Growth, water status and leaf characteristics of Brassica carinata under drought stress and rehydration conditions

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Growth, water status, and leaf characteristics of *Brassica carinata* under drought and rehydration conditions

Azamal Husen · Muhammad Iqbal · Ibrahim M. Aref

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**Abstract** This study has analyzed the response of Ethiopian mustard (*Brassica carinata* A. Braun) to induced drought stress and subsequent rehydration in terms of plant growth, water status, chlorophyll concentration, chlorophyll fluorescence ($F_v/F_m$), net photosynthetic rate ($P_n$), transpiration rate ($E$), and stomatal conductance ($g_s$). Potted plants were subjected to drought stress for 15 days and then to a daily irrigation for 5 days with 100 \% field capacity (FC), and then extent of possible loss due to stress and gain due to rehydration was analyzed. The control population was maintained on normal watering schedule with 100 \% FC. Drought stress reduced the growth rate of root and shoot, number of leaves, dimensions (width, length, and area) of leaves, and the biomass accumulation in different plant parts. With increase in drought stress, the relative water content, chlorophyll content, $F_v/F_m$, $P_n$, $E$, and $g_s$ were reduced. After rehydration, a complete or partial recovery was seen for all the parameters studied. On the whole, *B. carinata* employs a morpho-physiological drought-avoidance strategy.

**Keywords** *Brassica carinata* · Chlorophyll fluorescence · Drought stress · Dry mass · Relative water content · Stomatal conductance

**Introduction**

Developing countries in the tropical and sub-tropical Asia and Africa, characterized with a high growth rate of human population, are at a risk of food shortage due to abiotic factors (Lobell et al. 2008; Ortiz et al. 2008; Wang and Frei 2011). Abiotic stresses (drought, salinity, extreme temperature, chemical toxicity, and oxidative stress), reducing the average yields of most of the major crop plants by over 50 \% (Bray et al. 2000), are the primary cause of the worldwide crop loss, and pose a major threat to global food security in the twenty-first century (Battisti and Naylor 2009). Moreover, the global climate change will likely add to water scarcity making it a greater limitation for plant productivity over a vast area of land. Extreme weather conditions, particularly in terms of temperature and precipitation, may render fluctuations in water availability more common, and thus pave the way of frequent drought episodes (Saxe et al. 2001). Ethiopia, Eritrea, Djibouti, and Somalia represent the horn of Africa; where the available land is drought-prone (Ezra 2001). Water deficit disturbs various physiological and biochemical traits and has adverse bearing on plant growth and productivity (Bartels and Sunkar 2005; Anjum et al. 2008; Husen 2010; Aref et al. 2013; Lipiec et al. 2013). Stomatal functioning, including the stomatal response to soil water potential and fluctuating environmental conditions, provides/maintains leaf turgor under drought stress. Photosynthesis inhibition due to reduced CO$_2$ diffusion and mesophyll conductance, in consequence of stomatal closure, is a primary
physiological impact of drought (Grassi and Magnani 2005; Du et al. 2010). Plants protect their cellular and subcellular systems, including the photosynthetic apparatus, from cytotoxic effects of active oxygen radicals with the help of antioxidant enzymes (Anjum et al. 2008, 2012). The mechanism adopted by plants to withstand water stress often induces accumulation of osmolyte compounds, such as inorganic ions (especially K⁺), sugars, and amino acids (e.g., proline) (Elsheery and Cao 2008). Chlorophyll fluorescence (the maximum quantum yield \( F_\text{v}/F_\text{M} \)) has been used as a diagnostic tool in the studies of environmental stress (Ashraf et al. 2004; Yin et al. 2005), genotypic variation (Janssen et al. 1995), altitudinal variation (Husen et al. 2004a), and species-specific diurnal changes (Husen et al. 2004b), and thus forms a significant character to determine the seedling-stock quality (Kauser et al. 2006; Husen 2009, 2013).

