The Effect of Water Stress on Total-Phenolic Content of Barley

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ABSTRACT

Little is known about the relation between water stress and the accumulation of phenolics in plant tissues. The present study aimed to investigate the effect of water stress and maturation on the production of total-phenolics (TP) by four barley (*Hordeum vulgare* L.) varieties ('Manel', 'Martin', 'Rihane', 'Espérance'). During three phenological stages (S-8, S-10.5, S-11), following Feekes scale, whole barley plants were pulled out of the field and separated into roots, stems, and leaves. Water extracts were prepared from plant parts and their TP contents were determined by spectrophotometer. To determine periods of water deficit (WD) at field, climatic characterization of the region was carried out. TP accumulated in barley plant and its parts under the influence of water deficit essentially at S8, which coincided with barley spring growth. However, TP content decreased when WD became more pronounced at the following stages. This response may be explained, partially by the biosynthesis of lignin from free phenols when the plant approached maturity. Results suggest that water stress stimulates the synthesis and accumulation of TP in barley tissues during active growth periods (spring growth) at S-8. This response doesn't persist until the critical periods of WD where barley maturity favors a decrease in TP content for all plant parts. Regardless of growth stage and WD, barley accumulates preferentially phenolics in above-ground plant parts. The evolution of phenolic accumulation under water stress showed the same trends for the tested barley varieties, indicating a genetic control of phenolic production and their partitioning across plant parts.

Keywords: *Barley, Maturation, Phonological stages, Total-phenolics, Water deficit.*

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Highlights of this paper

- Relation-ship between water stress and phenolics accumulation in barley tissues was investigated, during three phenolgical stages.
- Phenolic accumulation in barley tissues decreased under water deficit.
- A decrease that could be explained in part by the biosynthesis of lignin when reaching maturity.
- Phenolics accumulate preferentially in above-ground parts of barley.
- Phenolic accumulation in barley under water deficit showed the same trends for all tested local barley varieties, indicating a genetic control.

1. INTRODUCTION

Phenolics are the largest category of phytochemicals and the most widely distributed in the plant kingdom [1]. They are secondary metabolites characterized by an aromatic ring with one or more hydroxyl groups. The structural classes of phenolics include polyphenolics (hydrolysable and condensed tannins) and monomers such as ferulic acid and catechol [2, 3]. Phenolic compounds are involved in many interactions of plants with their biotic and abiotic environment. These substances accumulate in plant tissues and cells during ontogenesis and under the influence of various environmental stimuli. Phenolics were shown in cell walls, vacuoles and were associated with cell nuclei in leaves of monocotyledonous and dicotyledonous plants [4]. Several phenolic compounds, especially phenolic acids, and free phenols, were identified in many crop tissues such as sorghum (*Sorghum bicolor* L. Moench) [5], wheat (*Triticum aestivum* L.) [6-8], rice (*Oryza sativa* L.) [9], oat (*Avena sativa* L.) [10], rye (*Secale cereale* L.) [11] and barley (*Hordeum vulgare* L.) [12-14].

Secondary metabolites in plants are known to contribute to the defense mechanism against herbivores and disease factors. Concentrations of these compounds are often enhanced by biotic and abiotic stress [15]. Nitrogen deficiency causes a great increase in the concentrations of chlorogenic and isochlorogenic acids in sunflower (*Helianthus annuus* L.) tissues [16]. Also tolerant rice cultivars to UV-B irradiation accumulated relatively higher levels of phenolics than susceptible ones [17]. When exposed to water stress, total-phenols of cowpea (*Vigna unguiculata* L.) seedlings increased by 19 % [18]. Drought stress increased phenolic acid production in edible amaranth (*Amaranthus tricolor*) [19]. It's known that phenolics play a protective role in response to drought stress by neutralizing the reactive oxygen species behind oxidative stress [20]. On the contrary, Caliskan, et al. [21] found that drought stress did not cause significant changes in phenolic (chlorogenic acid, rutin, hyperoside, isoquercetine, quercitrine, quercetine) contents of *Hypericum pruinatum* plantlets.

Since little is known about relationships between water stress and accumulation of phenolics in barley tissues, the present work was undertaken to: i) determine water deficit periods during the growing season 2002/03 and ii) study the impact of characterized water deficit on total phenolic production in barley plant parts of four local barley varieties ('Manel', 'Martin', 'Espérance', 'Rihane'), in the semi-arid conditions.

