DIVERSITY FOR SEEDLING VIGOR IN WILD BARLEY (HORDEUM VULGARE L. SUBSP. SPONTANEUM) GERmplasm

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Abstract

Seedling vigor is important for improving stand establishment of barley crops, particularly in arid regions and areas where the soil temperature is low at sowing time. Three hundred and fifteen wild barley accessions from the Wild Barley Diversity Collection (WBDC) were evaluated for nine seedling vigor traits in a polyhouse and growth chamber under hydroponic conditions. The accessions exhibited significant differences for all traits investigated. Traits showing greatest phenotypic variation were seedling visual score, plant height, shoot fresh weight, shoot dry weight and shoot length. Seed weight exhibited the least variation. Seed weight was significantly correlated with visual seedling score and shoot and seedling fresh and dry weight. Correlation analysis showed that the visual seedling score was a reliable method for estimating seedling vigor in wild barley. The first three principal components (PC) explained 82.3% of the variation present in the WBDC with PC1 (54.0%) associated with shoot fresh weight, shoot dry weight, seedling dry weight, seedling fresh weight, shoot length and seedling length. Accessions from the southwest portion of the Fertile Crescent, like WBDC020 (Turkey), WBDC238 (Jordan) and WBDC244 (Jordan) exhibited the highest positive values for most of the plant vigor traits investigated. These wild barley accessions likely carry alleles that will be useful for the improvement of plant vigor traits in cultivated barley.

Introduction

Barley (Hordeum vulgare L.) is an important crop often grown in areas with low rainfall where other crops such as wheat fail to grow (Whabi & Gregory, 1989). Barley exhibits some genetic variation in early vigor (Grando & Cecarelli, 1995); however, due to decades of intensive breeding, the genetic background of cultivated barley is rather narrow. Wild relatives constitute a potential rich source of genetic variation for crop improvement (Feldman & Sears, 1981). Despite the fact that their overall performance is agronomically less desirable, favorable alleles are present in wild relatives (Ellis et al., 2000). Wild barley, Hordeum vulgare L. ssp. spontaneum Koch., is the progenitor of cultivated barley, H. vulgare L. and is widespread across the Near East Fertile Crescent (Zohary, 1969). Wild barley is a diploid, predominantly self-pollinated annual that harbors great genetic variation (Gunaskera et al., 1994; Petersen et al., 1994; Volis et al., 2001). The genetic heterogeneity of wild populations has been recognized for its importance in adaptation to the environment (Volis et al., 2002, 2005, 2010). This richness of genetic diversity in wild barley and its occurrence over a wide range of habitats in the region suggest that the genetic resources of wild barley in the Fertile Crescent and Central Asia can be exploited for improvement of cultivated barley (Jana & Pietrzak, 1988). The diversity of regional landraces, local cultivars and related species can have a beneficial role in crop improvement (Feuillet et al., 2007). Exploiting biodiversity for genetic gain is not a new concept. Many major genes from wild relatives have been transferred into the cultivated gene pools of many crops (Hajjar & Hodgkin, 2007).

The performance of genotypes at germination, heterotrophic growth, and early autotrophic growth is collectively referred to as seedling vigor and early vigor (Revilla et al., 1999; Hund et al., 2004). Early vigor is considered an essential component of plant development under most environmental conditions (Ludlow & Muchow, 1990). The early growth of seedlings is crucial for their establishment and hence for their eventual success in terms of biomass production or seed output. In arid environments, crop varieties with early seedling vigor and good stand establishment tend to maximize use of available soil water, resulting in increased dry matter accumulation and improved grain yield. In temperate climates where lower soil temperature and higher moisture conditions often prevail at the time of planting, early sowing and use of minimum tillage exacerbate germination and seedling growth problems (Keim & Gardner, 1984). Seedling tolerance to low soil temperature is enhanced by vigorous seedling growth (Keim & Gardner, 1984).

Seedling vigor is a major breeding target in barley and other crops (Grando & Cecarelli, 1995; Karrel et al., 1993; Redoña & Mackill 1996a; Trachsel et al., 2010) because it is closely associated with crop growth and yield (Ellis, 1992). Simple traits such as seedling or plant height, seedling dry weight and kernel weight (Acevedo et al., 1991; Regan et al., 1992; Trachsel et al., 2010), rapid shoot growth, shoot dry weight and shoot length...
Williams & Peterson 1973; Sasahara et al., 1986; Grando & Ceccarelli 1995) have been identified as good indicators of a good seedling and early vigor in barley.

