

# Effects of Waterlogging on Growth and Development of Bread Wheat Genotypes

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**Abstract:** Bread wheat (*Triticum aestivum* L.) is a widely cultivated crop. Annually 10–15 million ha of this crop are affected by flooding leading to large production losses (20 to 50%). Intensive and unpredictable rainfall episodes are expected to increase due to global warming and more adapted varieties may help to cope with climatic changes. This work focused the effects of waterlogging on growth and development of four bread wheat genotypes from different origins. Plants were grown in climatized chambers. Waterlogging was imposed at tillering stage, and maintained for two weeks. Phenological observations through Zadoks scale, and relative chlorophyll content (SPAD) measurements, were performed during stress. Stress promoted different responses such as growth arrest, early senescence and no fertile tiller production, or growth enhancement through increased main shoot height and stable SPAD values, reflecting maintenance of photosynthetic ability. The genotypes capacity to contain progressive senescence induced by waterlogging was assessed through senescent biomass after stress and recovery.

**Keywords:** *Triticum aestivum*; germplasm; flooding; leaf senescence

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## 1. Introduction

The number of waterlogging episodes has been increasing globally, resulting mainly from more intense and unpredictable rainfall associated with climate change [1]. These extreme events affect farmlands causing significant production losses in many staple foods, such as wheat (*Triticum aestivum* L.), the third most cultivated cereal in the world.

Waterlogging lowers oxygen availability in the soil impairing roots functioning [2]. Constraints in the root system impacts the physiological responses of the shoots as the capture and transport of water and nutrients to the aerial part may be affected [3].

In waterlogging susceptible plants, negative effects on photosynthesis and respiration may induce alterations in sugar metabolism leading to severe energy deficiency [4]. Such changes may compromise plants growth, influencing the life cycle, lowering dry matter accumulation and affecting tillering [3]. Waterlogging can affect survival of the productive tillers, causing reductions of the spike number and yield.

One of the first visible signs of waterlogging is leaf chlorosis which is particularly evident in basal wheat leaves. This premature leaf senescence is related to chlorophyll degradation due to the remobilization of N to the younger leaves [3,5,6].

Wheat tolerance to waterlogging depends on several factors, including the genotype, growth stage [7], stress duration [8,9] and environmental conditions [3].

The study of plant responses under waterlogging allows the identification of features that contribute to tolerance. There is an urgent need to obtain wheat varieties better adapted to this stress in order to maintain/increase productivity in the context of climate change.

Considering the importance of bread wheat for human nutrition, growth and development responses to waterlogging (14 days) were evaluated in four wheat genotypes from different origins.

## 2. Material and Methods

### 2.1. Germplasm and Growth Conditions

Four bread wheat (*Triticum aestivum* L.) genotypes were analyzed: (G1)—Portuguese landrace; (G2)—Post Green Revolution, with Rht genes; (G3)—Australian germplasm and (G4)—Advanced line from the National Cereal Breeding Program (INIAV, I.P.).

Plants were grown in 5 L pots in walk-in growth chambers (EHHF 10000, ARALAB, Portugal), under controlled temperature (22/15 °C, day/night), irradiance (ca. 500–600  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), relative humidity (75%), photoperiod (14 h) and  $\text{CO}_2$  (400  $\mu\text{L L}^{-1}$ ).

Plants were maintained with a field capacity of ca. 85% [10], except in stressed plants during the waterlogging period.

### 2.2. Waterlogging Imposition

At tillering stage (Zadoks scale 22 to 25—Z22 to Z25) [11], and for each genotype, half of the pots were kept at ca. 85% field capacity (control plants) and the remaining were subjected to waterlogging (W). For this, pots were placed in plastic boxes and flooded until ca. 0.5 cm of a water film above the ground surface. After 14 days, water stress was suspended by removing the pots from the boxes and maintained in the same conditions as control plants (C) until harvest.

