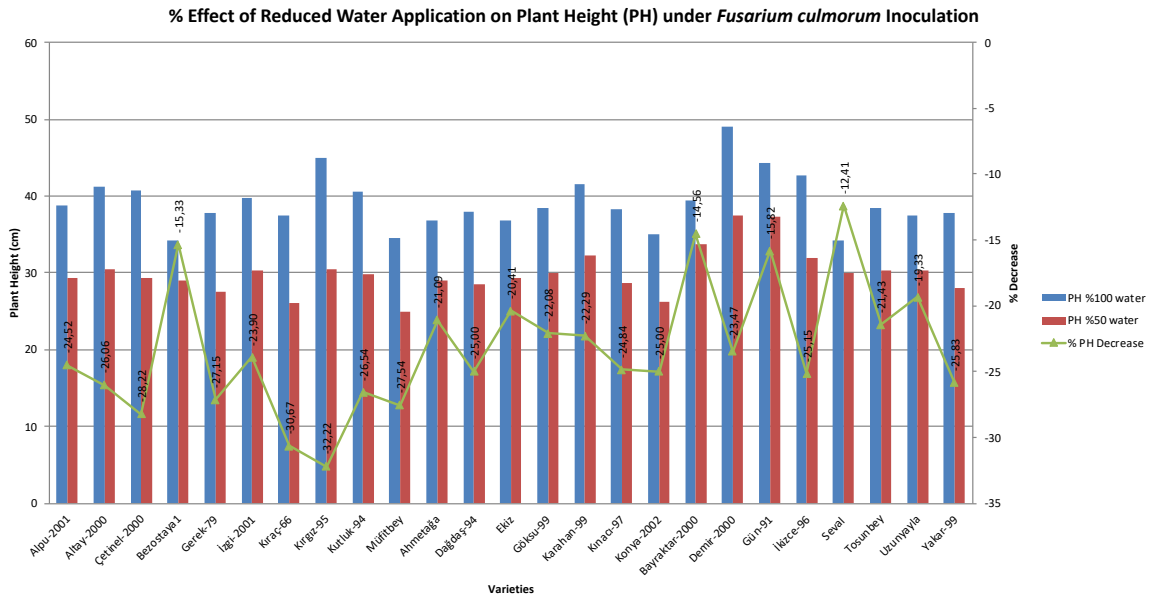


Figure 6. Percentage effect (%) of reduced water application (50% and 100% irrigation) on PH under *Fusarium culmorum* inoculation