The coleoptile or shoot length plays an important role in stand establishment, particularly in rain-fed Mediterranean environments where cereals are often sown when the soil profile is dry. Deep sowing can minimize the risk of germination and subsequent death of the seedlings if drought immediately follows rainfall (Acevedo & Naji, 1987). However, deep sowing may have a negative effect on crop establishment if the coleoptile is not long enough to ensure the emergence of the first leaf.

Seed mass also can cause differences in biomass. Seed mass is more important than the relative growth rate (RGR) in determining early plant biomass in wild barley (Van Rijn et al., 2000). In a study of the major factors responsible for variation in early vigor in barley, wheat, and oat, embryo size was found to be most important (López-Castaneda et al., 1996). Jurado and Westoby (1992) concluded that, among 28 native species from central Australia, seed size is more important than RGR or germination rate in determining seedling size 10 d after imbibition. Cisse & Ejeta (2003) reported that visual seedling scoring is a reliable method for estimating seedling vigor in sorghum. This trait also is important in other crops like maize (Revilla et al., 1999) and rice (Shhapit & Witcombe, 1998).

For genetic improvement of early seedling growth, information on genetic variation in traits related to early seedling vigor and also knowledge concerning the relationships among various seedling vigor traits are necessary. Therefore, the objectives of this study were to (1) quantify the effect of seed weight on early seedling vigor, (2) assess the relationship among the traits, and (3) assess the extent and structure of genetic diversity for these traits in an ecogeographically diverse collection of wild barley.

Materials and methods

**Plant materials:** Three hundred and fifteen wild barley accessions were assembled for the Wild Barley Diversity Collection (WBDC) by Dr. Jan Valkoun (Barley Curator, ICARDA, Aleppo, Syria) and B. Steffenson (University of Minnesota, USA) (Steffenson et al., 2007). The WBDC accessions were selected based on various ecogeographic characters (e.g. longitude/latitude, elevation, high/low temperature, rainfall, soil type) and were from 19 different countries, mostly in the Fertile Crescent (243/315 or 77.1%), but also from Central Asia (50/315 or 15.7%), North Africa (12/315 or 3.8%), and the Caucasus region (10/315 or 3.1%) (Roy et al., 2010).

**Dormancy break:** The wild barley accessions were sown and grown to maturity in a polyhouse at the experimental farm at Chonbuk National University in Jeonju, Korea in 2007-2008. Seeds were harvested and stored at 4°C before analysis. The grains of all 315 accessions were stored at 40°C for one month before sowing and at the time of sowing at 4°C for 72 h to break possible dormancy. Grains of uniform and average size were selected for each accession per replication, and ten grains were used for seedling vigor assessments in the laboratory experiments.

**Polyhouse experiment:** An experiment employing a randomized complete block design with three replications was conducted in 2009-2010 in the polyhouse at Chonbuk National University to evaluate seedling vigor among the 315 accessions. Fertilization and other standard cultural practices recommended for barley cultivation at this location were used. Single row plots, each 1 m long and with 50 cm between rows, were used. Each plot was drill-seeded in the soil. Seeds were planted on 30 October in 2009 within the range of planting dates considered optimal for barley in South Korea. The average minimum and maximum soil temperatures at the 5-cm depth for the growing period were 4.3 and 13.5°C, respectively. The accessions were visually scored for seedling vigor on a scale of 1 (most vigorous) to 9 (least vigorous). These assessments were made at the 3- to 4-leaf stage, when differences in seedling vigor among accessions were the greatest. The visual scoring system is a relative evaluation based on the range of variation observed for seedling size in the collection and attempts to integrate seedling height, length and width of individual leaves. The observation on plant height in cm was taken on five arbitrarily selected plants at the same time.

**Laboratory experiments:** The hydroponic method of Myhill & Konzak (1967) was employed for seedling cultivation. Seeds were sown with the embryo side down between two sheets of wet thick filter paper (thickness 0.75 mm, weight 400 g/m²), Schleicher and Schuell, Dassel, Germany) 6 cm high and 8 cm wide. After rolling up the sheets, the two distal edges were tied up with rubber bands to prevent the seeds from falling out. The resultant seed germination “sandwiches” were held up vertically in a plastic rack placed inside of a plastic box. Thus, ten seeds were located at the top of each filter paper sandwich, with the bottom half of the sandwich immersed in water. The plastic boxes were placed in a growth chamber in darkness at a temperature of 4°C for 72 h and then subjected to a photoperiod of 14 h at 20±2°C. The intensity of lighting was 350 μmol PAR m⁻²s⁻¹. On the 8th day after sowing, five uniform and representative seedlings were selected and measured for seedling length (mm); seedling fresh and dry weight (without seed) (mg); shoot length (mm); and shoot fresh and dry weight (mg). The seedling (without grain) and shoot were placed in individual coin envelopes and dried in an oven at 70°C until a constant weight was achieved. One hundred arbitrarily selected grains per accession were weighed to obtain the individual grains weight.