2. MATERIALS AND METHODS

2.1. Collection of Plant Material

Four local barley varieties ('Manel', 'Martin', 'Espérance', 'Rihane') were sown in November 2002 at the experimental station of Ecole Supérieure d'Agriculture du Kef (ESAK) located in the semi-arid zone of Tunisia, on an alkaline (pH = 7.5) soil with sandy-clay-loamy (48 % clay, 34 % sand, 18 % silt) texture and 2 % of organic matter. From soil preparation to harvest, standard cultural package adapted to the semi-arid zone was applied. For field experiments, the experimental design was a Complete Randomized Block Design (CRBD) with four replications in six-row plots of 12 m each. The seeding rate was 120 Kg/ha. Whole barley plants were randomly

pulled out of the field at three growth stages: stage 8 (S-8: flag leaf visible), stage 10.5 (S-10.5: heading complete), and stage 11 (S-11: ripening) following Feekes scale [22].

2.2. Determination of Water Deficit Periods

Climatic data (monthly rainfall, monthly means temperature, monthly evapotranspiration) relative to the growing season, were collected from a neighboring meteorological station Table 1.

Table-1. Climatic data* relative to the biological cycle of barley growing seasons.

Month	Rainfall (mm)	ETP** (mm)	Water balance (mm)
November	117.1	61.0	56.1
December	53.2	30.0	23.2
January	235.9	31.0	204.9
February	66.8	51.0	15.8
Mars	19.6	78.0	-58.4
April	90.0	100.0	-10.0
May	25.5	138.0	-112.5
Total	608.1	489.0	119.1
Mean/month	86.9	69.9	17.0

Source: Meteorological Station of Boulifa/Kef, adjacent to the experimental site.
Note: Evapotranspiration potential.

The evapotranspiration (ETP) was calculated using Espinar Formula [23]. It allows locating water deficit (WD) periods [24]. Each time that ETP exceeds rainfall, the period is considered in WD.

2.3. Preparation of Water Extracts

Roots were washed with tap water to remove soil and the whole plant was gently washed with distilled water, dried between two paper towels, and separated into roots, stems, and leaves. All plant components were chopped into 1 cm pieces and dried at 50 °C for 24 h. A 5 g portion of each plant part was placed in 100 ml distilled water and agitated on a horizontal shaker for 24 h at 200 rpm. Water extracts were passed through four layers of cheesecloth, stored at 5 °C, and centrifuged at 12500 rpm for 20 min prior to total-phenolics analysis.

2.4. Determination of Total-Phenolics

The Folin-Denis method was used for total-phenolic (TP) analysis [25] with tannic acid (TA) used as the standard, a procedure described by Makkar [26]. Folin-Denis Reagent is a mixture of 10 g sodium tungstate, 2 g phosphomolybdic acid and, 5 ml phosphoric acid in 75 ml distilled water. The mixture was refluxed for 2 hr, cooled, and diluted to 100 ml with distilled water. A Sodium-Carbonate saturated solution was obtained by adding 40 g anhydrous sodium carbonate to 150 ml distilled water, dissolved for 1 hr at dark and, adjusted to 200 ml. TA standard solution was obtained by dissolving 50 mg of TA in 100 ml distilled water. Aliquots of 0, 20, 40, 60, 80 and, 100 µl of the standard TA solution were dispensed into tubes containing 0.5 ml Folin-Denis reagent and 2.5 ml saturated sodium carbonate solution. The standards were diluted to 4 ml with distilled water and quickly shaken. Absorbance was determined after 35 min in dark at 750 nm by spectrophotometry.

Determination of TP for each barley water-extract was made by adding 0.5 ml Folin-Denis reagent and 2.5 ml saturated sodium carbonate solution to 1 ml barley water-extract. Absorbance was determined and the TP content was obtained using the standard curve. Units of TP were expressed in mg of TA equivalents/ml extract and then multiplied by 20, based on an extraction ratio of 1: 20 (w/w), to express it in mg of TA equivalents/g of plant-tissue.

3. RESULTS

3.1. Water Deficit Periods

The distribution of the rainfall and the ETP (Figure 1) permitted the determination of WD periods that tested barley varieties were submitted to. A long period, fitting the biological cycle of barley was observed, starting from March to June, the date of harvest. The severity of WD increased markedly from April to June.

The three stages (S-8, S-10.5, S-11) when barley plants were collected for TP analysis were made during a water deficit period. The first collection was carried out on March 15 during a water deficit period of 42 mm. The second and the third collections were made during a more pronounced water deficit period, which were 132 and 226 mm, respectively Figure 1.

