*Article*

# Effects of Low Water Availability on Root Placement and Shoot Development in Landraces and Modern Barley Cultivars

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**Abstract:** Early vigor has been proposed as a favorable trait for cereals grown in drought-prone environments. This research aimed at characterizing early stage shoot and root growth of three Spanish barley landraces compared with three modern cultivars. Genotypes were grown in an automated phenotyping platform, GrowScreen-Rhizo, under well-watered and drought conditions. Seminal and lateral root length, root system width and depth were recorded automatically during the experiment. Drought induced greater growth reduction in shoots (43% dry weight reduction) than in roots (23% dry weight). Genotypic differences were larger under no stress, partly due to a more profuse growth of landraces in this treatment. Accession SBCC146 was the most vigorous for shoot growth, whereas SBCC073 diverted more assimilates to root growth. Among cultivars, Cierzo was the most vigorous one and Scarlett had the least root dry weight of all genotypes, under both conditions. Root growth was redirected to lateral roots when seminal roots could not progress further in dry soil. This study reveals the presence of genetic diversity in dynamics of early growth of barley. The different patterns of growth observed for SBCC073 and SBCC146 should be explored further, to test if they affect field performance of barley in drought-prone environments.

**Keywords:** stress tolerance; drought; high-throughput phenotyping; assimilate partitioning

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## 1. Introduction

Drought is one of the major causes for yield losses of barley worldwide [1]. The influence of recent changes in precipitation and temperature on barley production worldwide has been estimated as a decrease of 10.20 MT year$^{-1}$ [2]. Particularly, barley growth in the Mediterranean often occurs under drought and high temperatures, and large inter-annual changes in environmental conditions [3–5]. Plants respond and adapt to drought stress by the induction of various morphological, biochemical, and physiological responses. However, their relation with an improved agronomic performance depends on the timing and duration of the stress, and on climate, soil, and watering regime features of each agricultural system [6].

Landraces grown in drought-prone regions represent an important source of breeding material for unfavorable environments [7]. In fact, barley landraces have demonstrated yield advantage over

modern cultivars in experiments carried out in stressful environments in the West Asia North Africa (WANA) region [8] and in Spain [9]. This advantage disappeared when the experiments were carried out in environments that were more favorable [8,9]. Unravelling the mechanisms that confer agronomic advantage to these landraces in stress environments is a sensible research goal with potential impact on plant breeding.

Landraces have been acknowledged as a source of favorable traits for barley breeding [10–15]. Early vigor, defined as fast development of leaf area and/or crop biomass has been proposed as one of the traits responsible for the good performance of landraces in drought-prone environments [16]. This is a complex trait associated with many seedling characteristics [17]. A vigorous early development results in plants covering faster the soil, thus reducing the direct water evaporation and increasing water transpired by the plant canopy. This feature may be particularly useful in Mediterranean conditions, where rainfall peaks coincide with the early developmental stages of the plant and the water lost by evaporation from uncovered soil may be important [18]. A vigorous root growth (in terms of root density, root hair length) leads to a greater contact between roots and soil, which in turn enhances the uptake of water and nutrients [19]. Deeper root systems allow accessing water in deeper soil layers that might otherwise be unavailable. An option to improve root depth may be to identify genotypes with faster-growing roots, a trait presenting large genetic variation [20]. The result of improving this kind of trait would be an enhanced efficient use of water (EUW), a concept presented by Blum [21], which challenges the classical focus on water use efficiency, and could result in enhanced resilience of crops. In wheat, it has been reported that a large root system contributes to increased water and nitrogen capture early in the crop season and, at later stages, may facilitate the uptake of additional water for grain filling [22]. However, there are trade-offs involved, and it is not possible to generalize on the effect of root system architecture on grain yield under water deficit, as it depends on environmental and genetic contexts [23], including precipitation (amount and distribution), soil properties, rooting characteristics, and plant architecture [24]. There are evidences that domestication and breeding have caused a reduction of crop root systems, likely because these processes sought to optimize biomass allocation towards the grain [23]. However, breeding has not been equally successful everywhere. In the Mediterranean region, "breeding has not made much progress in low-yielding environments" [25] and, therefore, there is ground to further explore the relationship of phenotypic traits with grain yield for this type of environments. It is well established that plant development is hampered by low water availability in the soil [23]. However, cereals show a variety of responses. In wheat, for instance, there are differential genotypic responses of root growth to drought conditions. A genotype with high vigor showed increased root growth ability under drought, which resulted in higher water use, water use efficiency and grain yield than a low-vigor one [26]. This differential behavior was already evident during early development, in which water and nutrient capture depend largely on seminal and lateral roots [27].

Some Spanish landraces have shown an outstanding agronomic performance in field trials with low average productions, in which drought was the main limiting factor [9]. We want to investigate whether this adaptation is related to early growth traits including early-vigor and root traits which could improve their ability to extract water from the soil. The objective of this work was to study three promising Spanish landraces with good agronomic performance in stress-prone environments, with a focus on their early shoot and root growth, and compare these phenotypic characteristics with three modern cultivars.

## 2. Materials and Methods

### 2.1. Plant Materials

Six barley genotypes were tested in this study: landrace-derived lines SBCC042, SBCC073, and SBCC146, and cultivars Cierzo, Orria, and Scarlett. SBCC042, SBCC073, and SBCC146 are inbred lines derived from Spanish landraces that showed high grain yield when evaluated in sites with low average

production [9]. Additionally, SBCC146 has shown early vigorous growth during field trials over the years (E. Igartua, unpublished). Orria is a Spanish cultivar, selected from a CIMMYT nursery, and one of the outstanding parents used in the national public barley breeding program [28], characterized by high productivity. Cierzo is a highly productive elite cultivar, currently cultivated in Spain, released by the national public barley breeding program. Scarlett is an elite malting cultivar, very productive and widely grown in Europe. All are six-rowed genotypes, except Scarlett, which is two-rowed.

