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Water Stress Influence on The Vegetative Period Yield Components of Different Maize Genotypes

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Abstract: Maize is an important food staple in many countries, and is useful in animal feed and many industrial applications. Its productivity is highly sensitive to drought stress, which may occur at any period during its growth cycle. The objective of this work was to compare the water stress influence on the performance of different maize genotypes in critical vegetative stages. Four genotypes of maize (namely a single-cross hybrid (AG 9045), a double-cross hybrid (AG 9011), a triple-cross hybrid (AG 5011), and a variety (AL Bandeirante)) were subjected to a 10-day period without irrigation in the vegetative stages that determine the number of kernel rows and the plant's ability to take up nutrients and water (V4, V6 and V8). The impact of low water availability was assessed by analyzing plant height, height of the first ear insertion, stem diameter, yield per plant, and number of rows per ear, evincing that the yield per plant was the most sensitive parameter in all the stages. With regard to the influence of the genotype, the single-cross hybrid was demonstrated to be the most resilient to water shortage.

Keywords: drought stress; genotype; grain yield; *Zea mays* L.

1. Introduction

Corn is one of the most important cereals as a source of energy for humans and animals, standing out as the fifth most produced commodity in the World according to The Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) [1]. World production in 2017/2018 exceeded 1000 million tons, out of which ca. 370 million tons were produced in the U.S., followed by China (215 million tons) and Brazil (85 million tons) [2]. According to the USDA (United States Department of Agriculture) [3], the 2018/2019 area dedicated to corn production in the world has been forecasted at 184 million hectares, with an average yield of 5.72 tons per hectare.

The yield of a corn crop is the result of a combination of the genetic potential of the cultivar, the management of the crop and the environmental conditions of the cultivation area. As regards the latter, water availability and drought episodes have become one of the main factors affecting C_4 crop yields, representing a serious threat to agricultural production worldwide. Potential impacts

of ongoing climate change will change agricultural conditions and will spur more frequent, severe, and likely prolonged episodes of drought [4].

Drought impacts include growth, yield, membrane integrity, pigment content, osmatic adjustment, water relations, and photosynthetic activity [5–7], and it triggers physiological and biochemical processes that may enable the plants to tolerate and adapt to such conditions with less reduction in economic yield [8]. In the case of maize, Kumar, et al. [9], on the basis of data from 38 cultivars evaluated at 10 environments, found that the average reduction in grain yield due to drought stress was 52%. Similar results were reported by Menkir, et al. [10] for top-cross maize hybrids under managed drought stress, with 53%–64% annual average yield reductions, and by Žalud, Hlavinka, Prokeš, Semerádová, Balek and Trnka [4], who found that precipitation totals explained 64% of the yield variability observed across stations.

In order to alleviate the negative impacts of abiotic stresses and sustain and increase agriculture production under future challenging environments, it is essential to use integrated approaches that improve the efficiency of agricultural water use and to develop stress tolerant plant varieties. In fact, identifying plant germplasm with superior drought tolerance traits has become one of the top priorities for maize breeding programs [11,12], such as the Drought Tolerant Maize for Africa Project, focused on the development, testing and deployment of drought tolerant maize varieties and hybrids adapted to production environments in sub-Saharan Africa [13].

Since the yield loss depends not only on the duration and the severity of the stress, but also on when the stress occurs, it is of foremost importance to assess the effects of water availability during the whole crop cycle. [14]. Drought tolerance of maize hybrids has been screened at the seedling stage by, for instance, Ali, et al. [15] and Akinwale, et al. [16]. Likewise, the tolerance in relation to grain weight and nutritional quality of maize during grain-filling stages for different hybrids has been studied by Barutcular, et al. [17] and El Sabagh, et al. [18].

There are indications that there is some degree of plasticity in the tolerance of corn to water limitation, depending on the growth stage [19–21]. Early season drought reduces plant growth and inhibits plant development [22]. Drought that occurs at the V8 to V17 stages has a significant impact on plant growth, architecture, ear size, and kernel numbers [23,24]. Drought occurring between two weeks before and two weeks after the silking stage can cause significant reductions in kernel set and kernel weight [25], resulting in an average of 20% to 50% yield loss [26]; and Kamara, et al. [27] reported that water deficit reduced biomass accumulation by 37% at silking, by 34% at grain filling period, and by 21% at maturity.