### 2.3. Plants Evaluation and Measurements

Wheat growth stages were accessed through Zadoks scale [11], at the beginning (T0) and after 14 days (T14) of waterlogging, and after 7 and 14 days of recovery (T7R and T14R, respectively) in 12 plants per treatment.

Main shoot height was obtained at T14, T7R and T14R in 12 plants per treatment.

Number of tillers was recorded in 12 plants per treatment at T0, T14, T14R and at the end of the growth as productive tillers.

Senescence was evaluated in the 2nd top leaf of the main shoot through relative chlorophyll content, measured with a SPAD-502 portable device (Minolta, Japan), and by quantification of the percentage of chlorotic leaves (dry weight) in the plant. Those evaluations were performed in 12 and 3 plants, respectively, at T14, T7R and T14 R.

### 2.4. Statistical Analysis

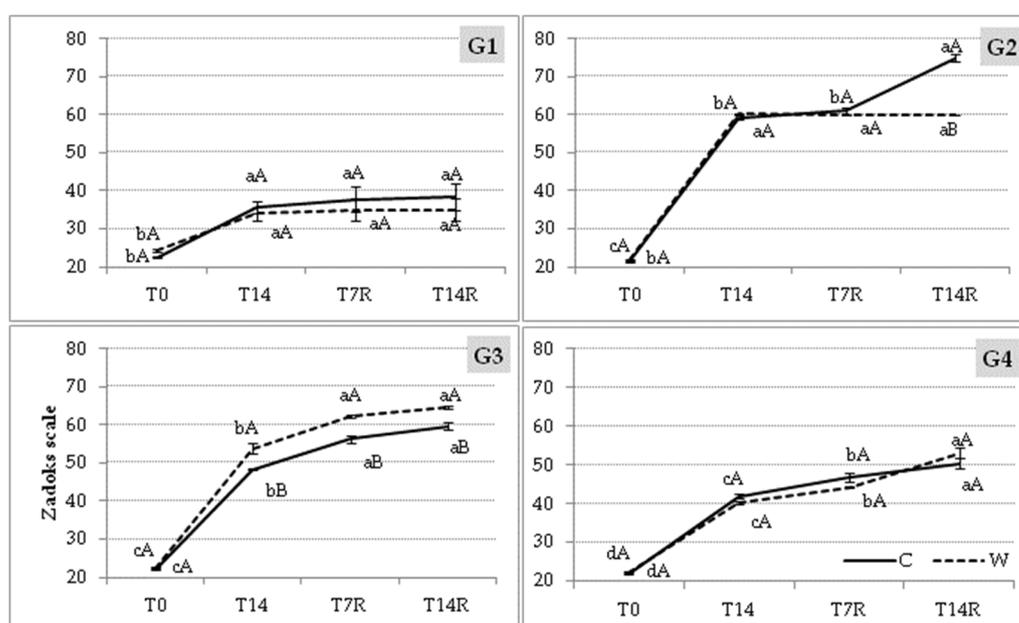
Data were analyzed using a two way ANOVA to evaluate the differences between water treatments (C or W), between time of treatments (T0, T14, T7R and T14R), and their interaction, followed by a Tukey's test for mean comparisons. A 95% confidence level was adopted for all tests, which were performed independently for each genotype.

## 3. Results

### 3.1. Growth Stages

Phenological stages didn't show significant differences between control (C) and waterlogged plants (W) in the end of treatment (T14) except in G3 (Figure 1). In that genotype waterlogging seems to promote plants development with C plants reaching