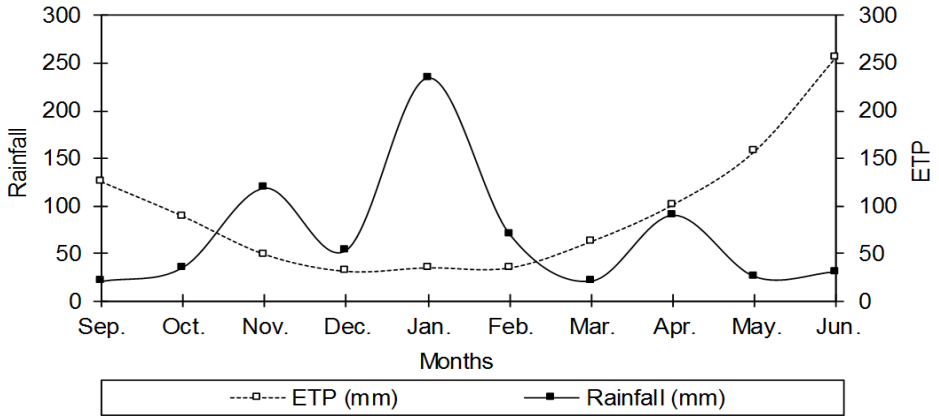

Figure-1. ETP and Rainfall evolutions during 2002/03 growing season.

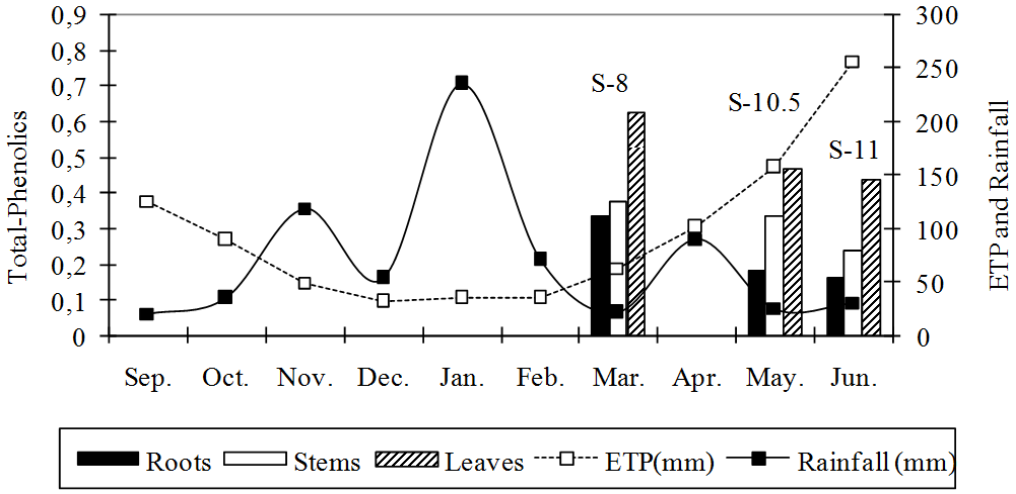

Figure-2. Evolution of total-phenolic content (mg TA/g) of barley plant parts during three phenological stages (S-8, S-10.5, S-11), all varieties confounded.

3.2. Evolution of Total Phenolic Contents of Barley Plant Parts

The TP content of each plant part was the mean of all field observations relative to the corresponding plant part at a studied growth stage. Evolution of TP contents of plant parts (roots, stems, leaves) during the three phenological stages was studied using the following equation: [[(Total-phenolics/Stage-ii – Total-phenolics/Stage-i) / (Total-phenolics/Stage-i)] x 100]. The three plant parts, all varieties confounded, expressed the most important TP contents during S-8 when the water deficit was relatively low. This content decreased afterward at S-10.5 and S-11, although WD became more pronounced. At S-10.5, when WD became more

pronounced, phenolic content in roots decreased by 45 % (0.33 mg TA/g vs 0.18 mg TA/g) were that of stems and leaves decreased respectively by 11 % and 25 %. Compared to S-10.5, TP contents in roots stems and leaves at the S-11, decreased respectively by 11 %, 30 %, and 6 %. From S-8 to S-11, TP content in roots registered the most important decrease (52 %) (0.33 mg TA/g vs 0.16 mg TA/g) when compared to that of stems (38 %) (0.37 mg TA/g vs 0.23 mg TA/g) and leaves (30 %) (0.62 mg TA/g vs 0.44 mg TA/g) Figure 2. When barley plants approach physiological maturity (S-10.5 and S-11), TP content in all plant parts decreases Figure 2.

At all studied growth stages (S-8, S-10.5, S-11), barley plant parts have invariably the same ranking, based on TP content: (leaves > stems > roots), indicating that barley accumulates phenolics preferentially in above-ground plant parts.