*2.2. Experimental Design*

Barley genotypes were grown during May and June 2014, for four weeks under control and drought conditions in the phenotyping platform GrowScreen-Rhizo [29], located in the PhyTec Experimental Greenhouse at the institute IBG-2: Plant Sciences, Forschungzentrum Jülich GmbH, Germany. Daylength was set at 16 h, and supplemental illumination (SON-T AGRO 400, Philips, Amsterdam, The Netherlands) was automatically turned on when the ambient light intensity outside the greenhouse was <400 mmol m$^{-2}$ s$^{-1}$, between 06:00 and 22:00 h local time. Day/night temperatures were set at 24 °C and 18 °C.

One-hundred sieved seeds (>2.5 cm long) per genotype were weighted and surface sterilized by immersion in 10 mL of a 1% NaClO solution, under stirring movement for 15 min, and then rinsed 10 times with 10 mL of sterile water each. Subsequently, seeds were placed inside closed Petri dishes (between two filter papers imbibed with 1 mL of sterilized water), and kept in the dark in the laboratory for 2 days. Seedlings with homogenous root length were chosen for transplantation. The weight of one hundred sieved seeds was recorded, and used as covariate in the analyses of variance, to eliminate any influence seed size could have on traits measured during early plant development.

Plants were grown in 72 rhizotrons (outer dimensions: 90 × 70 × 5 cm, inner dimensions 85 × 60 × 3.4 cm, volume 18 L) slanted in an angle of 43° and filled with manually sieved black peat soil (Graberde; Plantaflor Humus, Vechta, Germany; containing N, ~120 mg L$^{-1}$; P$_2$O$_5$, ~20 mg L$^{-1}$, and K$_2$O, ~170 mg L$^{-1}$) and compacted with the same person power (Figure S1A,B). This black substrate provides a good color contrast with roots, and has been used successfully for drought studies [30]. Rhizotrons were filled with the substrate to the same level (2 cm from the rim) and then the substrate was covered with ion exchange resin beads to prevent water evaporation. The set of 72 rhizotrons was divided into two groups, half for control and the other half for the drought treatment. At the beginning of the experiment the soil of control treatment was set to a water content of 71% (expressed as percentage of field capacity), whereas the soil for the drought treatment was sun dried in the greenhouse until it reached 35% water content.

The treatments were distributed alternatively over rhizotrons (one control followed by one drought) across the four sections of the facility (18 rhizotrons in each section), which were distributed over the four quadrants of the greenhouse. Two seedlings were transplanted to each rhizotron, each of a different genotype. Each genotype was paired twice with each of the other genotypes and with itself per treatment, so each genotype was replicated 24 times in total, 12 per treatment, 6 times per quadrant, of which three were under drought and 3 under control conditions. Watering was ensured by 4 drippers installed on the top of each rhizotron. All plants were similarly watered for two weeks from transplant, on a daily basis (700 mL over the first 3 days; 100 mL per day since day 4). Since then, water was withdrawn from the drought treatment, which received only one additional irrigation (100 mL) in the 17 day until the end of the experiment (day 27), whereas irrigation continued daily at the control treatment.

*2.3. Traits*

The experiment lasted 4 weeks, after which the plants were harvested and several traits were recorded. This duration was calculated based on previous evidence, to avoid plant roots reaching the bottom of the rhizotrons. During the experiment, automatic imaging of the visible part of the root system was performed 2–3 times a week for a total of 8 measurements (Figure S1C,D). Maximum depth and

width of the root system, total root length, seminal and lateral root length were evaluated through image analysis. Root system images acquired at the GrowScreen-Rhizo facility were retraced with different colors for seminal and lateral roots, then analyzed using the software tool GROWSCREEN-Root [31], modified by [29]. Leaf and tiller number were manually counted at six dates, twice a week for each plant during the experiment. Leaf area (LA) was estimated by measuring manually the length and the maximum width for each measurable leaf using a ruler, twice per week, then using the following formula to calculate leaf area:

$$\text{Leaf area} = \text{Width} \times \text{Length} \times k \qquad (1)$$

where $k$ is the shape factor, which is 0.69 for barley leaves [32].

Chlorophyll fluorescence was measured using a pulse-amplitude-modulated Mini-PAM fluorometer (MINI-PAM, Heinz Walz GmBH, Effeltrich, Germany), according to the manufacturer's instructions, to determine the effects of drought on electron transport system of photosystem II, on the youngest fully expanded leaf. The maximum quantum yield of photosystem (PS) II (Fv/Fm), was obtained by dividing variable fluorescence (Fv) on maximal fluorescence (Fm) after 20 min of dark adaptation on the middle part of the leaves, prior to a saturating light pulse. Chlorophyll content was estimated using SPAD chlorophyll meter (SPAD-502) (Minolta Corp., Ramsey, NJ, USA), at the end of the experiment. Measurements were taken at the base, at the middle and at the top of the last expanded leaf per plant, and then an average value per leaf was calculated. Stomatal conductance was measured on three genotypes (Orria, SBCC073, Scarlett) with a leaf porometer (Decagon Devices Inc., Pullman, WA, USA), on day 21 of the experiment, one measurement per plant on the last fully expanded leaf, on all plants of those genotypes. At harvest, shoot fresh weight per plant was immediately taken. After opening the rhizotrons, roots were carefully washed, removed from the soil, thereafter fine washed to eliminate substrate traces. Shoot and root dry weight were recorded after oven drying at 65 °C for 72 h.