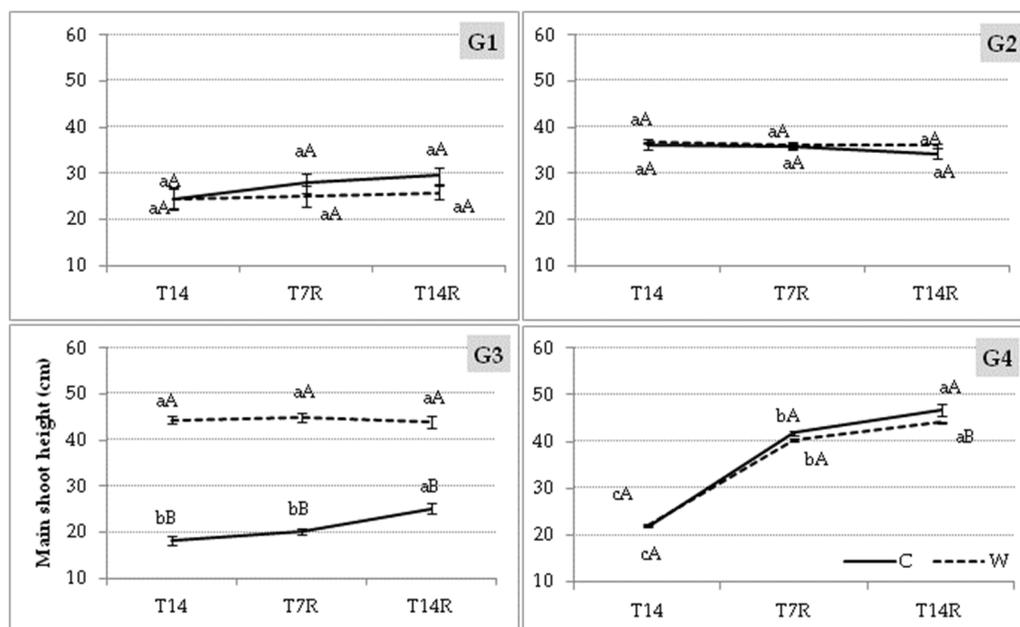
stage Z48 (Booting—Flag leaf sheath opening) and W plants stage Z54 (Ear emergence—One-half of ear emerged) at the end of the water stress. During recovery a similar performance was observed with significant higher values of Zadoks scale in W plants (Z65—Flowering half-way complete) when compared with C plants (Z60—Beginning of flowering). Genotypes G1 and G4 (Portuguese landrace and Portuguese advanced line, respectively) remained unaltered along the recovery period when comparing C and W plants. In the G2 genotype (Post Green-revolution), and despite similar development during water stress, W plants remained at Z60 (Beginning of flowering) from T14 to T14R. On the other hand, C plants continued to develop, reaching stage Z75 (Milk Development—Medium milk) at the end of the recovery period (T14).



**Figure 1.** Impact of 14 days waterlogging on the development of four *Triticum aestivum* L. genotypes using the Zadoks scale. (G1)—Portuguese landrace; (G2)—Post Green Revolution, with reduced height (Rht) genes; (G3)—Australian germplasm and (G4)—Advanced line from the National Cereal Breeding Program (INIAV, I.P.). C—Control plants. W—Waterlogged plants. Observations were performed at the beginning (T0) and at the end (T14) of water treatment imposition, and after 7 (T7R) and 14 days (T14R) of recovery. Different letters express significant differences between water stress treatments for the same day of observation (A, B), or between days of observation for the same water treatments (a, b, c), always separately for each genotype.

### 3.2. Main Shoot Height

At the end of waterlogging, in G1, G2 and G4 genotypes, no significant differences were observed between C and W plants concerning main shoot height (Figure 2). In G3, waterlogged plants presented values 1.4 fold higher than C plants. During recovery this genotype showed similar trends with 1.2 and 0.8 higher values at T7R and T14R respectively. Although G4 did not show differences at T14 at T7R, at the end of the recovery period W plants presented a small, but significant, decrease in the main shoot height.



**Figure 2.** Impact of 14 days waterlogging on the main shoot height (cm) of four *Triticum aestivum* L. genotypes. (G1)—Portuguese landrace; (G2)—Post Green Revolution, with reduced height (Rht) genes; (G3)—Australian germplasm and (G4)—Advanced line from the National Cereal Breeding Program (INIAV, I.P.). C—Control plants. W—Waterlogged plants. Observations were performed at the beginning (T0) and at the end (T14) of water treatment imposition, and after 7 (T7R) and 14 days (T14R) of recovery. Different letters express significant differences between water stress treatments for the same day of observation (A, B), or between days of observation for the same water treatments (a, b, c), always separately for each genotype.

