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THE EFFECT OF FLAG LEAF DEFOLIATION ON GRAIN MORPHOLOGY IN WHEAT AFTER DROUGHT STRESS

UTJECAJ DEFOLIJACIJE LISTA ZASTAVIČARA NA MORFOLOGIJU ZRNA PŠENICE NAKON SUŠNOG STRESA

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ABSTRACT

It is known that flag leaf influences grain yield potential in wheat (*Triticum aestivum* L.). However, little is known regarding the influence of defoliation of flag leaf after drought stress. The effect of flag leaf defoliation and drought stress on the grain's weight, area, width, length, and circularity was investigated in six bread wheat varieties. Drought treatment, genotypes and their interaction significantly influenced investigated traits while weight and length of the grain were not significantly affected by flag leaf defoliation. However, the interaction of drought treatment and flag leaf defoliation showed a significant effect only on grain circularity. The results showed that drought applied after anthesis significantly reduced mature grain weight in wheat, whilst the effect of flag leaf defoliation after anthesis was insufficient to cause significant differences. However, drought tolerance of some varieties (Bubnjar) might mitigate the drought and defoliation effects as they could have a compensatory increase in the photosynthesis of spikes. Other morphological traits of grain showed less pronounced differences under stress treatments that were also genotypic-specific characteristics. Generally, genetic influence was observed among wheat varieties when they were imposed on source restriction (flag leaf defoliation) and drought stress.

Keywords: defoliation, drought, flag leaf, grain morphology, wheat

SAŽETAK

Poznato je da list zastavičar utječe na potencijal uroda zrna pšenice (*Triticum aestivum* L.). Međutim, malo se zna o defolijaciji lista zastavičara nakon suše. U šest sorata krušne pšenice ispitivan je učinak defolijacije lista zastavičara i sušnog stresa na težinu, površinu, širinu, duljinu te kružnost zrna. Tretman u suši, genotipovi i njihova interakcija značajno su utjecali na ispitivana svojstva, dok na težinu i duljinu zrna nije značajno utjecala defolijacija lista zastavičara. Međutim, interakcija tretmana sušom i defolijacije lista zastavičara pokazala je značajan učinak samo na kružnost zrna. Rezultati su ukazali da je tretman sušom primijenjen nakon cvatnje značajno smanjio težinu zrelog zrna pšenice, dok je učinak defolijacije lista zastavičara nakon cvatnje bio nedostatan da proizvede značajne razlike. Međutim, tolerantnost nekih sorti na sušu (Bubnjar) mogla bi ublažiti učinke suše i defolijacije jer bi mogla kompenzacijski povećati fotosintezu klasova. Ostala morfološka svojstva zrna pokazala su manje izražene razlike u stresnim tretmanima što je bila genotipska specifičnost. Općenito, genetski utjecaj uočen je među sortama pšenice kada su podvrgnute defolijaciji i sušnom stresu.

Ključne riječi: defolijacija, suša, list zastavičar, morfologija zrna, pšenica

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important staple foods in the world that is widely produced under diverse environmental conditions. Due to global warming, it has been projected that climate change has induced the global temperature to increase in the range of 0.6–4°C by 2050, thus showing an anomaly which is significantly higher than the maximum allowable increase (Wang et al., 2023). Climate change has further changed the occurrence of droughts, making them more frequent, longer, and more severe. A model was developed showing that global warming causes serious drought in 60% of wheat-growing areas of the world (Elahi et al., 2022). Drought is especially dangerous during the flowering stage of wheat growth when it can shorten the grain filling period and decrease the grain yield (Zhang et al., 2022). Further, during that period, drought stress may result in partial or complete sterility of the florets (Alghabari et al., 2013). Grain formation occurs 12-14 days after anthesis and fertilization of the florets. This is also the time when most of the endosperm is formed which corresponds to the cell division phase. It is evident that high post-anthesis temperatures or drought reduce mature grain weights in wheat (Kino et al., 2020). Thus, drought can influence the fullness of wheat grains during the grain filling stage (Duvnjak et al., 2023).