*2.4. Statistical Analysis*

Regression and analyses of variance were performed using the REML (restricted maximum likelihood) and the ANOVA procedures implemented in Genstat software, version 18 [33]. First a REML analysis was run, in which genotypes, treatments and their interaction were considered fixed factors. The four sections or compartments of the facility, and the position of the rhizotron in each section (row, nested within sections) were considered random. To test whether differences between landraces and cultivars (a fixed factor named "type", for type of cultivar) were significant, REML-derived genotypic means were used for a second analysis of variance, using the ANOVA procedure. "Type" and "type by treatment" interaction were tested against the residual variances (not explained by type and type by treatment) for genotypes and genotype by treatment, respectively. This is a very conservative test, as true genotypic variance was allowed into the residual term. In the regression analysis, the genotype SBCC146 was used as reference for comparison with the other genotypes.

## 3. Results

*3.1. Control vs Drought*

A comparison of the overall results for the two treatments provides an insight on the general responses of barley to drought. Stomatal conductance differed significantly between treatments, with the control average (225 mmol m$^{-2}$ s$^{-1}$) more than doubling the average for the drought treatment (102 mmol m$^{-2}$ s$^{-1}$). Shoot biomass was more affected by drought than root biomass. Shoot biomass (SDW) decreased by 45% due to drought, whereas root biomass (RDW) decreased only by 24%, and leaf area suffered a 40% reduction, while total root length was reduced only by 10%. Total leaf number on the main tiller and tillering ability were also severely reduced by drought (Table 1; Table 2, Figure 1).
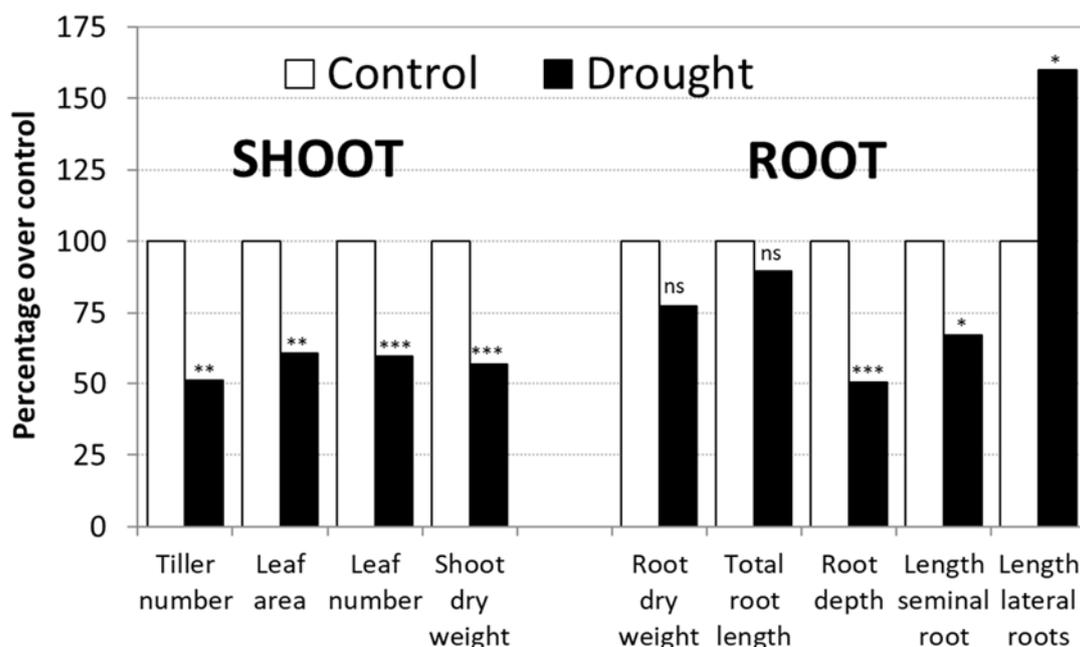
**Figure 1.** Averages of relative shoot and root variables for six barley genotypes grown in rhizotrons under drought, compared to the control treatment (100%). Each data point represents the average of up to twelve replicates (single plants). *, **, ***, ns, significant differences between treatments for $p < 0.05$, $p < 0.01$, $p < 0.001$, and non-significant, respectively.

Root growth under well-watered conditions started at a fast pace, and then slowed down after approximately 15 days (Figure 2A). At the drought treatment, the general trend was opposite. It started slowly but, after about 15 days, the rate of growth increased and equaled the rate during the first two weeks compared with the control (Figure 2A). At the end of the experiment, total root length was almost equal at both treatments, although growth patterns were different. Under drought, the root system developed less in depth and seminal root development was arrested while lateral root growth increased markedly (Figure 2B).

Under both conditions, seminal roots were longer overall than lateral roots. Under drought, however, the difference was smaller due to the remarkable growth increase of lateral roots (64%) and the reduction of seminal roots (42%) compared to the control treatment (Figure 2B). At 13 days after sowing, plants grown under drought conditions produced already longer lateral roots than under control conditions and the difference between drought and control grown plants increased over time (Figure 2B). The trade-off between the growth of seminal roots in depth and the lateral root resulted in similar total root length under control and drought conditions (Figure 1).