Nonetheless, the information regarding the effect of water stress during earlier vegetative stages and its influence on yield components of maize plants is still very limited. To the best of the authors knowledge, maize vegetative and yield parameters from V2 to V12 stages have only been studied in detail in relation to nitrogen, low light, and weed stresses by Moriles, et al. [28], and under progressive drought by withholding irrigation for varying lengths of time from jointing or tasseling [29]. Thus, the aim of this study was to fill this research gap by evaluating the response of four maize genotypes to drought stress in V4, V6, and V8 vegetative stages, with a view to identifying the most stable ones, with potential for use in environments with and without water restriction. A parallel aim was to determine in which of the three aforementioned periods these genotypes were most vulnerable to water availability shortages.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

The work presented herein was conducted at the Laboratório Didático de Análise de Sementes (LDAS) and in the greenhouse facilities of the Faculty of Agronomy Elisha Maciel (FAEM) at the Universidade Federal de Pelotas, Capão do Leão, RS, Brazil (31°48′13.0″ S, 52°24′51.5″ W), in the 2009/2010 season.

In the naturally-lit greenhouse, the cover of the enclosures reduced total radiation by ~20%–30%, but total daily radiation inside was generally well above 10 MJ·m^{-2}. The experiment was conducted under semi-controlled conditions (air temperature (26 ± 2)/(20 ± 2) °C, relative air humidity (50 ± 5)/(80 ± 5) % day/night), using evaporative coolers to control daytime temperatures.

The seeds were sown in December, within the recommended time for the state of Rio Grande do Sul. Pots were filled with 14 kg of soil collected from the A1 horizon of a solodic eutrophic Haplic Planosol, pertaining to the Pelotas mapping unit, which had previously been dried and sieved through a 5 mm mesh.

The loamy soil characteristics were as follows: pH (H$_2$O), 5.5; SMP pH, 7.1; OM, 14 g·kg^{-1}; P, 8.2 mg·kg^{-1}; K, 76 mg·kg^{-1}; Al, 0.4 cmolc·kg^{-1}; Ca, 1.9 cmolc·kg^{-1}; Mg, 0.7 cmolc·kg^{-1}; apparent density, 1.31 g·cm^{-3}; clay, 170 g·kg^{-1}; silt, 370 g·kg^{-1}; sand, 460 g·kg^{-1}; field capacity, 17.8%; permanent wilting point, 9.6%; available water, 8.2%.

Fertilization was performed on the basis of existing levels of N, P, and K determined from soil tests and according to Commission of Soil Fertility and Soil Chemistry for the States of Rio Grande do Sul and Santa Catarina (CFQS RS/SC) recommendations [30] to ensure no nutrient deficiency: a nitrogen dose of 130 kg·ha^{-1} was split in two applications, one before sowing and another in coverage; and a phosphorus (140 kg·ha^{-1}) and potassium (90 kg·ha^{-1}) application was conducted 14 days before sowing. Liming (1.6 tons·ha^{-1}) was applied 30 days before sowing.

Four commercial maize genotypes were assessed, chosen on the basis of their widespread use in the South of Brazil: a single-cross hybrid (AG 9045), a double-cross hybrid (AG 9011), a triple-cross hybrid (AG 5011) and a variety (AL Bandeirante). Six seeds per container were sown, the healthiest seedlings were chosen for the study based on water deficiency, and these were left to grow to full size and thinned by removing the plants between them, leaving only one plant per pot at the end of the thinning process.

Treatments consisted of combinations of different water conditions and maize genotypes, in a factorial scheme of 4 × 4, that is, four water conditions (with water restriction (WR), in which plants were subjected to water stress in the 1–10 days after emergence (DAE), 11–20 DAE or 21–30 DAE periods, and without water restriction (WWR), in which the plants were irrigated keeping the soil at field capacity) by four maize genotypes (single-cross hybrid (SH), double hybrid (DH), triple hybrid (TH) and variety (VAR), totaling 16 treatments with four replications (64 experimental units).