The most effective adaptation strategies for climate change include the development of new genetic wheat varieties with improved drought tolerance (Duvnjak et al., 2024a). One of the ways to increase grain yield is the breeding for enhanced grain size as it is directly related to 1000 kernel weight, the component of grain yield. Grain size provides the space for grain filling and can be spliced into grain length, grain width, grain thickness, and grain surface area. Brinton et al. (2017) reported that the 5A locus increased grain weight with a primary effect on grain length. Along with that is the fact that grain length is genetically controlled and stable across environments and has a pleiotropic effect on grain width in a later stage of grain development and is more variable across environments.

Average grain weight and final grain yield depend on the formation, translocation, partitioning and accumulation of assimilates during the grain filling of the post-anthesis period (Hafsi et al., 2000). It was found that wheat flag leaf contributes to grain filling more than 50%, while its defoliation generated grain yield losses of 18 to 30%. Photosynthates and assimilates are transported to the developing grain after photosynthesis in the flag leaf and from pre-anthesis reserves in tissues such as the stem and the ear. Duvnjak et al. (2024b) showed that under drought the loss of chlorophyll content in the flag leaves can be the first sign of the photosynthesis inactivation. Further, during the grain-filling stage, drought stress accelerates the accumulation of reactive oxygen species, the decomposition of chloroplasts, and reduces the accumulation of assimilates in wheat flag leaves (Racz et al., 2022). Thus, defoliation of flag leaf will reduce photosynthetic area, negatively affecting overall plant vitality which can result in lower production. The objective of the current research was to evaluate to what extent drought stress, in the period of 14 days after anthesis and defoliation of flag leaves after that, influences morphological traits of wheat grain.

2. MATERIALS AND METHODS

2.1. Plant material and experimental layout

In the current study, six winter wheat varieties (Rujana, Silvija, Fifi, Bubnjar, Anđelka, and Pepeljuga) of the Agriculture Institute Osijek were examined at drought stress in the anthesis stage for two weeks (Duvnjak et al., 2024b) in the year 2022. After germination, wheat seedlings were placed in a plant growth chamber to undergo a six-week vernalization period. After that, an experiment was set up in a greenhouse (Gis Impro d.o.o., Vrbovec, Croatia) where the experiment included two treatments: control treatment (C) consisted of plants with regular irrigation where volumetric soil moisture content (VSMC) was maintained at 30-35%, while in drought treatment water content was reduced by 65%. Soil moisture measuring device (TDR 150 Soil Moisture Meter, Spectrum Technologies, Aurora, USA) was used daily for the measurement of the total amount of water present in the soil. After two-week drought treatment, flag leaves were removed from plants in one group, and the second group kept the flag leaves on the plants.

2.2. Grain morphology

Morphological grain traits (weight, area, width, length, and circularity) were evaluated by the MARVIN precision system for efficient seed analysis (MARViTECH GmbH, Wittenburg, Germany). The grains were distributed on the measuring surface. A digital camera recorded the images, and image processing software analysed the images. The results were displayed in a log table. The analysis procedure was followed using control images.

2.3. Statistical analysis

For grain morphology analysis, six biological replications represented the mean values for sub-treatments (with or without flag leaf) under each control and drought treatment. A combined ANOVA was calculated across treatments and flag leaf defoliation. Fisher's Least Significant Difference (LSD) test ($\alpha = 0.05$) was used to evaluate whether the observed difference in performance between treatments and flag leaf defoliation for each genotype separately (control plants vs plants in drought in sub-treatments with plants with or without flag leaf) was significant (Statsoft Inc., Tulsa, OK, USA). Error bars represent standard errors.