*3.2. Genotypic Differences*

Genotypes were significantly different for all measured traits with the exception of Fm, Fv, and $F_0$ (Table 1). Differences between treatments were also significant for most traits, except for total root length, SPAD, and Fm. Genotype by treatment interaction, however, was significant only for leaf area, shoot and root dry weight (Table 2, Figure S2).
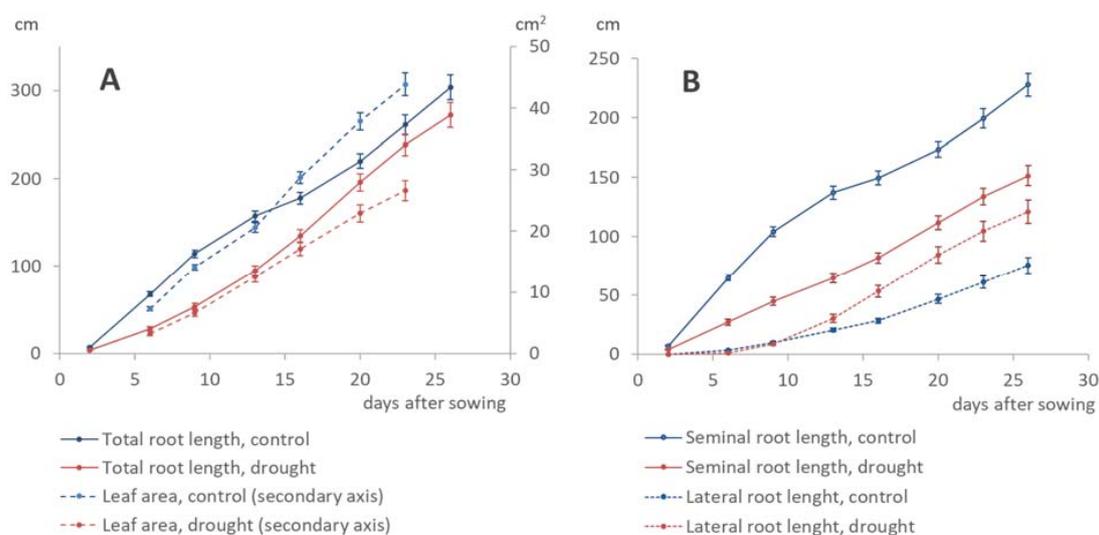
**Figure 2.** Dynamics of growth of roots and leaves during the duration of the experiment, averaged over genotypes, for the control and drought conditions (bars represent ± standard errors of the means). (**A**) Total root length averaged (primary axis) and total leaf area (secondary axis). (**B**) Seminal and lateral root lengths.

SBCC146 had a larger leaf area under both control and stress conditions and produced more shoot fresh weight, significantly only under control conditions (Table 2). In addition, it had greater shoot dry weight, albeit not significantly different from SBCC073, SBCC042, and Cierzo. Regarding roots, SBCC073 ranked the first by producing significantly more root dry weight, only under control conditions, as it was the genotype most affected by drought. SBCC042 produced more tillers under both conditions and was the genotype least affected by drought. For leaf number, SBCC042 also ranked first under control conditions, followed by the other two landraces (Table 2). At the individual genotype level, the responses observed were not homogeneous within the cultivar and landrace groups, indicating the presence of genetic variation in both groups.

Overall, there were no significant differences between landraces and modern cultivars (Table 2). However, there were significant type (i.e., cultivar vs landraces) by treatment interactions for shoot variables LN, LA, fluorescence parameters including Fv/Fm, and SDW. In all cases, landrace values were significantly higher than cultivar values under control, but not under drought. Landraces were more vigorous in general at the control treatment but the differences were less pronounced under the drought treatment (Table 2, Figure S2). It is worth mentioning that two landraces (SBCC042 and SBCC146) produced more tillers than cultivars under drought conditions (Table 2, Figure S2). There was significant genotypic variation for both SPAD and Fv/Fm, both within cultivars and landraces. The highest and lowest overall values for these two traits corresponded to two landraces (SBCC146 low, SBCC073 high), indicating that even in this reduced sample of genotypes there was remarkable phenotypic variability.

Significant "Genotype by Treatment" and "Type × Treatment interactions" were detected for several traits (Table 1). LA and SDW followed parallel trends (Figure S3), indicating that genotypes Cierzo and Scarlett presented the lesser reduction for these traits under drought, i.e., had a more resilient response. Similarly, cultivars conserved LA better than landraces under drought. Regarding RDW, the interaction seemed driven by the opposite trends shown by genotypes SBCC073, which showed a steep RDW reduction under drought, and Scarlett that presented a slight increase of RDW in this condition (Figure S3).

**Table 1.** Analyses of variance of traits measured in six barley genotypes, under control and drought conditions in rhizoboxes, at the end of the four weeks' experiment.

| Source of Variation | df | TN | LN | LA | $F_0$ | Fv | Fm | Fv/Fm | SPAD | SFW |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatments | 1 | ** | *** | ** | * | ** | * | *** | ns | *** |
| Genotypes | 5 | *** | ** | *** | ns | ns | ns | ** | *** | *** |
| Type (cultivars vs landraces) | 1 | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Genotype × Treatment | 5 | ns | ns | * | ns | ns | ns | ns | ns | ns |
| Type × Treatment | 1 | ns | *** | * | ns | ** | ** | * | ns | ns |
| | df | SDW | RDW | S/R | RSD | RSW | TRL | SRL | LRL | |
| Treatments | 1 | *** | ns | ** | *** | * | ns | * | * | |
| Genotypes | 5 | *** | *** | *** | *** | ** | *** | *** | ** | |
| Type (cultivars vs landraces) | 1 | ns | ns | ns | ns | ns | ns | ns | ns | |
| Genotype × Treatment | 5 | * | * | ns | ns | ns | ns | ns | ns | |
| Type × Treatment | 1 | * | ns | ns | ns | ns | ns | ns | ns | |

TN: tiller number. LN: leaf number. LA: leaf area. $F_0$: initial fluorescence, Fv: variable fluorescence. Fm: maximum fluorescence, Fv/Fm (maximum quantum yield): ratio of Fv to Fm. SPAD readings, SFW: shoot fresh weight. SDW: shoot dry weight. RDW: root dry weight. S/R: shoot/root dry weight ratio. RSD: root system depth. RSW: root system width. TRL: total root length. SRL: seminal root length. LRL: lateral root length. *, **, ***, ns represent differences statistically significant for $p < 0.05$, $p < 0.01$, $p < 0.001$, and non-significant, respectively.