Ethiopian mustard (Brassica carinata A. Braun) is a suitable crop for semi-arid climatic regions. Flowering of vegetable cultivars (when leaves/shoots are eaten as vegetable) is delayed by regular harvesting of leaves or young shoots. Time of flowering often depends on the cultivar and the environmental conditions. If grown in dry regions, it flowers earlier and produces ripe seeds within 4 months from sowing. Plants grown with adequate moisture produce seeds in 5–6 months. It is the third most important oil crop, next to noug (Guizotia abyssinica) and linseed (Linum usitatissimum), in the highlands of Ethiopia (CSA 2003). Compared with other oil crops occupying the same ecological niche in Ethiopia, it gives the highest yield (Hiruy et al. 1983). Its leaves are eaten and cooked as vegetable, while seeds are used to oil the baking plate for making ‘Injera’, a popular local food item. Because of high erucic acid content of seeds, it is used in numerous non-food applications such as biodiesel, biopolymers, lubricants, soaps, and surfactants (Velasco et al. 2003). Under Ethiopian climatic conditions, this plant is usually sown with maize in April, preferentially after the first rain. Occasionally, sowing is delayed for the absence of rain, because water deficit during early growth stages leads to wilting and finally death of plants. Brassica species and their cultivars give their best under normal soil and environmental conditions; their growth, seed yield, and oil production are markedly reduced under stressful conditions such as drought, water logging, salinity, extreme temperature, ultraviolet-B radiation, and nutrient deficiency or excess, etc. (Ashraf and McNeill 2004; Ghobadi et al. 2006; Siddiqui et al. 2008; Sangtarash et al. 2009; Kumar et al. 2009; Pace and Benincasa 2010; Benincasa et al. 2013) Cultivars of B. juncea and B. napus facing water stress at earlier growth stages exhibited recovery, but the stress during pod development severely reduced most of the yield components (Gan et al. 2004). Water stress accelerates the process of flower and fruit drop and decreases seed yield (Gan et al. 2004; Sinaki et al. 2007). It decreases chlorophyll contents and increases proline accumulation, as in canola plants (Gibon et al. 2000; Din et al. 2011). Water deficiency is also associated to high salinity levels in the soil, and salinity amplifies the effect of low water potential (Ashraf and McNeil 2004). The adverse effects of moisture stress on water status, photosynthesis, and growth of Brassica species were mitigated by elevated CO₂ level in the atmosphere. B. carinata was found by Uprety et al. (1995) to be less sensitive to moisture stress than B. campestris and B. nigra etc., but Ashraf and Mehmoood (Ashraf and Mehmoood 1990) regarded it to be the most sensitive to drought stress, possibly due to osmotic adjustment. In another study, the changes in stomatal conductance (\( g_s \)) or chlorophyll content could not explain fully the reduction in net photosynthetic rate (\( P_n \)) induced by moisture stress in B. carinata hybrids and their parents (Voleti and Uprety 1997). A positive relationship between \( P_n \) and seed yield suggests that \( P_n \) can be used as an important tool for the selection of new strains with high seed yield (Pan et al. 2011).

Based on the preliminary observations, one can assume that drought stress should alter various growth parameters and leaf characteristics of B. carinata, but the level of assumed recovery on termination of drought stress and restoration of water-sufficient condition may not be predicted. Therefore, this study was carried out to examine the effect of drought stress and rehydration on B. carinata in terms of plant growth and leaf characteristics such as water status, chlorophyll concentration, \( F_\text{v}/F_\text{M}, P_n, \) transpiration rate (\( E \)), and \( g_s \) of B. carinata in order to determine its level of tolerance to water deficit condition.

**Materials and methods**

**Experimental site**

The experiments were conducted in Botanical Sciences Research Laboratory (Department of Biology) at Tewodros campus of the University of Gondar located at \( 12^\circ35'14.19''\text{N}, 37^\circ26'29.53''\text{E} \) at 2,143 m above mean sea level. Annual average of the maximum temperature at Gondar lies around 27 °C, while that of the minimum temperature around 16 °C. March, April, and May are the hottest months, with an average maximum temperature of 29 °C. The average annual precipitation in Gondar lies about 1,161 mm and the annual average of relative humidity (RH) is about 56 %. On a monthly scale, the lowest (40 %) RH occurs in January and February and the highest (79 %) in July. During the entire experimental
period, RH was 50 %, the maximum and the minimum daily temperature was recorded 29 ± 1 and 18 ± 1 °C, respectively, and no rainfall was observed.

Plant material

Seeds of *B. carinata* cultivar ‘Merawi’ obtained from Gondar Agricultural Research Centre, Gondar, Ethiopia, were surface sterilized with 0.2 % HgCl₂ solution, with frequent shaking for 5 min, rinsed many times with distilled water and then transferred for germination to plastic trays containing 50 % sand soil and 50 % clay and being watered regularly. 15-day-old seedlings were transferred separately to plastic pots (8 cm width × 16 cm height) filled with 75 % sandy loam (1.5 kg) and 25 % compost (FYM) (500 g) and irrigated daily with tap water for 10 days, supposedly a period of plant acclimatization. Sandy loam (62.56 % sand, 14.88 % clay, and 22.56 % silt), with pH 7.32, and EC 0.69 ms/cm was used.