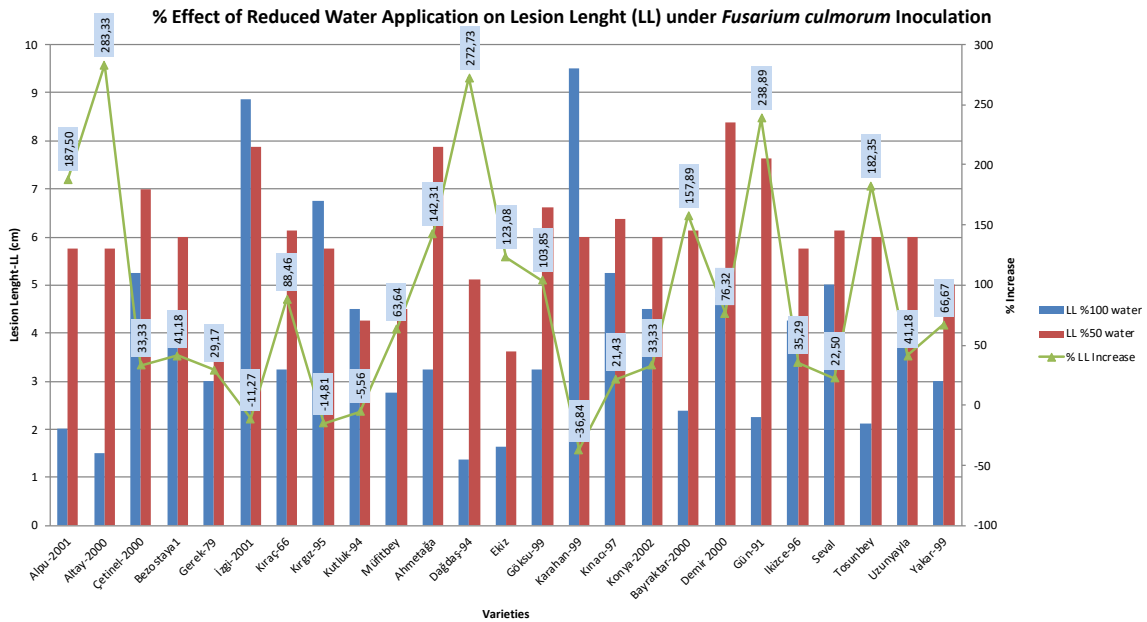


Figure 7. Percentage effect (%) of reduced water application (50% and 100% irrigation) on LL under *Fusarium culmorum* inoculation

Altay-2000 and Dağdaş-94 were used as drought and disease tolerant cultivars and compared to other cultivars, especially Dağdaş-94 suppressed and tolerated disease more than other cultivars at 50% and 100% irrigation levels and limited disease symptoms (0-10 scale). In % disease change rates, the varieties with the highest increase in disease score with decreasing irrigation level were Altay-2000 (487,5%), Gün-91 (340,9%) and Ahmetağa (229,7%), while the varieties with the lowest increase in disease score were Gerek-79 (41,7%), Bezostaya-1 (45,1%), and Yakar-99 (56,4%), respectively (Figure 5). The results revealed that Altay-2000, used as a control for drought and disease tolerance, did not provide the expected resistance to drought stress and was even the variety with the highest proportional increase in disease symptoms. However, when CS values are analysed in both conditions, it stands out as one of the varieties with the lowest total CS

of all varieties. The overall CS scores (%100 CS + %50 CS) show that varieties with high drought tolerance such as Tosunbey (3,70), Dağdaş-94 (3,80), Ekiz (4,48), Yakar-99 and Bayraktar-2000 are at the forefront.

The effect of reduced water application on PH under *Fusarium culmorum* inoculation showed that the reduction in PH of the varieties was more similar to each other compared to CS, ranging from a minimum of 12,41% to a maximum of 32,22%. Drought and disease tolerant varieties Altay-2000 and Dağdaş-94 showed 26,06% and 25% reductions in plant height respectively; the highest reduction in PH was observed in Kırgız-95 (32,22%), Kıraç-66 (30,67%), Çetinel-2000 (28,22%) and the lowest reduction in PH was observed in Seval (12,41%), Bayraktar-2000 (14,56%) and Bezostaya-1 (15,33%) (Figure 6).