**Table 2.** Means and means' comparison for different traits measured in six barley genotypes, under control and drought conditions in rhizoboxes, at the end of the four weeks' experiment. *Cultivars* is an average of Cierzo, Orria, and Scarlett. *Landraces* is an average of SBCC042, SBCC073, and SBCC146.

| | TN | LN | LA | $F_0$ | Fv | Fm | Fv/Fm | SPAD | SFW | SDW | RDW | S/R | RSD | RSW | TRL | SRL | LRL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | **Control** | | | | | | | | | | |
| Cierzo | 2.09a | 5.74bc | 45.1b | 321.1a | 853.9a | 1175a | 0.727b | 37.68bc | 2.027b | 0.232a | 0.064bc | 3.64bc | 51.04ab | 15.11a | 354.7a | 250.3ab | 107.0 |
| Orria | 1.17b | 5.43c | 34.9cd | 286.8a | 833.6a | 1124a | 0.742b | 39.30b | 1.432cd | 0.166b | 0.052c | 3.16cd | 43.33b | 11.79a | 263.0b | 228.2ab | 35.4c |
| Scarlett | 1.96a | 6.07bc | 27.9d | 313.4a | 838.5a | 1152a | 0.728b | 37.74bc | 1.177d | 0.149b | 0.035d | 4.39a | 46.41b | 14.41a | 214.0b | 147.6c | 65.4abc |
| SBCC042 | 2.57a | 7.62a | 48.5b | 316.6a | 870.8a | 1187a | 0.734b | 37.78bc | 1.922b | 0.242a | 0.065bc | 3.70bc | 45.85b | 12.26a | 263.1b | 201.9b | 59.6bc |
| SBCC073 | 2.29a | 6.68ab | 43.7bc | 288.1a | 989.8a | 1277a | 0.775a | 44.38a | 1.667bc | 0.249a | 0.091a | 2.85d | 47.89b | 12.98a | 364.8a | 270.2a | 95.2ab |
| SBCC146 | 2.25a | 6.86ab | 61.4a | 306.4a | 853.5a | 1160a | 0.736b | 33.50c | 2.496a | 0.281a | 0.068b | 4.18ab | 58.61a | 14.34a | 351.9a | 266.1a | 83.9ab |
| Cultivars | 1.74A | 5.75B | 36.0A+ | 307.1A | 842.0A+ | 1150A+ | 0.732A+ | 38.24A | 1.545A | 0.182B | 0.050B | 3.73A | 46.93A | 13.77A | 277.2A | 208.7A | 69.3A |
| Landraces | 2.37A | 7.05A | 51.2A+ | 303.7A | 904.7A+ | 1208A+ | 0.748A+ | 38.55A | 2.028A | 0.257A | 0.075A | 3.58A | 50.78A | 13.19A | 326.6A | 246.1A | 79.6A |
| | | | | | | | **Drought** | | | | | | | | | | |
| Cierzo | 1.14abc | 3.96a | 34.0ab | 286.5a | 986.2a | 1270a | 0.777ab | 34.84bc | 1.116a | 0.155a | 0.054ab | 2.70ab | 27.25a | 14.90a | 336.3a | 176.2a | 161.8a |
| Orria | 0.39c | 3.49a | 17.7c | 270.5a | 941.5a | 1212a | 0.777ab | 39.18ab | 0.546c | 0.083b | 0.033c | 2.44ab | 19.95a | 08.83c | 202.1b | 126.9a | 74.4c |
| Scarlett | 0.89bc | 4.26a | 24.3abc | 282.1a | 1006.0 a | 1284a | 0.783a | 41.94a | 0.893a | 0.125ab | 0.040bc | 3.12a | 28.12a | 14.16ab | 283.5ab | 151.3a | 129.4ab |
| SBCC042 | 1.95a | 4.29a | 25.8abc | 252.4a | 900.8a | 1154a | 0.781ab | 40.68a | 0.951a | 0.123ab | 0.054ab | 2.37b | 21.22a | 10.38bc | 269.9ab | 136.1a | 134.3ab |
| SBCC073 | 0.65bc | 3.03a | 21.2bc | 268.6a | 999.6a | 1261a | 0.793a | 43.59a | 0.732a | 0.118ab | 0.052abc | 2.29b | 18.20a | 09.94bc | 236.7b | 146.1a | 91.6bc |
| SBCC146 | 1.27ab | 3.76a | 35.6a | 300.4a | 880.4a | 1180a | 0.746b | 32.63c | 1.056a | 0.144a | 0.057a | 2.59ab | 33.09a | 12.78abc | 293.7ab | 174.1a | 123.0abc |
| Cultivars | 0.81A | 3.90A | 25.3A+ | 279.7A | 977.9A+ | 1255A+ | 0.779A+ | 38.65A | 0.852A | 0.121A | 0.042A | 3.12A | 25.11A | 12.63A | 2740A | 151.5A | 121.9A |
| Landraces | 1.29A | 3.69A | 27.5A+ | 273.8A | 926.9A+ | 1198A+ | 0.773A+ | 38.97A | 0.913A | 0.128A | 0.054A | 2.42A | 24.17A | 11.03A | 266.8A | 152.1A | 116.3A |

TN: tiller number. LN: leaf number. LA: leaf area. $F_0$: initial fluorescence, Fv: variable fluorescence. Fm: maximum fluorescence, Fv/Fm (maximum quantum yield): ratio of Fv to Fm. SPAD readings, SFW: shoot fresh weight. SDW: shoot dry weight. RDW: root dry weight. S/R: shoot/root dry weight ratio. RSD: root system depth. RSW: root system width. TRL: total root length. SRL: seminal root length. LRL: lateral root length. Means followed by a different small-case letter within each column and treatment are not significantly different according to a least significant difference (LSD) for $p < 0.05$. Means separation (LSD, $p < 0.05$) between cultivars and landraces are indicated with capital letters. A "+" sign indicates that, although there was no difference between cultivar and landrace means under both conditions separately, there was a significant type by treatment interaction taking into account the sum of differences under both conditions.