Drought stress treatments

All seedlings were irrigated to 100 % field capacity (FC) before the commencement of the drought treatment. Of a total of 200 potted seedlings, 50 % were grown under drought stress, while the remaining 50 % were allowed to grow under normal watering schedule (at 100 % FC). Drought stress was created for a period of 15 days by watering the plants at 5-day interval (on day 0, 5, 10, and 15 of the experiment). Thereafter, regular watering was performed for 5 days with 100 % FC (from 15 to 20th day), thus creating a stress-relief phase (Fig. 1). The first sampling of 15 seedlings was done just before starting the drought treatment. In each of the subsequent samplings (on the 5th, 10th, 15th, and 20th day of the drought treatment), 15 plants from the stressed lot and 15 from the control were collected and analyzed for parameters mentioned below. Thus in five collections, a total of 75 control plants, 45 drought-affected plants, and 15 rehydrated plants were measured, using leaf area meter (AM 300, ADC Bio Scientific Limited, UK). Five replications were taken for each parameter.

Leaf water status

Water status of leaf was determined in the control and drought-stressed plants on days 0, 5, 10, 15, and 20 of the experiment, by measuring the relative water content (RWC) in fully developed leaves (obtained from middle portion of plant). Normal watering schedule was maintained till the 20th day in the control plants, whereas the drought-affected ones were rehydrated with 100 % FC from 15 to 20th day. Leaf samples were weighed immediately after harvesting, to obtain fresh weight, and then placed overnight in distilled water at 5 °C in the dark, before obtaining their turgid weight (TW). The material was then oven-dried at 80 °C for 24 h, and dry weight (DW) obtained. The relative water content was calculated as

\[
RWC = \left\{ \frac{(FW - DW)}{(TW - DW)} \right\} \times 100.
\]