**Data analysis:** Principal Components Analysis (PCA) was used to reduce the dimensionality, and extract the maximum variance for all the traits. The scores of derived variables calculated for all the accessions were used to visualize the accession seedling vigor in 2- and 3-D PCA scatter plots. Relationships between pairs of measured traits were analyzed by the Pearson product-moment correlation. The PCA and Pearson correlation analyses were performed with SPSS (SPSS Inc., Chicago, Illinois, USA).
Results

The range, mean, standard error (SE) and coefficient of variation (CV) for all traits are given in Table 1 and show high among-accession variation for visual seedling vigor score and the other traits studied. The traits exhibiting the greatest variation were visual score (CV=33.0), plant height (CV=32.9), shoot fresh weight (CV=32.2), shoot dry weight (CV=30.7) and shoot length (CV=29.9). Seed weight exhibited the least variation (CV=16.0). The other traits showed intermediate levels of variation with CVs ranging from 23.4 to 26.8.

Relationship among seedling vigor traits: Highly significant correlations were detected among all the seedling traits (Table 2). However, seed weight showed only weak or no correlation with seedling traits (Table 2). The highest correlation coefficients ($r$=0.76-0.94) were observed in pairwise comparisons with seedling fresh weight, seedling dry weight, shoot fresh weight, shoot dry weight, and shoot length. Visual seedling score was significantly correlated with all the traits (albeit with relatively low $r$ values of 0.10-0.53), except seedling length (Table 2).

Principal component analysis: The first three principal components explained 82.3% of the observed variation. PC1 accounted for 54.0% of variation and showed the largest loading values for shoot fresh weight, shoot dry weight, seedling dry weight, seedling fresh weight, shoot length and seedling length. PC2 and PC3 accounted for 17.1% and 11.1% of the observed variation, respectively. Plant height and visual seedling score contributed the most to PC2 and seed weight to PC3 (Table 3). Accessions WBDC020 (Turkey), WBDC238 (Jordan) and WBDC244 (Jordan) showed the highest seedling vigor among accessions on the basis of all traits combined (Fig. 1). Accessions with the highest positive values for most traits originated from the southwest portion of the Fertile Crescent, primarily in the countries of Jordan, Turkey, Israel, and Syria (Fig. 2). The PCA revealed a noticeable trend, viz. a overlap (despite a certain degree of separation) of accessions from the northern and southern part of the Fertile Crescent (Fig. 3). The accessions from Israel and Jordan were quite well separated from Turkey, Syria, Iran and Iraq on PC2. Among the Fertile Crescent countries, accessions from Lebanon showed the highest overlap between the two sub-regions (Fig. 3).

Table 1. Range of variation, mean, SE and CV for seedling vigor traits of 315 accessions from the Wild Barley Diversity Collection.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>SE</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedling length (mm)</td>
<td>42.00</td>
<td>290.00</td>
<td>157.00</td>
<td>0.534</td>
<td>23.40</td>
</tr>
<tr>
<td>Seedling fresh weight (mg)</td>
<td>3.30</td>
<td>23.10</td>
<td>11.18</td>
<td>0.043</td>
<td>26.81</td>
</tr>
<tr>
<td>Seedling dry weight (mg)</td>
<td>0.2800</td>
<td>1.4400</td>
<td>0.8176</td>
<td>0.002</td>
<td>24.19</td>
</tr>
<tr>
<td>Shoot length (mm)</td>
<td>20.00</td>
<td>111.00</td>
<td>57.84</td>
<td>0.251</td>
<td>29.91</td>
</tr>
<tr>
<td>Shoot fresh weight (mg)</td>
<td>1.10</td>
<td>14.10</td>
<td>5.67</td>
<td>0.026</td>
<td>32.19</td>
</tr>
<tr>
<td>Shoot dry weight (mg)</td>
<td>0.1400</td>
<td>0.9400</td>
<td>0.4296</td>
<td>0.001</td>
<td>30.67</td>
</tr>
<tr>
<td>Visual seedling score</td>
<td>1</td>
<td>9</td>
<td>4.76</td>
<td>0.022</td>
<td>33.00</td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>6.00</td>
<td>43.00</td>
<td>23.27</td>
<td>1.112</td>
<td>32.86</td>
</tr>
<tr>
<td>Seed weight (g)</td>
<td>2.40</td>
<td>7.40</td>
<td>4.90</td>
<td>0.011</td>
<td>16.07</td>
</tr>
</tbody>
</table>