The results of the regression analyses over time (Table S1), using SBCC146 as the baseline, showed that under control conditions most variables had negative coefficients (meaning reduced growth compared to SBCC146). In many cases, these were significantly lower than 0, with the exception of genotype Cierzo for all variables, and root system width, which was not different from 0 (i.e., different from SBCC146) for any genotype. Under drought, differences among genotypes' sloped were less marked, with the exception of Orria, which showed clearly reduced growth over time compared to SBCC146.

### 3.3. Dynamics of Shoot–Root Partitioning

Over the first days of the drought treatment, growth slowed down without visible differences in shoot-root partitioning. Then, between 20–23 days after sowing, the plants in the drought treatment switched to a different response, with more assimilates apparently diverted towards the root, compared to the control treatment. The exact moment in which plants started to diverge in growth patterns between treatments may have depended on the frequency and timing of irrigations that occurred in this experiment and, therefore, is probably circumstantial. The relevant observation is that growth with limited water triggered some mechanism that affected assimilate partitioning. There were differences between the genotypes too, which are better appreciated in a combined graph (Figure 3).

The different genotypes displayed different growth patterns, with SBCC042 and SBCC146 devoting relatively more growth to shoots under control than SBCC073, which did the opposite. In SBCC073 and, to a lesser degree in Orria and SBCC146, the growth under drought was strongly reduced compared to control conditions. Cierzo and Scarlett presented the most balanced shoot and root growth between treatments, although with different growth rates between the two genotypes (Figure 3). The relationship between leaf area and total root length was in general linear for both conditions (control and drought). However, while the differences between genotypes were clear under control, they almost disappeared under drought.

At the control treatment, landraces SBCC042 and SBCC146 behaved differently from SBCC073 since very early on. SBCC073 invested more in root than in shoot. On the other side, SBCC042 and SBCC146 followed a similar pattern, devoting relatively more resources to shoot growth, with SBCC042 lagging behind in time, after SBCC146 (Figure 3A). For cultivars (Cierzo, Orria and Scarlett), their growth lines were mostly encompassed between the most extreme lines of the landraces, with Cierzo producing more biomass overall, and Scarlett less (Figure 3A).

Under drought (Figure 3B), SBCC146, which was relatively more vigorous, was clearly distinguishable from the other genotypes. Cierzo outperformed the two other cultivars only at the end of the experiment, by producing more biomass.
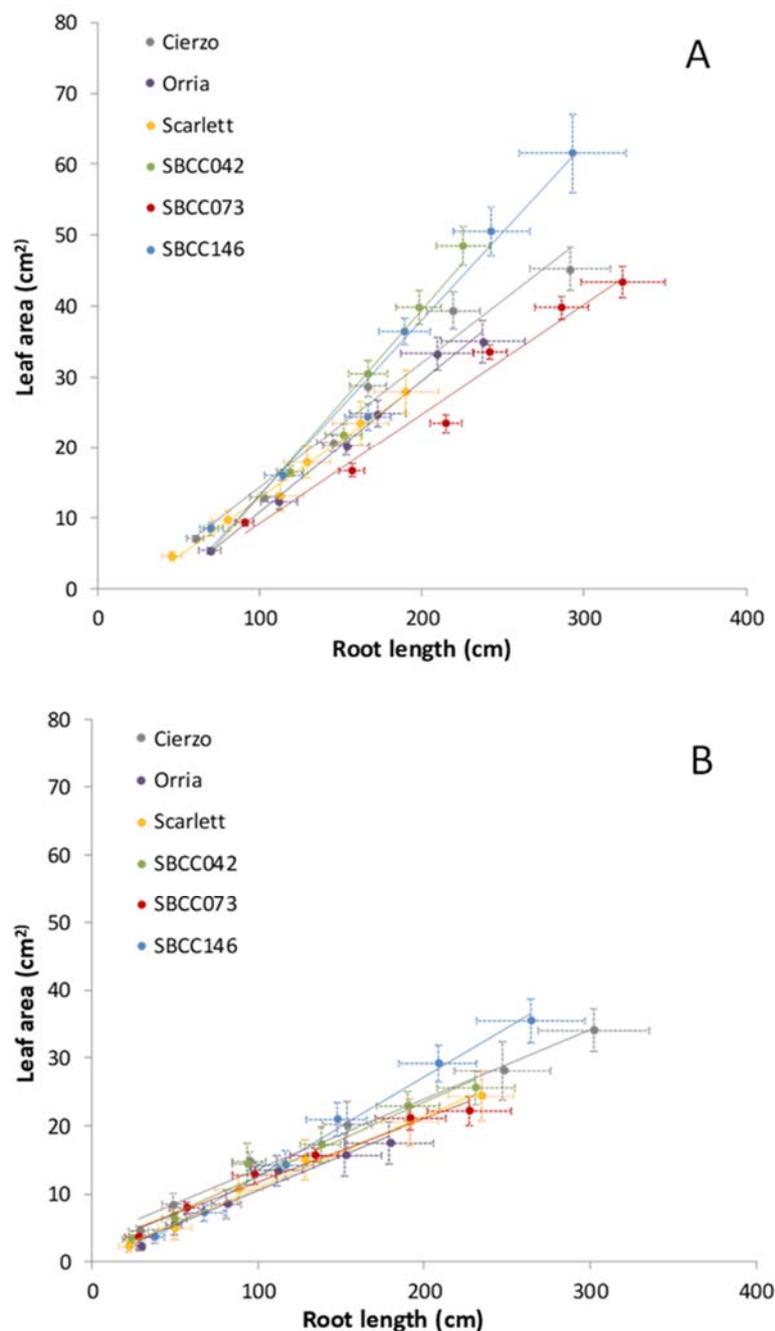
**Figure 3.** Total root length vs leaf area under control (**A**) and drought (**B**) conditions. Bars represent ± standard error of the mean of variables represented on both axes.