Chlorophyll concentration

Chlorophyll concentrations in leaves were analyzed on each sampling day. Following the method of Hiscox and Israelstam (1979), test tubes containing 0.5 g of green-leaf tissue in 10 ml of dimethyl sulfoxide (DMSO) were kept in oven at 65 °C for 2 h. Optical density (OD) of the extract
<table>
<thead>
<tr>
<th>Growth and leaf parameters</th>
<th>Day 0 Mean ± SE</th>
<th>Day 5 Mean ± SE</th>
<th>Day 10 Mean ± SE</th>
<th>Day 15 Mean ± SE</th>
<th>Day 20* Mean ± SE</th>
<th>% Variation (day 0 vs day 15)</th>
<th>% Variation (day 0 vs day 20)</th>
<th>% Variation (day 15 vs day 20)</th>
<th>% Variation (C vs RHD on day 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root length (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.00 ± 0.08d</td>
<td>3.90 ± 0.45e</td>
<td>4.10 ± 0.52b</td>
<td>5.21 ± 0.18a</td>
<td>6.11 ± 0.15a</td>
<td>73</td>
<td>103</td>
<td>17</td>
<td>30</td>
</tr>
<tr>
<td>Treated</td>
<td>3.10 ± 0.75e</td>
<td>3.13 ± 0.06d</td>
<td>3.18 ± 0.08c</td>
<td>4.25 ± 0.07b</td>
<td>6</td>
<td>41</td>
<td>33</td>
<td></td>
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<tr>
<td>Shoot length (cm)</td>
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<tr>
<td>Control</td>
<td>2.88 ± 0.05d</td>
<td>3.12 ± 0.36e</td>
<td>4.78 ± 0.15b</td>
<td>5.10 ± 0.14a</td>
<td>5.44 ± 0.28a</td>
<td>77</td>
<td>88</td>
<td>6</td>
<td>33</td>
</tr>
<tr>
<td>Treated</td>
<td>2.90 ± 0.06d</td>
<td>3.08 ± 0.05c</td>
<td>3.15 ± 0.10c</td>
<td>3.60 ± 0.09b</td>
<td>9</td>
<td>25</td>
<td>14</td>
<td></td>
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<tr>
<td>Number of leaves</td>
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<td></td>
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<tr>
<td>Control</td>
<td>3.50 ± 0.78f</td>
<td>4.11 ± 0.82d</td>
<td>5.34 ± 0.34c</td>
<td>7.22 ± 0.22b</td>
<td>8.74 ± 0.34a</td>
<td>109</td>
<td>149</td>
<td>21</td>
<td>45</td>
</tr>
<tr>
<td>Treated</td>
<td>3.76 ± 0.05f</td>
<td>3.93 ± 0.07d</td>
<td>4.05 ± 0.06d</td>
<td>4.73 ± 0.75e</td>
<td>15</td>
<td>35</td>
<td>16</td>
<td></td>
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<tr>
<td>Basal Diameter (mm)</td>
<td></td>
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<td></td>
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<tr>
<td>Control</td>
<td>0.30 ± 0.07c</td>
<td>0.34 ± 0.02d</td>
<td>0.41 ± 0.03c</td>
<td>0.47 ± 0.08b</td>
<td>0.52 ± 0.04a</td>
<td>56</td>
<td>73</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>Treated</td>
<td>0.31 ± 0.05e</td>
<td>0.32 ± 0.06c</td>
<td>0.34 ± 0.04d</td>
<td>0.40 ± 0.02c</td>
<td>13</td>
<td>33</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf area (mm²)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>710.00 ± 4.16e</td>
<td>1234.43 ± 3.21c</td>
<td>1456.82 ± 4.37c</td>
<td>1878.59 ± 7.23b</td>
<td>2412.31 ± 6.31a</td>
<td>164</td>
<td>239</td>
<td>28</td>
<td>17</td>
</tr>
<tr>
<td>Treated</td>
<td>1129.14 ± 5.83d</td>
<td>1196.00 ± 8.59d</td>
<td>1323.00 ± 6.30d</td>
<td>1988.00 ± 5.56b</td>
<td>86</td>
<td>180</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf width (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>26.95 ± 1.12d</td>
<td>38.43 ± 1.18d</td>
<td>47.67 ± 2.22d</td>
<td>55.34 ± 2.10b</td>
<td>72.45 ± 4.71a</td>
<td>105</td>
<td>168</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>Treated</td>
<td>32.00 ± 2.01e</td>
<td>34.65 ± 1.18d</td>
<td>37.40 ± 3.34d</td>
<td>46.75 ± 2.11c</td>
<td>38</td>
<td>73</td>
<td>25</td>
<td></td>
<td></td>
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<tr>
<td>Leaf length (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>39.25 ± 2.29d</td>
<td>48.92 ± 3.12d</td>
<td>65.23 ± 3.56d</td>
<td>70.12 ± 4.87b</td>
<td>80.23 ± 3.22a</td>
<td>78</td>
<td>104</td>
<td>14</td>
<td>23</td>
</tr>
<tr>
<td>Treated</td>
<td>44.60 ± 1.24d</td>
<td>48.60 ± 1.23d</td>
<td>50.05 ± 1.29c</td>
<td>61.50 ± 2.20c</td>
<td>27</td>
<td>56</td>
<td>22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The controls were maintained with daily watering (100 % FC), while the stressed seedlings were watered on the 5th, 10th, and 15th day of experiment. Observations on the 20th day (after five days of daily watering—100 % FC) exhibit the subsequent recovery. Values followed by the same letter indicate no significant differences at $P < 0.05$ level according to the Tukey’s test. Each value represents the mean ± SE of five replicates. C Control and RHD Rehydrated plants on the 20th day.
from each test tube was then read at 645 and 663 nm, using a T60 UV–Vis spectrophotometer (PG Instruments Limited, England). The contents of chlorophyll \( a \), chlorophyll \( b \), and total chlorophyll were estimated using the formulae given by Duxbury and Yentsch (1956) and MacLachlan and Zalick (1963), respectively.

### Physiological measurements

Leaf gas exchange was measured between 10 and 11 a.m. on each sampling day. Stomatal conductance \((g_s)\), net photosynthetic rate \((A)\), and transpiration rate \((E)\) were measured using a portable leaf gas exchange system (ADC Bio Scientific Limited, UK) on fully expanded attached leaves. Chlorophyll fluorescence of leaves was also recorded for each treatment with the help of portable Multi-Mode OS5p Chlorophyll Fluorometer (Opti-Sciences, Inc., USA). Prior to fluorescence measurements, the upper surface of leaf was pre-darkened with leaf clips for 30 min to ensure complete relaxation of all reaction centers. The basal non-variable chlorophyll fluorescence \((F_0)\), the maximal fluorescence induction \((F_M)\), and variable fluorescence \((F_V)\) were determined. The maximum quantum yield \((F_V/F_M)\) was also calculated.