3.3. Evolution of Total-Phenolic Contents of Barley Varieties

For each tested barley variety, TP content was considered as the sum of those of roots, stems, and leaves. 'Manel' TP content at S-8, was about 1.35 mg of TA/gram when WD was not pronounced. The TP content in this variety decreased by 26 % at S-10.5 (1.35 mg TA/g vs 0.99 mg TA/g), although WD became more pronounced (132 mm). At S-11, the water deficit became more accentuated (226 mm) and the TP content decreased again by 14 %. The global decrease of the TP content in 'Manel' tissues, registered from S-8 to S-11 (1.35 mg TA/g vs 0.86 mg TA/g), was about 36 % Figure 3.

TP content in 'Martin' tissues was equal to 1.26 mg of TA/g at S-8. At S-10.5, the TP content decreased by 29 % (1.26 mg TA/g vs 0.89 mg TA/g) and decreased again by 13 % from S-10.5 to S-11 (0.89 mg TA/g vs 0.78 mg TA/g). The global decrease registered by this variety was about 38 % (Figure 3). The TP content in 'Espérance' decreased firstly by 35 % from S-8 to S-10.5 and secondly by 5 % from S-10.5 to S-11. Consequently, the global decrease was about 38 % (1.41 mg TA/g vs 0.87 mg TA/g) Figure 3.

Like 'Manel', 'Martin' and 'Espérance', 'Rihane' registered the highest TP content at S-8 (1.32 mg TA/g). TP in 'Rihane' decreased as WD became pronounced. This decrease was 31 % between S-8 and S-10.5 (1.32 mg TA/g vs 0.91 mg TA/g) and 9 % between S-10.5 and S-11 (0.91 mg TA/g vs 0.83 mg TA/g). The global decrease registered by the variety 'Rihane' from S-8 to S-11 was about 37 % Figure 3.

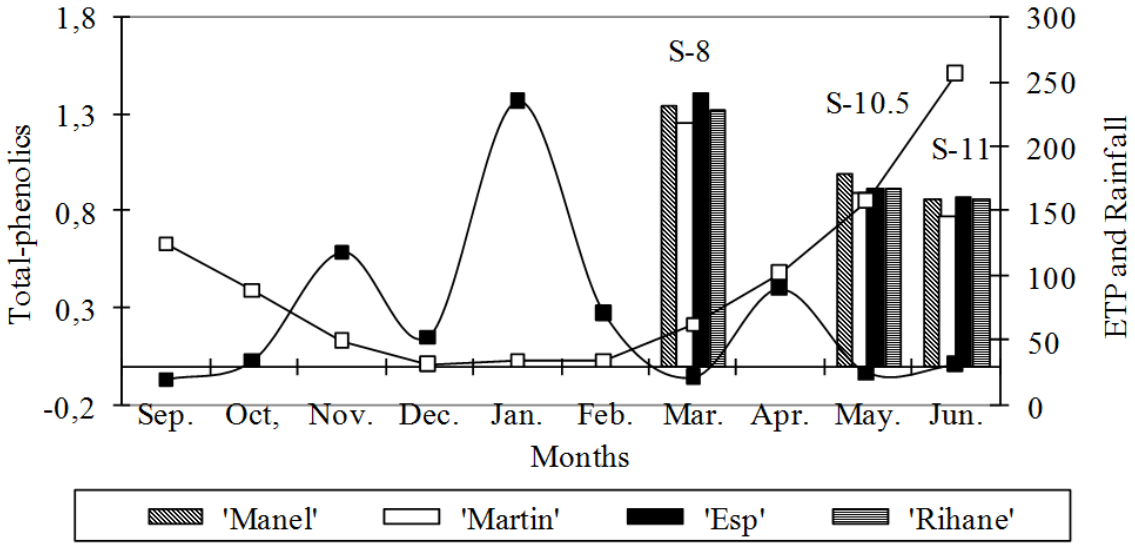

Figure-3. Evolution of total-phenolic content (mg TA/g) of four barley varieties ('Manel', 'Martin', 'Espérance', 'Rihane') during three phenological stages (S-8, S-10.5, S-11), all plant confounded.

TP contents in the four tested barley varieties showed the same evolution profile. Between S-8 and S-10.5, the TP contents in all studied varieties registered an important decrease and a less pronounced decrease between S-10.5 and S-11.