3. RESULTS

3.1. Analysis of variance

The results of the combined analysis of variance for genotype (G), flag leaf defoliation (F), treatment (T), and their interactions are shown in Table 1. Significant effects of genotype (G), treatment (T), and their interaction were found for all five traits studied. The effect of flag leaf defoliation (F) was significant for area, width and circularity of the grain, the G × F interaction was significant for weight, length, and circularity of the grain, the T × F interaction was significant for circularity of the grain only, while the G x T x F interaction was significant for weight, length and circularity of the grain.

Table 1 Mean squares (MS) of the combined analysis of variance for morphological traits of grain (weight, area, width, length, and circularity)

Tablica 1. Sredina kvadrata (MS) kobinirane analize varijance za za morfološka svojstva zrna (težina, površina, širina, duljina i kružnost)

Source of variation Izvor varijance	DF	MS				
		Weight Težina	Area Područje	Width Širina	Length Duljina	Circularity Kružnost
Genotype (G) / Genotip (G)	5	0.6295***	24.57***	0.523***	1.487***	0.0126***
Treatment (T) / Tretman (T)	1	5.5264***	14.14***	0.773***	0.091*	0.0167***
Flag leaf defoliation (F) Defolijacija lista zastavičara (F)	1	0.0153	7.15**	0.795**	0.031	0.0133***
GxT	5	0.0890**	4.50***	0.113***	0.055*	0.0012**
GxF	5	0.0638*	0.37	0.018	0.055*	0.0014**
TxF	1	0,0144	0.40	0.047	0.022	0.0122***
GxTxF	5	0.1301***	0.60	0.013	0.055*	0.0013**
Error / Pogreška	120	0.0242	0.79	0.020	0.020	0.0004

*, **, *** F test of corresponding mean squares significant at the 0.05, 0.01, and 0.001 probability levels, respectively / F test sredina kvadrata na 0.05, 0.01, and 0.001 značajnosti. DF-degrees of freedom / stupnjevi slobode

3.2. Grain morphology in control and drought treatments with and without flag leaf

The highest mean values for grain weight were 1.45 g (control with flag leaf) and 1.47 g (control without flag leaf), while the lowest mean values were 0.59 and 0.56 g, recorded in drought for specific genotypes. All genotypes, except Bubnjar, showed significantly larger grain weight in control, especially in sub-treatment with flag leaf, followed by control without flag leaf.

The highest mean grain area values were recorded in drought with flag leaf (15.44 Ø), while the lowest were found in drought without flag leaf (11.48 Ø). Rujana and Silvija did not show any significant differences between grain areas under treatments or flag leaf existence. Only Bubnjar showed a significant increase in grain area in drought with flag leaf, compared to control with flag leaf.

The maximum mean values for grain width were 3.34 mm (control with flag leaf), 3.38 mm (control without flag leaf), 3.43 mm (drought with flag leaf), and 3.37 mm (drought without flag leaf). None of the genotypes had significant differences in control with or without flag leaf, while Fifi and Silvija significantly decreased grain width in drought with or without flag leaf, compared to control, as well as Anđelka without flag leaf.