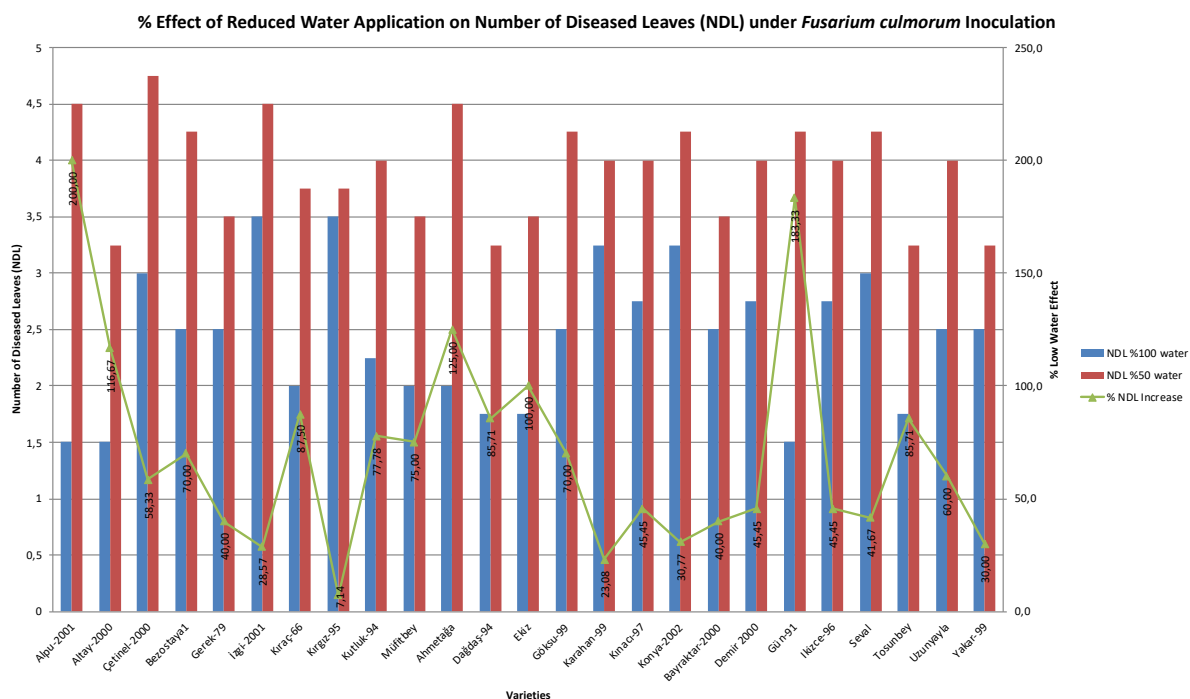


Figure 8. Percentage effect (%) of reduced water application (50% and 100% irrigation) on the NDL under *Fusarium culmorum* inoculation

The percentage effect of *Fusarium culmorum* inoculation with reduced water application on LL was observed to increase in almost all cultivars, while a decrease was observed in Karahan-99, İzgi-2001, Kırgız-95 and Kutluk-94. The varieties with the highest increase in LL were Altay-2000 (283,33%), Dağdaş-94 (272,73%) and Gün-91 (238,89%); the varieties with the lowest increase were Kınacı-97 (21,43%), Seval (22,50%) and Gerek-79 (29,17%). In addition to all these evaluations, although the control varieties Altay-2000 and Dağdaş-94 showed the highest increase in lesion rate, these varieties were among the lowest-scored varieties in CS and total CS. As a result, it can be concluded that disease tolerance was maintained and LL was suppressed under irrigation and limited irrigation conditions (Figure 7).

When examining the effect of artificial drought with reduced water application on NDL under disease stress, it was found that the number of diseased leaves increased from outside to inside in all varieties under artificial drought. Under full and semi-irrigated conditions, Alpu-2001 (200%), Gün-91 (183.33%) and Ahmetağa (125%) showed the highest increase in NDL, while the lowest increases were observed in Kırgız-95 (7,14%), Karahan-99 (23,08%) and İzgi-2001 (28,57%). In evaluating the number of diseased leaves, it can be stated that the control varieties Altay-2000 and Dağdaş-94 had the lowest values in terms of NDL in both conditions, except for the % change, and this situation is due to the disease and drought tolerance of the varieties (Figure 8).

Conclusion

The results showed that although all the varieties in the study had different characteristics (red-white grain, developed for dryland-irrigated areas, tall-short PH, earliness-lateness, drought tolerant, tolerant to *Fusarium culmorum*, etc.), it was observed that CS, LL and NDL

increased and PH shortened in all varieties inoculated under reduced water application. Considering the overall study, this can be explained by the fact that the plants exposed to drought are already weakened under stress and therefore more easily and severely infected by pathogens as previous studies like Papendick and Cook (1973), Cariddi and Catalano (1990) and Alahmad et al., (2018). Decreases in yield factors such as plant growth, volume and PH under limited irrigation conditions (artificial drought, disease and combination) are extremely important for wheat production. The fact that agricultural production, which has become problematic as a result of climate change, is also affected by disease means that the problem of production will be exacerbated. These findings highlighted the importance of studying resistance to multiple biotic and abiotic factors together under the same conditions (especially for Central Anatolia) in breeding and resistance breeding studies. The combination of resistance to drought stress and root-crown rot diseases was found to be particularly important for the region, as they are connected.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Fatih Özdemir: Validation, Writing - original draft, Methodology, Investigation, Conceptualization, Validation, Review and editing, Methodology, Investigation, Conceptualization, Validation, Writing - original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Formal analysis, Data curation, Formal analysis, Data curation.

Ethical approval

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Data availability

Not applicable.

Consent for publication

Not applicable.

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