### Experimental design and statistical analysis

A completely randomized design was used for this experimentation. Five replications, each of three plants, were prepared from each sample of the treated and control plants for further analysis. Using version 13.0 of Statistical Package for Social Sciences (SPSS) software (SPSS Inc., Illinois, USA), data were subjected to one-way analysis of variance (ANOVA) to determine the significant difference among drought treatments. Means were compared using the Tukey’s test at significance level \(P \leq 0.05\) (values marked with the same letter within a row or column are not significantly different at \(P > 0.05\) level).

### Results

**Plant growth and leaf characteristics**

Data showing the effects of drought stress and subsequent rehydration on plant growth and leaf characteristics are presented in Table 1. Compared to their growth level on day 0 of the experiment, the controls attained 73 % of their root length, 77 % of shoot length, 109 % of number of leaves, 56 % of shoot basal diameter, 164 % of leaf area, 105 % of leaf width, and 78 % of leaf length by the 15th day of experiment. The root length, shoot length, and leaf dimensions decreased progressively in stressed plants with

### Table 1

<table>
<thead>
<tr>
<th>Characteristics of Brassica carinata</th>
<th>Day 0</th>
<th>Day 5</th>
<th>Day 10</th>
<th>Day 15</th>
<th>Day 20</th>
<th>Day 20*</th>
<th>% Variation</th>
<th>% Variation</th>
<th>% Variation</th>
<th>% Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry mass (g) leaves</td>
<td>0.0440 ± 0.007d</td>
<td>0.0480 ± 0.006c</td>
<td>0.0490 ± 0.008b</td>
<td>0.0500 ± 0.002a</td>
<td>0.0530 ± 0.003a</td>
<td>13</td>
<td>20</td>
<td>6</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
<td>0.0480 ± 0.007d</td>
<td>0.0490 ± 0.008b</td>
<td>0.0500 ± 0.002a</td>
<td>0.0510 ± 0.001a</td>
<td>0.0550 ± 0.004a</td>
<td>15</td>
<td>20</td>
<td>6</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td>0.0080 ± 0.001c</td>
<td>0.0080 ± 0.001c</td>
<td>0.0080 ± 0.001c</td>
<td>0.0080 ± 0.001c</td>
<td>0.0080 ± 0.001c</td>
<td>10</td>
<td>12</td>
<td>5</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Total plant weight</td>
<td>0.1000 ± 0.018</td>
<td>0.1050 ± 0.016</td>
<td>0.1100 ± 0.014</td>
<td>0.1170 ± 0.014</td>
<td>0.1240 ± 0.014</td>
<td>15</td>
<td>20</td>
<td>6</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

The controls were maintained with daily watering (100 % FC), while the stressed seedlings were watered on the 5th, 10th, and 15th day of the experiment. Observations on the 20th day (after five days of daily watering—100 % FC) exhibit the subsequent recovery. Values followed by the same letter indicate no significant differences at \(P > 0.05\) level according to the Tukey’s test.

Each value represents the mean ± SE of five replicates.

C Control and RHD Rehydrated plants on the 20th day.
increasing number of days since the commencement of drought. Drought stress imposition for 15 days caused a significant reduction of root length (6%), shoot length (9%), number of leaves (15%), shoot basal diameter (13%), and the area (86%), width (38%), and length (27%) of leaf in comparison with the control plants that received regular watering for 15 days. After 5 days of rehydration, the stressed plants exhibited significant partial recovery, as observed on the 20th day. They gained about 41% in root length, 25% in shoot length, 35% in number of leaves, 33% in shoot basal diameter, 180% in leaf area, 73% in leaf width, and 56% in leaf length, compared with the level of these features on the 15th day of experiment, i.e., under the maximum stress applied. When compared with the control, this variation on the 20th day was 30, 33, 45, 23, 17, 35, and 23% for root length, shoot length, number of leaves, shoot basal diameter, leaf area, leaf width, and leaf length, respectively, in the stressed plants (Table 1).