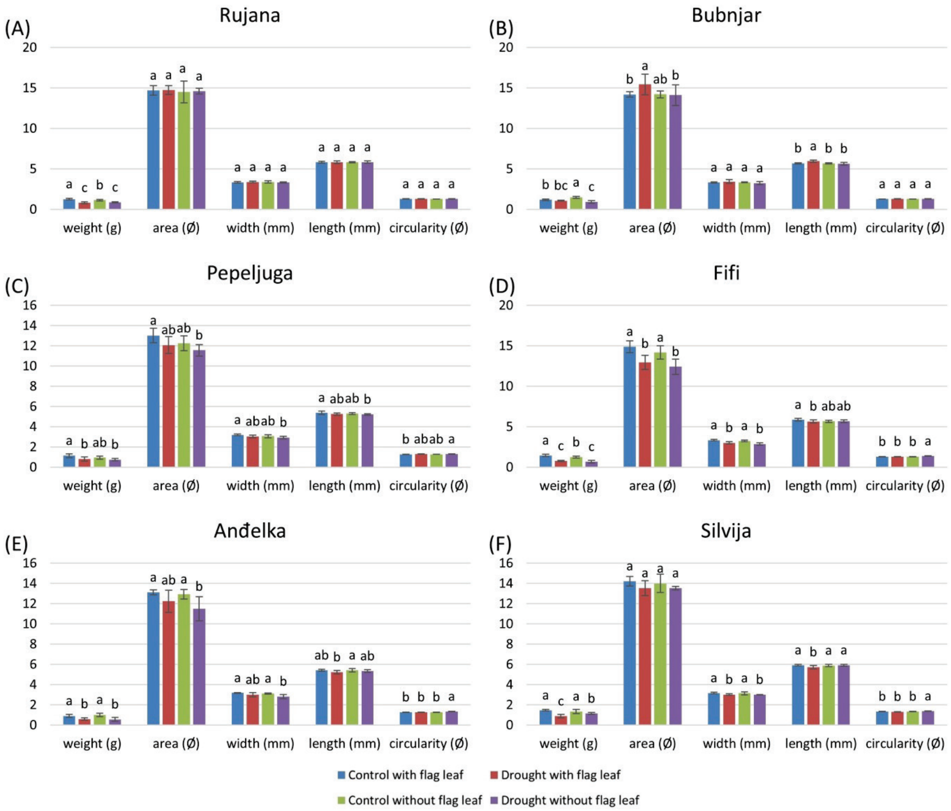

Figure 1 Morphological traits of grain in wheat varieties Rujana (a), Bubnjar (b), Pepeljuga (c), Fifi (d), Anđelka (e), and Silvija (f) at four treatments (control with flag leaf, drought with flag leaf, control without flag leaf, and drought without flag leaf). Trait means with the same letter do not significantly differ at p < 0.05

Slika 1. Morfološka svojstva zrna pšenice sorti Rujana (a), Bubnjar (b), Pepeljuga (c), Fifi (d), Anđelka (e) i Silvija (f) u četiri tretmana (kontrola sa listom zastavičarom, suša sa listom zastavičarom,kontrola bez lista zastavičara i suša bez lista zastavičara). Srednje vrijednosti svojstava s istim slovom ne razlikuju se značajno pri p < 0,05

The grain length mean values ranged from 5.30 mm (control without flag leaf) to 5.91 mm (control with flag leaf) and 5.22 (drought with flag leaf) to 5.95 mm (drought with flag leaf). Only Bubnjar significantly increased grain length in drought with flag leaf, compared to other treatments. Fifi significantly decreased grain length in drought with flag leaf, compared to control with flag leaf, while Silvija significantly decreased it in drought with flag leaf, compared to other treatments.

The variations in grain circularity ranged from 1.26 Ø (control with flag leaf) to 1.33 Ø (control with flag leaf) and 1.26 Ø (drought with flag leaf) to 1.36 Ø (drought without flag leaf). Grain circularity was significantly increased in drought without flag leaf in Fifi, Anđelka and Silvija, compared to other treatments, while Pepeljuga significantly increased it, compared to control with flag leaf.

4. DISCUSSION

Flag leaf is an important plant part, contributing to grain yield formation during grain filling. This importance might be differentially expressed under drought conditions. The current research showed that the effects of flag leaves on some morphological traits of grain depend on the interaction of wheat genotype with the environment. Considering that flag leaf defoliation treatments were applied two weeks after anthesis, and at this stage, pollination and grain number were not affected, it was evident that drought stress applied immediately after anthesis affected all investigated traits of grain more strongly than the effect of flag leaf defoliation. This may be because drought stress at the early stage during grain filling period reduces the number of endosperm cells and the number of starch granules per cell, which may be the reason for the decrease in the size of grain (Dhakal, 2021). Also, it was seen that drought tolerance of some varieties influenced grain traits during grain formation. Further, earlier defoliation of flag leaf could have caused more damage to the development and morphology of grain than it was in the current research. For example, it was reported that the absence of the flag leaf from the booting stage could reduce the grain yield by 30% in barley (Racz et al., 2022).