### 3.3. Tillers number

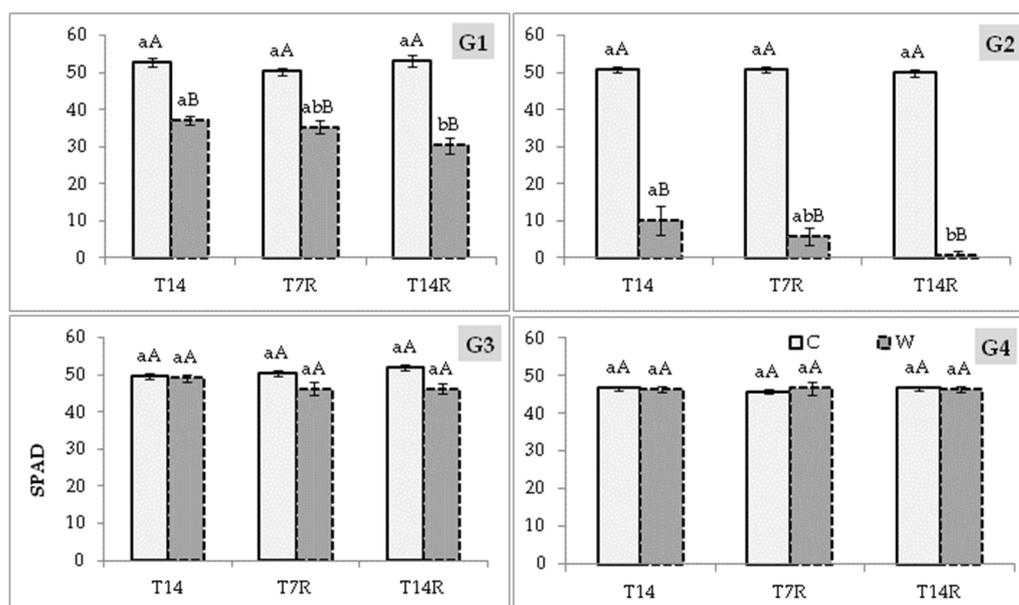
At the beginning of the stress treatment there were no differences between C and W plants in all genotypes (Table 1). Only G2 was negatively affected by waterlogging, showing no living tillers from T14 until the end of the growth cycle. After the recovery period (T14R) and despite a decreasing trend in G3 and G4, and an increasing trend in G1, the number of productive tillers at harvest did not show differences induced by waterlogging.

**Table 1.** Impact of 14 days waterlogging on tillers number of four *Triticum aestivum* L. genotypes. (G1)—Portuguese landrace; (G2)—Post Green Revolution, with reduced height (Rht) genes; (G3)—Australian germplasm and (G4)—Advanced line from the National Cereal Breeding Program (INIAV, I.P.). C—Control plants. W—Waterlogged plants. Observations were performed at the beginning (T0) and at the end (T14) of water treatment imposition, after 14 days of recovery (T14R) and at maturity (as fertile tillers). Different letters express significant differences between water stress treatments for the same day of observation (A, B), or between days of observation for the same water treatments (a, b, c), always separately for each genotype.