At the start of the drought simulation, all plants had 3–4 fully developed and completely green leaves. The drought stress was applied by withholding irrigation in the following growth stages, identified according to [31]: 1–10 DAE (V4 stage), 11–20 DAE (V6 stage), and 21–30 DAE (V8 stage). The soil moisture (determined by gravimetric method) reduced from field capacity of 17.8% to ca. 6.5%. Before and after the period of water-deficit stress, the experimental units were irrigated on a daily basis and were kept at field capacity, supplementing the water lost by evapotranspiration. The pots were weighed at the same hour every day using an electronic digital scale with a maximum capacity of 32 kg and a sensitivity of 1 g, and the weights obtained were used to determine the amount of water applied. The control was always kept at field capacity and no visual signs of drought stress occurred.

2.2. Measurements of Grain Yield Traits

The variables analyzed—at harvest—were: plant height (PH, in cm), height of the first ear insertion (FEI, in cm), stem diameter (SD, in mm), number of rows per ear (NRE) and yield per plant (Y, in g·plant^{-1}), in agreement with Balem, et al. [32]. PH was measured from the soil surface to the tip of the plant, with the aid of a millimeter ruler; FEI was obtained by measuring from the soil surface to the point of insertion of the first ear; SD was determined by measuring the section at the first internode above the plant collar with a calipers; NRE was obtained by counting the number of rows of each ear; and Y was determined by weighing on an analytical balance, correcting the kernel weight for 13% moisture, following a procedure analogous to that described by Zhou, et al. [33], using a moisture tester (Model 14998, Dickey-John Corporation, Auburn, USA) to determine the per cent grain moisture.

2.3. Data Analysis

Statistical analyses were conducted using Statistical Analysis System Version 2.0 for WinStat [34]. The experiment was laid out in a randomized block design and data were subjected to analysis of variance (ANOVA). Means were compared by Tukey's HSD test at the 5% significance level ($p \leq 0.05$).

3. Results and Discussion

3.1. Impact of Water Stress in The V4 Stage

In the assays in which the different maize genotypes were subjected to water stress during the 1–10 DAE period, a significant interaction was found between the PH, FEI, and Y variables and the water-deficit stress (Figure 1).
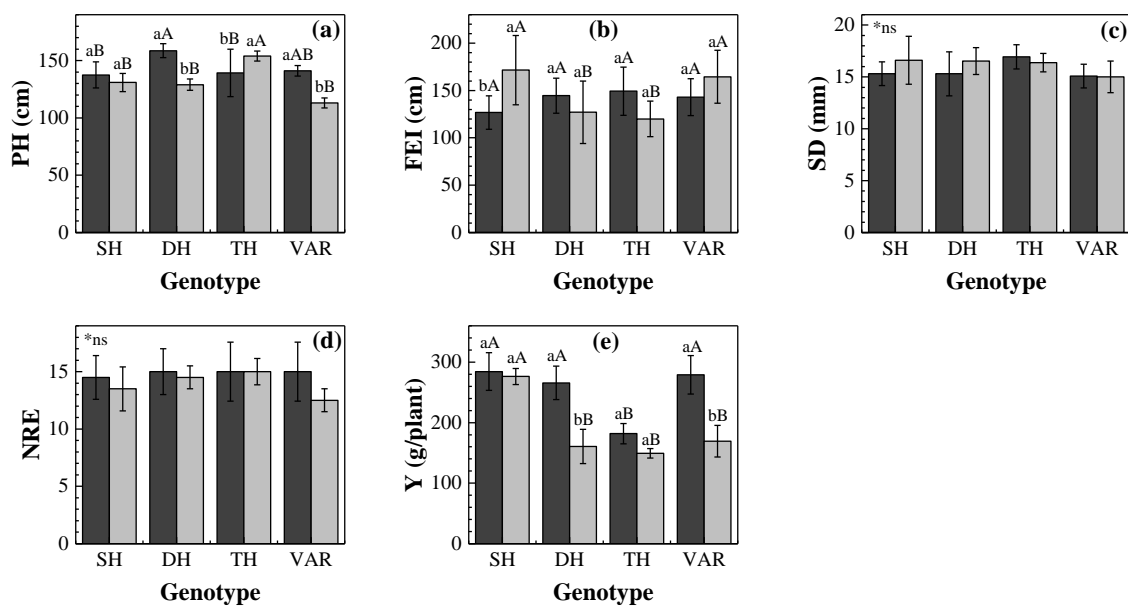