## 4. Discussion

### 4.1. Drought Effect on Barley Genotypes

In this experiment, drought caused a significant reduction of growth, expressed in all seedlings' traits, as reported in previous studies [34]. The lesser inhibition of root growth by low water potentials, compared to shoot growth also confirms previous observations [35]. A review of a large number of plant studies under controlled conditions, extended this observation to all situations in which the limiting factor affected the growth below ground (water, nutrient shortage). In some cases, the absolute root biomass of plants in water-limited environment can even increase compared to well-watered conditions [36,37].

Although the comparison of landraces vs cultivars has a limited scope, given the small sample size, it is worth noting that all the significant interactions found indicated a more vigorous growth of landraces at the control treatment, which was lost under drought.

Overall, the landraces showed higher root dry weight, leaf area and leaf number under control conditions compared to cultivars. Landraces, however, presented larger reductions in shoot growth under drought, causing significant interactions for leaf area, leaf number and shoot dry weight. This pattern indicates a more vigorous growth of landraces when water is not limiting, in a typical "water spender" strategy of risk-taking plants with anisohydric behavior [38,39].

Grando and Ceccarelli [40] reported deeper seminal root systems of landraces compared to modern cultivars of barley, which would indicate an enhanced ability to extract water from deeper levels. However, they found no difference for number of seminal root axes nor for seminal root dry weight.

This pattern observed for landraces is consistent with a dehydration avoidance strategy [41]. Actually, one of the landraces tested in this experiment, SBCC073, maintained stomatal conductance well above cultivar Scarlett when subjected to water stress in a pot experiment [42]. This behavior, which results in what breeders call "early vigor", was proposed as an adaptive mechanism to provide rapid soil cover and reduce water evaporation in arid zones [43]. This water-spending strategy may produce a yield advantage if combined with root system traits that confer optimized water uptake to buffer intermittent stress periods [44].

The water-spending strategy observed in the landraces is the most common response to drought found in crops [45]. A very similar growth pattern, combined with high stomatal conductance, was observed in durum wheat landraces [46]. A recent study with soybean landraces demonstrated their capacity to produce more above-ground biomass while maintaining higher rates of photosynthesis and transpiration to lower values of soil water content, compared to modern cultivars, which displayed more conservative water-use strategies [47].

### 4.2. Low Water Availability Causes Changes in Root System Architecture

We observed a shift in the pattern of root growth between treatments. After a few days of adjustment at the beginning of the experiment, growth was apparently diverted to the soil areas with higher water content. In the drought treatment, this occurred approximately in the upper half of the rhizotrons, the shallowest root part, as the little water provided comes from the top. In addition, at the drought treatment, seminal roots were much less developed compared to lateral ones, which grew profusely in the upper part of the rhizotrons. It seems that the drive of the plant to generate roots is very strong and, if it cannot progress with seminal roots due to lack of water, growth is shifted towards lateral roots, producing a compensation that results in a minor loss of total root length and biomass. The modification of rooting patterns (distribution, morphology, density) in response to changes in soil water status has been described for wheat. Root dry weight, length and density of wheat changed its vertical distribution in response to both early and late water stress [48–50], and a similar phenomenon of root compensation was observed [51] in a study with adult wheat plants grown in soils with contrasting humidity at the top and bottom soil layers. When the top layer was dry and the bottom one was wet, the plants had a larger root system than when grown in fully wet soils, which indicated enhanced root growth in the wet soil part when it was stimulated by water stress in another soil area.

This observation leads us to question if the production of more lateral roots in an area where more water is available at a particular moment could be an advantage in the long term. In principle, a denser shallow root system, compensating for the loss of capacity of exploration in depth, could be advantageous in drought-prone environments known by low and sporadic rain events. In particular, a vigorous root system may be of particular importance in Mediterranean environments, in which rain-fed crops depend on seasonal rainfalls. However, in environments where crops rely on stored soil water, a vigorous root system increases the risk of depleting the water stored before grain filling completion [22]. Another relevant hypothesis for this discussion proposes that localized root proliferation in response to

water availability could be disadvantageous under drought [52]. The rationale behind this reasoning is that, in environments characterized by unpredictable and scarce rainfall, water in the soil is a transitory resource, subjected to movement and depletion at a faster time scale than root growth and senescence, which implies a significant long-term cost in root maintenance [53]. In addition, a fast local response may impede constructing roots in soil areas with greater water availability over time [52]. Some experimental evidence already indicates the existence of different results for trade-offs involving rooting patterns and environmental conditions. In a durum wheat experiment under low moisture conditions, the deepest root genotype had a yield advantage of 37%–38%. In moisture-rich environments, however, yield of this same genotype was reduced by 20%–40%, compared with the shallowest root types [54]. The question posed above deserves further investigation, which should be optimally answered with field studies, complemented by phenotyping of the dynamics of root growth under controlled conditions.

### 4.3. Shoot vs. Root Growth

The study of the dynamics of leaf area vs. root length over time offers new insights on the general patterns of plant development. This perspective is possible thanks to sequential image analysis of root growth, and reveals different patterns of biomass partitioning among accessions (Figure 3). This was evident at the control treatment, in which fewer missing points resulted in better data quality, but was also confirmed at the drought treatment. All genotypes had a linear relationship between root and shoot growth (Figure 3), but there were differences in the genotypic slopes of the traits plotted against time (Table S1).