4. DISCUSSION

Barley accumulates phenolics in plant parts (roots, stems, leaves), during three phenological stages (S-8, S-10.5, S-11) which coincided with a prolonged period of WD. Del Moral [16], reported that water stress, induced by NaCl stress, increased concentrations of two phenolic acids (chlorogenic, isochlorogenic), in sunflower (*Helianthus annuus* L.) roots, stems, and leaves. The results of the present work are also in agreement with those mentioned by Brachet and Mousseau [27] and Balakumar, et al. [18] who found that water stress increased TP synthesis and accumulation, respectively in common heather (*Calluna vulgaris*) and cowpea (*Vigna unguiculata* L.). Also, Sarker and Oba [19] reported that drought stress increases phenolic acid production in edible amaranth (*Amaranthus tricolor*). Weidner, et al. [11], however, reported that cells of unripe rye grains reacted to an enforced dehydration treatment by lowering the level of TP compounds, and especially the content of phenolic acids (p-coumaric, ferulic, synapic, vanillic, caffeic). This discrepancy may be explained by the nature of the biological materials chosen for each study. Besides, the mechanisms involved in water stress tolerance may be different from one species to another.

Several authors reported that phenolic compounds could accumulate in different plant tissues under the influence of various environmental stresses like an attack by insects and pathogenic fungi [28], copper deficiency [29], and exposition to UV radiation [16, 18]. The accumulation of secondary metabolites under stress is an adaptive response to conditions under which the functions of these compounds become more important. Phenolics accumulation and their defensive action against predators and pathogens become more critical under stress [30]. This could explain the enhanced production of phenolics by barley plants at S-8 when the plants were still in full growth and submitted to a first water deficit.

Regardless of growth stage and water stress, the four barley varieties accumulated higher levels of TP in the leaves when compared to stems and roots. These results are in agreement with those found by Waniska, et al. [31] who reported that sorghum contains phenolic compounds at all stages of growth, with higher levels in leaves and glumes. Cherney, et al. [32] however claimed that in four sorghum varieties, phenolic compounds such as p-coumaric and ferulic acids were found in higher levels in the stem than in leaf blades or leaf sheaths. Ben-Hammouda, et al. [5] found that mature plants of three sorghum hybrids generally contained higher levels of TP contents in the leaves than in the roots, glumes, culms, or seeds. Seedlings of 58 wheat accession, showed that phenolic acids, such as p-hydroxybenzoic, vanillic, cis-p-coumaric, cis-ferulic, trans-p-coumaric, and trans-ferulic acids were more concentrated in roots than in shoots [7].

Although WD became more pronounced during S-10.5 and S-11, the total phenolic contents of the whole plant and its components decreased. In healthy plants of sorghum, it was shown that levels of phenolic acids always decrease as the plant matures Woodhead [28]. Weidner, et al. [11] reported also that phenolic acids and total phenolic compounds decreased considerably in unripe rye caryopses, at the final stage of grain maturation. The decrease of total phenolic content may be partially explained by the conversion of a fraction of total phenolics into lignin as the plant approach maturity. The induction of moisture stress for a period of 12 days at transplanting and pre-blooming stages in marigold (*Tagetes erecta* L.), provoked a sharp peak in the accumulation of phenols. The enhanced phenol metabolism led to the biosynthesis of lignin [33]. Water stress induced in three-week-old plants of maize (*Zea mays* L.), the biosynthesis of Caffeate-O-methyltransferase, a key enzyme in the biosynthesis of lignin monomers catalyzing the methylation of cinnamic acids [34]. Biosynthesis of lignin could act as a mechanism to

combat moisture stress [32]. Indeed, in sorghum subjected to severe water stress, the tolerance of roots to water deficits may be increased by changes such as lingo-suberization, which in turn could restrict the loss of liquid water and water vapor to the surrounding medium [35].

It appears clearly that phenolic accumulation in barley tissues occurs independently of the tested variety, and is strongly controlled by environmental conditions i.e. environmental stresses. But TP contents in the tested barley varieties registered the same evolution profile, this suggests that phenolic distribution between plant parts is genetically controlled in the barley varieties. These varieties appear to be sister lines.

The results of the present work demonstrated that water stress at barley spring growth (stage 8) was coupled with an accumulation of total phenolics in plant tissues. This suggests that water stress induces total phenolics synthesis and accumulation in barley, and these secondary metabolites may play a role in the water stress tolerance of barley. Total phenolics decreased than in all plant parts as the plant approached maturity, although, at the same time water stress became more pronounced. This suggests that a fraction of total phenolics was probably converted into lignin, which in turn, could combat water stress. Independently of growth stage and water deficit, barley accumulates preferentially phenolics in above-ground plant parts.

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