Figure 1. Impact of water-deficit stress on the four maize genotypes in the 1–10 DAE (days after emergence) period: (**a**) plant height (PH, cm); (**b**) height of the first ear insertion (FEI, cm); (**c**) stem diameter (SD, mm); (**d**) number of rows per ear (NRE); and (**e**) yield per plant (Y, g·plant^{-1}). Dark grey bars and light grey bars correspond to without water restriction (WWR) and water restriction (WR) conditions, respectively. Averages followed by the same uppercase or lowercase do not differ by the Tukey test at 5% probability. Lowercase letters compare the values of water regimes (WWR vs. WR) and capital letters compare values among genotypes. The bars represent the mean standard deviations. *ns = not significant.

In relation to the plant height variable, it could be observed that the triple-cross hybrid was the only genotype that was not negatively affected when subjected to water stress (see Figure 1a). While the double-cross hybrid showed the largest plant height in well-watered conditions (WWR), the triple-cross hybrid led to the largest PH under water deficit (WR).

As regards the height of the first ear insertion (Figure 1b), the single-cross hybrid genotype showed a significant increase under WR in comparison to WWR conditions. For the other three genotypes, the differences in the FEI variable in the 1–10 DAE period were smaller. Although the SH and VAR genotypes performed better than the DH and TH in terms of this variable, the behavior was reversed for the number of rows per ear (Figure 1d).

With regard to the stem diameter (Figure 1c), it was slightly larger for SH and DH under water stress and remained almost constant for the TH and VAR genotypes.

Apropos of the most relevant variable from the agricultural point of view (i.e., the yield per plant), remarkable differences on the water stress impact were evinced: while low water availability barely affected the yield of the SH (Figure 1e) and had a limited effect on that of the TH, the DH, and VAR genotypes demonstrated a high sensitivity to water deficit during the first 10 days after emergence, with a substantial decrease in kernel production. Under water stress in the 1–10 DAE period, the single-cross hybrid was found to lead to the highest yield per plant.

Working with maize, Westgate and Boyer [35] demonstrated that yield was particularly affected in this stage, when the plant begins to form and define the leaves and spikes that eventually will produce (i.e., this is considered the time when the plant provides the maximum number of grains and therefore the productive potential). The high sensitivity of anthesis and early grain fill to water stress can be explained by the lack of reserves, and this sensitivity of grain development to water stress would decrease as reproduction progresses (as a result from an increasing availability of reserves).

3.2. Impact of Water Stress in The V6 Stage

According to Magalhães and Durães [36], water stress in the V6 to V8 stages can inhibit the elongation of developing cells, affecting the length of the internodes of the stalk, and thereby decreasing the storage capacity of sugars in the stalk.