Table 2. Correlation among seedling vigor traits for 315 accessions from the Wild Barley Diversity Collection.

<table>
<thead>
<tr>
<th>Traits</th>
<th>PLANT</th>
<th>VISUAL SEEDLING</th>
<th>SHOOT DRY</th>
<th>SHOOT FRESH</th>
<th>SHOOT LENGTH</th>
<th>SEED WEIGHT</th>
<th>SEEDLING DRY WEIGHT</th>
<th>SEEDLING FRESH WEIGHT</th>
<th>SEEDLING LENGTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual seedling score</td>
<td>0.53***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot dry weight</td>
<td>0.24**</td>
<td>0.21**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot fresh weight</td>
<td>0.23**</td>
<td>0.22**</td>
<td>0.94***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot length</td>
<td>0.23**</td>
<td>0.10**</td>
<td>0.82***</td>
<td>0.84***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed weight</td>
<td>0.09</td>
<td>0.28**</td>
<td>0.14</td>
<td>0.15*</td>
<td>-0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seedling dry weight</td>
<td>0.17**</td>
<td>0.23**</td>
<td>0.84***</td>
<td>0.83***</td>
<td>0.65***</td>
<td>0.22**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seedling fresh weight</td>
<td>0.15**</td>
<td>0.23**</td>
<td>0.76***</td>
<td>0.81***</td>
<td>0.62***</td>
<td>0.23**</td>
<td>0.93***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seedling length</td>
<td>0.04</td>
<td>0.06</td>
<td>0.51**</td>
<td>0.55***</td>
<td>0.63***</td>
<td>-0.00</td>
<td>0.61***</td>
<td>0.68***</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Principal component analysis of seedling vigor traits in the Wild Barley Diversity Collection.

<table>
<thead>
<tr>
<th>Component contribution variable</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedling length</td>
<td>0.76</td>
<td>-0.08</td>
<td>-0.07</td>
</tr>
<tr>
<td>Seedling fresh weight</td>
<td>0.89</td>
<td>0.06</td>
<td>0.26</td>
</tr>
<tr>
<td>Seedling dry weight</td>
<td>0.90</td>
<td>0.10</td>
<td>0.23</td>
</tr>
<tr>
<td>Shoot length</td>
<td>0.86</td>
<td>0.14</td>
<td>-0.21</td>
</tr>
<tr>
<td>Shoot fresh weight</td>
<td>0.92</td>
<td>0.17</td>
<td>0.06</td>
</tr>
<tr>
<td>Shoot dry weight</td>
<td>0.91</td>
<td>0.18</td>
<td>0.05</td>
</tr>
<tr>
<td>Visual seedling score</td>
<td>0.08</td>
<td>0.80</td>
<td>0.30</td>
</tr>
<tr>
<td>Plant height</td>
<td>0.11</td>
<td>0.90</td>
<td>-0.09</td>
</tr>
<tr>
<td>Seed weight</td>
<td>0.06</td>
<td>0.11</td>
<td>0.93</td>
</tr>
<tr>
<td>Total variance explained (%)</td>
<td>54.07</td>
<td>17.11</td>
<td>11.18</td>
</tr>
<tr>
<td>Accumulated variance (%)</td>
<td>54.07</td>
<td>71.18</td>
<td>82.37</td>
</tr>
</tbody>
</table>
Fig. 1. Principal component analysis of seedling vigor traits in the Wild Barley Diversity Collection. Axes are the three principle components, PC1, PC2 and PC3. Key to outlying accessions 20=WBDC020; 238=WBDC238 and 244=WBDC244.

Fig. 2. Principal component analysis of seedling vigor traits in geographical origins (Fertile Crescent, FC; Central Asia, CA; North Africa, NA; and Caucasus region, CR). Axes are the three principle components, PC1, PC2 and PC3.
Fig. 3. Principal component analysis of seedling vigor traits of accessions from the Fertile Crescent. Axes are the two principle components, PC1 and PC2.