The shoot vs. root dynamics under control conditions (Figure 3) showed a remarkable shift towards shoot growth around the fourteenth day of the experiment, mainly for landraces, which was a result of appearance and expansion of leaves. On the contrary, plants under drought tended to invest more in root biomass for most genotypes, as revealed by the reduced S/R ratios in this condition (Table 2). Intra-specific variation for assimilate partitioning between roots and shoots has been found in wheat [55–57], generally with relatively larger roots in landraces [55,56]. In our case, we did not observe overall differences in S/R ratio between landraces and cultivars, but we did find genetic variability among the landraces for assimilate partitioning. In two of them, shoot growth was favored over root growth (SBCC042 and SBCC146) and in the other one (SBCC073) we observed the opposite. These observations reveal two different strategies of assimilate partitioning during early growth in landraces, with possible implications for adaptation to different environments. These strategies should be explored further. Actually, several populations were developed from crosses between SBCC073 or SBCC042, with cultivar Cierzo, and SBCC073 with cultivar Orria [15,58], and are amenable for future studies. The results of the present experiment led us to propose making a new population from the cross between SBCC073 and SBCC146, to test rooting features and shoot-root dynamics between these genotypes with contrasting partitioning features.

Our results are consistent with the optimal partitioning theory, which predicts that, in a scenario of reduction of precipitations caused by climate change, plants will allocate higher carbon portion to root growth to enhance the ability of root to access and acquire water [59]. Studies on whole plant growth patterns in response to different stresses have shown alteration of assimilate partitioning. Plants increased their root to shoot ratio under low nitrogen conditions [60], and under water stress [61].

Besides, several studies have found heritable variation and quantitative trait loci (QTL) affecting ratios describing assimilate partitioning. In a bread wheat study [62], three QTLs were detected for the ratio of root to shoot and two QTLs for root to whole plant ratio. Also, they found two colocalized QTLs for those two variables, and both ratios shared some QTLs with the maximum root length. Root to shoot ratio increased in rice cultivars subjected to water stress, in close association with the higher proportion of dry matter and soluble sugar in roots [61]. In barley, QTL for root traits with high heritability were discovered [63], and even candidate genes for shoot and root architecture traits at seedling stage [64].

There are examples of a positive effect of root system size with yield under drought in barley [65]. In another example, transgenic barley plants with an enlarged root system did not show a penalty for shoot growth or seed yield [66]. Assimilate partitioning between root and shoot, and its response to drought stress is an active area of research, which holds promise to contribute to plant breeding for stressful environments.

### 4.4. Usefulness of Physiological Traits

Chlorophyll content (or its surrogate SPAD measurements) has been proposed as a reliable indicator for screening barley genotypes for drought tolerance [67–69]. According to [70], high chlorophyll content indicates a low degree of photo-inhibition of the photosynthetic apparatus, therefore reducing carbohydrate losses for grain growth. It has also been associated with better nitrogen status of the plant, which may indicate better N uptake efficiency. In this study chlorophyll content, expressed as SPAD readings, had a higher average overall under drought than under control conditions, with SBCC073 consistently showing the highest value at both conditions. Higher greenness under drought was reported in some introgression lines derived from *H. vulgare* ssp. *spontaneum* [71]. However, those results differed from other studies in which higher SPAD values were reported under control conditions, measured in flag leaves at post-anthesis stage [67]. These differences may be related with the timing and method of measurement. In our case, SPAD readings were done on recently expanded leaves during vegetative growth. The differences found among genotypes seem constitutive, and it is open to question whether they bear any relation with agronomic performance. In general, a relationship between chlorophyll content and agronomic performance has been found post-anthesis. Therefore, our results should be validated at a later stage. In this respect, partial validation is provided by the high intrinsic SPAD values for genotype SBCC073 in a biparental mapping population, in which two QTLs for this trait were found [58].

Chlorophyll fluorescence has been advocated as a selection criterion for grain yield in wheat under drought, given its correlation with whole-plant mortality in response to environmental stresses [72]. We found no difference between landraces and cultivars under both conditions, as drought consistently increased Fv/Fm for all genotypes. Interestingly, as for chlorophyll content, SBCC073 again showed the highest Fv/Fm at both treatments. A similar situation was observed for barley cultivar Arta under a combination of heat and drought stress [69]. A study with a mapping population [67] also found differences for Fv/Fm between genotypes under drought conditions, and a correlation between chlorophyll fluorescence parameters and chlorophyll content, which was partially based on two common QTL. Finally, a study on tolerant and sensitive barley genotypes found an effect of drought on quantum yield, regardless of the growth stage [73]. This possible link between chlorophyll content and fluorescence could be explored in our plant materials, in populations already developed using some of the current genotypes as parents.

### 4.5. Perspectives

This kind of experiment under controlled conditions may not be directly predictive of effects in specific field conditions. However, it reveals differential responses that could hardly be measured in the field and helps to put the focus on specific plant features for further investigation.

This set of genotypes displayed root plasticity induced by drought. This behavior should be further investigated, and taken into account when breeding for drought tolerance based on root traits.

The different patterns of early growth shown by SBCC073 and SBCC146 should be explored further, to test agronomic implications. It is worth noting that SBCC073 was the highest yielding genotype at low yielding sites (<3 t ha$^{-1}$) in a series of field trials involving the 159 landraces of the SBCC and 25 old and new cultivars [9]. It was not as productive in trials averaging above 3 t ha$^{-1}$. It would be sensible to study and quantify whether the root-favoring dry matter partitioning system of SBCC073 provides an advantage at dry sites and, conversely, whether it induces a yield penalty at

less arid sites. As a result of this study, a population derived from the cross between these two most divergent genotypes in early partitioning is under development and will be studied in the near future.

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