A variability in the sensitivity of the different maize genotypes to drought stress in the 11–20 DAE period (vegetative stage V6) was also observed for plant height, height of first ear insertion, stem diameter and number of rows per ear (Figure 2a–d) parameters, with trends similar to those described in the previous section, albeit with no significant differences.
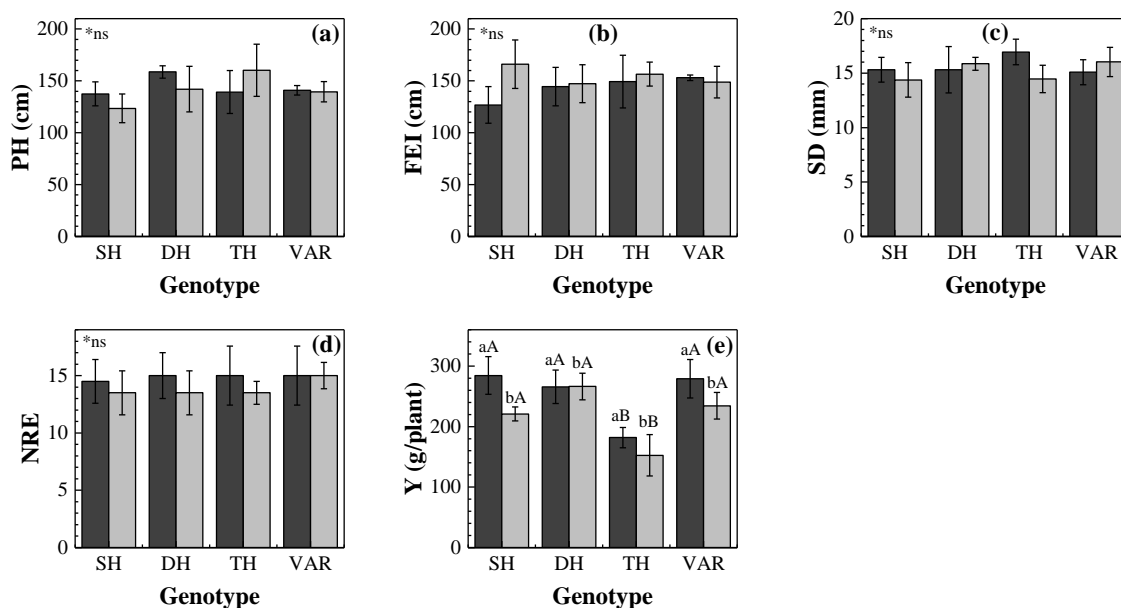

Figure 2. Impact of water-deficit stress on the four maize genotypes under study in the 11–20 DAE (days after emergence) period: (**a**) plant height (PH, cm); (**b**) height of the first ear insertion (FEI, cm); (**c**) stem diameter (SD, mm); (**d**) number of rows per ear (NRE); and (**e**) yield per plant (Y, g·plant^{-1}). Dark grey bars and light grey bars correspond to without water restriction (WWR) and water restriction (WR) conditions, respectively. Averages followed by the same uppercase or lowercase do not differ by Tukey test at 5% probability. Lowercase letters compare the values of water regimes (WWR vs. WR) and capital letters compare values among genotypes. The bars represent the mean standard deviations. *ns = not significant.

Regarding the influence of water-deficit stress in this period on the yield per plant (see Figure 2e), it was found that the single-cross hybrid and the double-cross hybrid were the most productive under

WWR and WR conditions, respectively. It is worth noting that, whereas no significant differences in yield were found for the DH, the TH was again the worst genotype in terms of yield performance. Assessing the physiology of maize under adverse conditions, Magalhães, et al. [37] reported that water deficit also resulted in thinner stems, smaller plants, and smaller leaf area, with an associated yield reduction in the 10% to 20% range.

3.3. Impact of Water Stress in The V8 Stage

Water stress in the 21–30 DAE period had a limited impact on the maize plants in terms of the height of the first ear insertion, stem diameter, and number of rows per ear variables (see Figure 3b–d), for which no significant variability was found. Conversely, it had a significant effect on plant height (Figure 3a) for DH, TH and VAR genotypes (but not for the SH). This would be in agreement with Aslam, et al. [38], who reported that the elongation of stem in maize under drought stress was reduced during the vegetative stage.
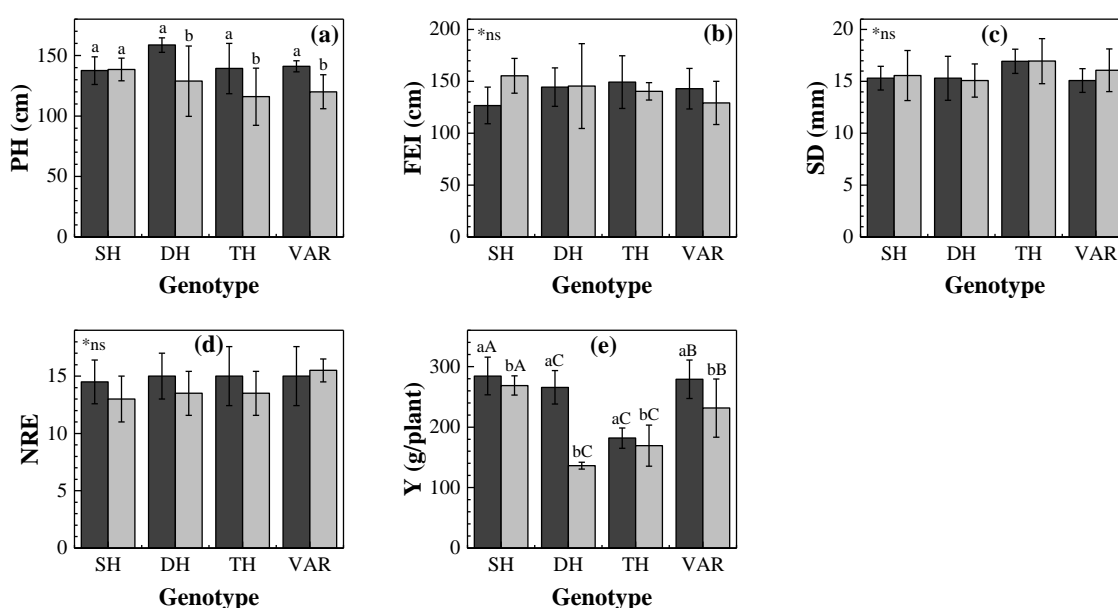