Genotype	Water Treatment	T0	T14	T14R	End of Growth Cycle
G1	C	4.9 ± 0.54 <sup>aA</sup>	4.7 ± 0.19 <sup>aA</sup>	6.7 ± 0.47 <sup>aA</sup>	1.4 ± 0.29 <sup>bA</sup>
	W	4.9 ± 0.34 <sup>bA</sup>	5.9 ± 0.62 <sup>abA</sup>	7.5 ± 1.06 <sup>aA</sup>	1.9 ± 0.48 <sup>cA</sup>
G2	C	2.1 ± 0.22 <sup>abA</sup>	2.6 ± 0.27 <sup>aA</sup>	2.6 ± 0.42 <sup>aA</sup>	1.8 ± 0.17 <sup>bA</sup>
	W	2.1 ± 0.08 <sup>aA</sup>	0.0 ± 0.00 <sup>bB</sup>	0.0 ± 0.00 <sup>bB</sup>	0.0 ± 0.00 <sup>bB</sup>
G3	C	2.4 ± 0.33 <sup>aA</sup>	1.9 ± 0.30 <sup>aA</sup>	3.1 ± 0.95 <sup>aA</sup>	2.0 ± 0.85 <sup>aA</sup>
	W	2.4 ± 0.15 <sup>aA</sup>	2.6 ± 0.23 <sup>aA</sup>	1.6 ± 0.31 <sup>aA</sup>	1.0 ± 0.25 <sup>aA</sup>
G4	C	2.3 ± 0.57 <sup>aA</sup>	2.1 ± 0.60 <sup>aA</sup>	2.9 ± 0.64 <sup>aA</sup>	1.3 ± 0.60 <sup>aA</sup>
	W	2.2 ± 0.21 <sup>aA</sup>	2.5 ± 0.29 <sup>aA</sup>	1.1 ± 0.36 <sup>ab</sup>	1.8 ± 0.28 <sup>aA</sup>

### 3.4. Chlorophyll Relative Content (SPAD Measurements)

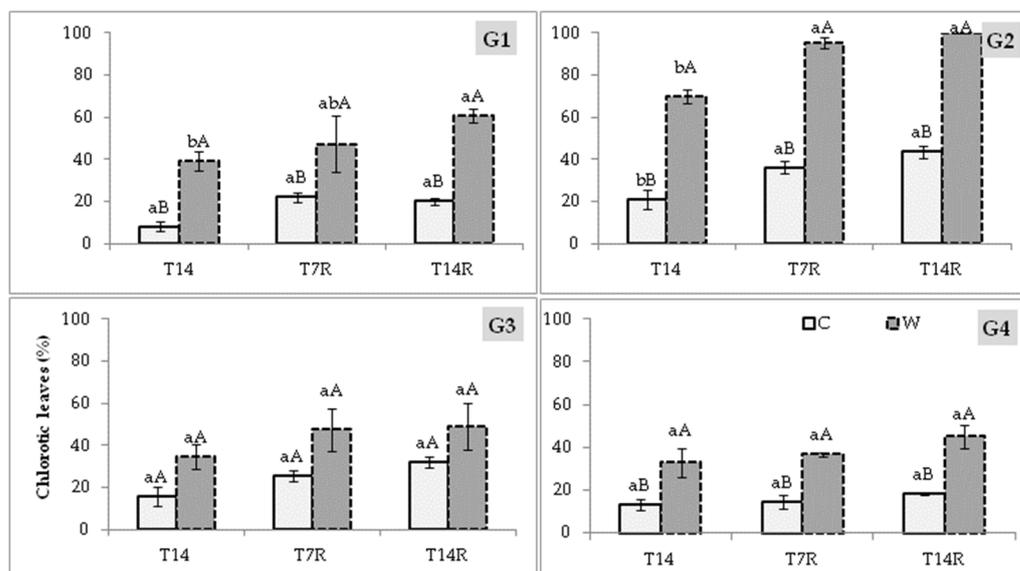
Chlorophyll relative content of the 2nd top leaf of the main shoot (Figure 3) showed that 14 days waterlogging did not induce a degradation of this pigment in two genotypes (G3 and G4). In fact, even during the recovery period C and W plants presented equal and stable values. In the Portuguese landrace G1, decreases of 29, 30 and 43% were found in T14, T7R and T14R, respectively, between C and W plants. Waterlogging affected severely the chlorophyll content of W plants in G2, which presented strong decreases of 80, 89 and 98% in T14, T7R and T14R, respectively, when compared with C plants.