Discussion

In this investigation, we observed a wide range of variation among the WBDC accessions for all seedling vigor traits examined. The level of variation observed was considerably higher than previously reported in wild barley. Grando and Ceccarelli (1995) observed low variation for mean seed weight (2.8 g with range of 1.9-3.6 g), while we found a higher mean (4.9 g) and wider range (range 2.4 g – 7.4 g). Similar results also were found for shoot dry weight. The low variation found by the previous investigators may have been due to limited number of accessions used (19) compared to the current study where 315 accessions from 19 different countries were utilized. High variations were also found for seedling vigor traits in sorghum (Cisse & Ejeta, 2003), rice (Cui et al., 2002) and maize (Trachsel et al., 2010).

The visual seedling score was significantly correlated with plant height, seed weight, shoot length and seedling and shoot fresh and dry weight. Significant correlation between seedling vigor scores and plant height as well as kernel weight were reported in corn (Revilla et al., 1999), while vigor scores in rice were significantly correlated with percentage germination, plumule vigor, and greening (Sthapit & Witcombe, 1998) and with seedling height and weight in sorghum (Cisse & Ejeta, 2003). Therefore, the visual seedling score can be a method for estimating seedling vigor in wild barley.

In this study, we found a positive correlation between seed weight and seedling dry weight. Seedling dry weight is known to correlate positively with seed weight (Van Andel & Biere, 1990; Van Rijn et al., 2000; Cui et al., 2002) because at the initial stage of seedling growth, growth is largely dependent on the seed reserve (Yoshida, 1981). Positive correlation between seed and seedling weight was observed in rice (Cui et al., 2002; Lu et al., 2007), but not in sorghum and wheat (Radford & Henzell, 1990; Mian & Nafziger, 1992). Revilla et al., (1999) found maternal effects for kernel weight on early field vigor and plant weight in maize, and recommended that accessions with larger kernel weight be used as the seed parent in breeding to improve early vigor. In maize, kernel weight was moderately correlated with leaf dry weight and shoot dry weight (Trachsel et al., 2010). Therefore, at least in some crops, seed weight can be used as a selection criterion for rapid seedling establishment.

The accessions from the Fertile Crescent (especially from Jordan, Israel, Turkey and Syria) showed good seedling vigor (Fig. 2). Jana and Pietrzak (1988) suggested that genetic resources of wild barley in the Fertile Crescent can be exploited for improvement of cultivated barley. Similarly, Feuillet et al., (2007) suggested the importance of regional landraces, local cultivars and related species as having a beneficial role for crop improvement. Many major genes have been transferred from wild relatives into the many cultivated crops as concrete examples of exploiting biodiversity (Hajjar & Hodgkin, 2007). ICARDA developed a drought tolerant variety by crossing a landrace with a wild barley line from Israel. This new drought-tolerant variety can produce 50% more grain yield than ordinary barley cultivars under dry land conditions (Ashraf, 2010).
Accessions WBDC020 from Turkey and WBDC238 and WBDC244 from Jordan exhibited the highest positive values among the wild barley accessions for most of the seedling vigor traits. These accessions may be useful as parents in breeding programs for seedling vigor in both arid environments and temperate regions. Ludlow & Muchow (1990) suggested that early vigor should be considered an essential component of crop plant development under most environmental conditions. The early growth of seedlings is crucial for their establishment and biomass production. In arid environments, crop varieties with early seedling vigor and good stand establishment tend to maximize use of available soil water, resulting in increased dry matter accumulation and improved grain yield. Keim & Gardiner (1984) reported that seedling tolerance to low temperature is enhanced by vigorous seedling growth.

Conclusions

Early seedling vigor is an important complex trait in barley and other field crops. This is the first study of early seedling vigor traits in a large and ecogeographically diverse collection of wild barley germplasm. Results show a wide variation for all the seedling vigor traits among the accessions. We found that a visual seedling score was a reliable method for estimating seedling vigor in wild barley. Accessions from the Fertile Crescent (especially from Jordan, Israel, Turkey and Syria) possessing good early seedling vigor traits may be useful as valuable genetic resources for the improvement of seedling vigor traits in cultivated barley. In particular, WBDC020 (Turkey), WBDC238 (Jordan) and WBDC244 (Jordan) had among the highest positive values for the most traits and may be useful as parents in transferring positive alleles for multiple seedling vigor traits.

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References


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