Figure 3. Impact of water-deficit stress on the four maize genotypes under study in the 21–30 DAE (days after emergence) period: (**a**) plant height (PH, cm); (**b**) height of the first ear insertion (FEI, cm); (**c**) stem diameter (SD, mm); (**d**) number of rows per ear (NRE); and (**e**) yield per plant (Y, g·plant^{-1}). Dark grey bars and light grey bars correspond to without water restriction (WWR) and water restriction (WR) conditions, respectively. Averages followed by the same uppercase or lowercase do not differ by the Tukey test at 5% probability. Lowercase letters compare the values of water regimes (WWR vs. WR) and capital letters compare values among genotypes. The bars represent the mean standard deviations. *ns = not significant.

On the other hand, the yield per plant proved to be—as in prior stages—the most sensitive variable to identify differences between treatments, clearly showing that water deficit had a significant influence on the different genotypes performance (Figure 3e). The SH was the most productive under both WWR and WR conditions. A very marked decrease under low water availability occurred for the DH, as in V4 stage, leading to a yield even lower than that of the TH (which again showed a poor performance under WWR conditions). In the case of the VAR genotype, the impact of water shortage in this period was more limited, in line with that for the V6 stage.

Limiting the availability of water in the soil during the pre-flowering stage affects the development of the vegetative structures by reducing the biomass production capacity. The occurrence of drought, as reported by Denmead and Shaw [39], would result in a decrease in maize production by 25% if it occurs prior to silking, by 50% at silking and by 21% after silking. This would also be in agreement

with the findings of Na, Fu, Yushu, Ruipeng, Shujie, and Yang [29], who showed that grain yield was significantly reduced (18.6%–26.2%) by progressive drought during the vegetative stage. Nonetheless, it is worth noting that withholding water at the vegetative stage may have some positive effects, as it has been reported to be effective in increasing protein, total amino acids, total soluble sugars, glucose, and sucrose contents in maize kernels [40].

4. Conclusions

The impact of water stress on several plant variables in different stages of the vegetative development of the plant was analyzed for four maize genotypes. The yield per plant, apart from being the most important variable from the agricultural point of view, also proved to be the most sensitive parameter to low water availability in all the stages under study (V4, V6 and V8). With regard to the influence of the maize genotype, the single-cross hybrid genotype was found to be the most resilient to water shortage, consistently leading to good yields, very close to those attained without water restriction. Conversely, the triple-cross hybrid genotype systematically had the lowest associated yields. The double-cross hybrid and the variety showed different sensitivities to water stress depending on the vegetative stage: while the yield was significantly affected if water stress occurred in the V4 stage, the sensitivity in the V6 stage was very limited, and the behavior in the V8 stage differed: the double-cross hybrid was affected and the variety was not. Consequently, the single-cross hybrid can be deemed as physiologically superior to the other genotypes and would be the preferred option in water restriction conditions in the vegetative stages.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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