**Figure 3.** Impact of 14 days waterlogging on the SPAD values of the 2nd top leaf of the main shoot of four *Triticum aestivum* L. genotypes. (G1)—Portuguese landrace; (G2)—Post Green Revolution, with reduced height (Rht) genes; (G3)—Australian germplasm and (G4)—Advanced line from the National Cereal Breeding Program (INIAV, I.P.). C—Control plants. W—Waterlogged plants. Observations were performed at the beginning (T0) and at the end (T14) of water treatment imposition, and after 7 (T7R) and 14 days (T14R) of recovery. Different letters express significant differences between water stress treatments for the same day of observation (A, B), or between days of observation for the same water treatments (a, b, c), always separately for each genotype.

### 3.5. Percentage of Chlorotic Leaves

Waterlogging negatively affected G1, G2 and G4 growth, as verified by the rise of the percentage of chlorotic dry matter in W plants when compared with C plants in T14, T7 and T14R (Figure 4). Although water stress also induces an increased chlorosis in G3, this was not statistically different from C plants. Among the genotypes under study, C plants did not show pronounced variations from T14 to T14R except in G2 where a significant increase was observed during the recovery period. At the end of the waterlogging treatment (T14), G2 was the most affected, showing 70% of senescent leaves that reached 100% at T14R.



**Figure 4.** Impact of 14 days waterlogging on the main shoot height of four *Triticum aestivum* L. genotypes using the Zadoks scale. (G1)—Portuguese landrace; (G2)—Post Green Revolution, with reduced height (Rht) genes; (G3)—Australian germplasm and (G4)—Advanced line from the National Cereal Breeding Program (INIAV, I.P.). C—Control plants. W—Waterlogged plants. Observations were performed at the beginning (T0) and at the end (T14) of water treatment imposition, and after 7 (T7R) and 14 days (T14R) of recovery. Different letters express significant differences between water stress treatments for the same day of observation (A, B), or between days of observation for the same water treatments (a, b, c), always separately for each genotype.

#### 4. Discussion

Different responses of wheat to waterlogging were reported previously [3,12] with adverse modifications on physiology, plant growth and plants development [3].

Several authors report that abiotic stresses can change wheat life cycle [13] and that waterlogging significantly reduces shoot growth [3,8,14–16] due to a decrease of energy generation. Other authors [17] refer a rapid elongation of plant apical meristems as an adaptation of plants to waterlogging. In the present work, as regards growth stages, the most striking changes were observed in G2 and G3, where waterlogging arrested (G2) or enhanced (G3) plant development. Main shoot height was promoted in G3 waterlogged plants along the study.

In wheat, not all tillers produce spikes, with many of them aborting before anthesis [18]. Several authors report a tiller reduction in wheat subjected to waterlogging. However, in some genotypes, no differences were found in the number of fertile tillers of stressed plants compared to plants maintained in optimal conditions [8,9,14,15]. In the genotypes under study, G2 produced no productive (fertile) tillers under waterlogging due to above referred growth arrest, while in the other genotypes the number of productive tillers at the end of cycle was similar when compared to controls.

Chlorophylls are crucial for photosynthetic activity and photoassimilation, and may be used as an indicator of leaf senescence [19,20], which was the case in genotypes under study. Monitoring of relative chlorophyll content in 2nd top leaf showed unaffected G3 and G4 stressed plants, but revealed an early and strong senescence in G2. Global senescence in whole plants (dry mass of chlorotic leaves) was more pronounced in G1 and G2. Such stress response mainly results from premature senescence of basal leaves, corresponding to chlorophyll degradation for N remobilization to younger leaves [3,5,6].

## 5. Conclusions

Variability in response to waterlogging was identified among the germplasm under study. Some observed traits were related to an enhanced survival ability, and may contribute to obtained new varieties of wheat better adapted to extreme climate events.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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