In the current research morphological traits of grain were investigated as it is known that grain size enhancement is an objective for high-yield wheat. Thus, the focus of the current research was based on changes in morphological traits of wheat grain in non-lethal drought stress during 14 days after anthesis stage after which plants were subjected to defoliation of flag leaf. Previous studies focused on increasing grain yield by increasing the number of grains, but grain yield can also be increased by increasing grain size (Ahmed et al., 2023). The same authors reported that grain width contributed to increased grain weight. Hence, grain weight is one of the key grain yield components, in combination with grain numbers. Among main grain yield components (spike number per unit area, kernel number per spike, and 1000 kernel weight), 1000 kernel weight has the highest heritability and is determined mainly by grain size, including grain width and grain length. Simmonds et al. (2014) reported that grain width on chromosome 6A was driven primarily by increased grain weight, suggesting that enhancing grain size could contribute to the genetic improvement of wheat yield. In the current research, it was observed that grain weight in most of the varieties was larger in control than in drought conditions, especially when flag leaf was present. Also, all traits were genotype-specific. This was expected, as previous research showed that genotype x defoliation of flag leaf interaction was significant for grain yield and biomass yield suggesting that the effect of defoliation was not proportionate in all genotypes possibly due to differences in contribution and compensation behaviour and is also subjected to environmental changes (Rosyara et al., 2005). Racz et al. (2022) showed that the genetic constitution of each studied variety had a specific response to the lack of the flag leaf for both leaf photosynthetic pigments and main grain yield components.

Flag leaf is an important plant organ for photosynthetic assimilation during the jointing stage and anthesis. Photosynthates and other assimilates are formed, translocated, portioned and accumulated in the endosperm cells with the largest rate of filling occurring between 14-28 after anthesis (Shewry et al., 2012). The fact that defoliated flag leaf showed significant influence on some traits (area, width, and circularity of the grain) and had no significant or minimal effects on the weight and length of the grain when defoliated, may be an indication of amplification of the photosynthetic activity of the other remaining parts of the plant (El Wazziki et al., 2015). Berwal et al. (2018) observed that flag leaf removal reduced the phytate deposition in developing grains of pearl millet but at the maturity stage, it might be synthesized and/or transported to the developing grains from the other plant parts like stems/sheath or other leaves. However, when drought occurs after anthesis, the rates of grain fill (grams of dry matter accumulated in the kernel per day) and grain weight, as in the current research, are significantly decreased. We hypothesise that the cause is decreased photosynthesis activity. In addition to less dry matter being accumulated in the grain per day, drought triggers early senescence. Bijanzadeh and Emam (2010) reported that different intensities of leaf removal in the beginning stage of sink capacity formation cause a significant reduction in grain weight in different wheat varieties. Further, defoliation at anthesis led to a more severe loss of grain weight than the corresponding treatments performed 14 days after anthesis (Esmaeilpour-Jahromi et al., 2012). Flag leaf defoliation at anthesis caused up to 34.5% of grain yield reduction (Mahmood and Chowdhry, 1997). In the current research flag leaf defoliation was performed two weeks after anthesis and this timing could be the reason why significant changes in the current research were not highly pronounced for all morphological traits of the grain.

5. CONCLUSION

The results showed that drought applied after anthesis had a larger effect on the morphology of wheat grain than the effect of flag leaf defoliation after anthesis. However, the effect of both stresses was genotype specific, especially considering drought tolerance. The weight and length of the grain were not significantly affected by flag leaf defoliation.

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