**Biomass**

In the control plants, 15 days of regular watering increased the leaf DM by 13%, shoot DM by 6%, root DM by 10%, and total DM by 15% over the status of day 0 of the exposure. Drought stress significantly affected dry mass (DM) of leaves, stems, roots, and the whole plant with reference to control (Table 2). Imposition of drought stress for 15 days caused significant reduction in leaf DM (15%), shoot DM (10%), roots DM (14%), and the total DM (16%) of the plant. After rehydration, the stressed plants exhibited little recovery on the 20th day; and the difference between the DM of leaves, shoot, roots, and the whole plant obtained on the 15th and the 20th day was 16, 12, 9, and 14% respectively. However, on the 20th day, variation between the control and the stressed plants was 18, 16, 12, and 17% for the leaf, shoot, root, and whole plant DM, respectively.

**Relative water content (RWC)**

Relative water content (RWC) on day 0 and day 15 was insignificant in both the samples. With reference to the control, RWC in the leaves of stressed plants progressively decreased with increase in the number of drought days (Fig. 2). Drought stress for 15 days reduced RWC significantly; as it was 76% on day 0 and 60% on day 15 of the experiment. After rehydration, the stressed plants showed a perfect recovery of leaf water status, and the difference between RWC values for days 0 and 20 was only 0.88%. In addition, very little variation (6%) was recorded between the control and treated plants on the 20th day.

**Photosynthetic pigments**

In control plants, regular watering for 15 days caused a 3, 6, and 4% improvement in the chlorophyll $a$, chlorophyll $b$, and total chlorophyll contents, respectively. On the contrary, 15-day exposure to drought stress markedly affected these variables (Table 3), leading to a 28, 54, and 39% decline in the three chlorophyll contents, respectively. After rehydration, the stressed plants recovered in chlorophyll contents almost fully. The difference between values of chlorophyll $a$, chlorophyll $b$, and total chlorophyll obtained on the 0th and the 20th day was 1.47, 1.44, and 1.46%, respectively. Besides, the difference between controls and the treated plants in values of chlorophyll $a$, chlorophyll $b$, and total chlorophyll, as recorded on the 20th day, was 5, 7, and 6%, respectively.

**Physiological parameters**

Chlorophyll fluorescence ($F_{v}/F_{m}$) in the control plants with 15 day regular watering exhibited insignificant (only 0.85%) variation. However, it varied significantly with
Characteristics of *Brassica carinata*

<table>
<thead>
<tr>
<th>Photosynthetic pigments of <em>Brassica carinata</em> seedlings as obtained on days 0, 5, 10, 15, and 20 of the drought stress experiment</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>Day 5</td>
<td>Day 10</td>
<td>Day 15</td>
<td>Day 20</td>
<td>% Variation (day 0 vs day20)</td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>Control</td>
<td>6.80 ± 0.18a</td>
<td>6.67 ± 0.16a</td>
<td>6.68 ± 0.14ab</td>
<td>5.12 ± 0.30a</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>6.85 ± 0.16b</td>
<td>7.01 ± 0.21a</td>
<td>6.88 ± 0.16a</td>
<td>5.12 ± 0.22b</td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>Control</td>
<td>4.85 ± 0.16b</td>
<td>4.35 ± 0.14b</td>
<td>4.24 ± 0.13b</td>
<td>4.21 ± 0.13b</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>4.88 ± 0.16b</td>
<td>4.35 ± 0.14b</td>
<td>4.24 ± 0.13b</td>
<td>4.18 ± 0.14a</td>
</tr>
<tr>
<td>Total chlorophyll</td>
<td>Control</td>
<td>11.65 ± 0.36b</td>
<td>11.12 ± 0.14b</td>
<td>10.82 ± 0.16b</td>
<td>8.20 ± 0.16b</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>11.12 ± 0.14b</td>
<td>10.82 ± 0.16b</td>
<td>8.20 ± 0.16b</td>
<td>7.28 0.58 7</td>
</tr>
</tbody>
</table>

The controls were maintained with daily watering (100 % FC), while the stressed seedlings were watered on the 5th, 10th and 15th day of experiment. Observations on the 20th day (after five days of daily watering — 100 % FC) exhibit the subsequent recovery. Values followed by the same letter indicate no significant differences at *P* < 0.05 level according to the Tukey’s test. Each value represents the mean ± SE of five replicates.

Discussion

The present study reveals that drought stress adversely affects the vegetative growth of *B. carinata* cultivar ‘Merawi’ seedlings, as is evident from the decreased length of root and shoot, leaf size, and the dry mass of different plant parts in comparison with controls. Some other *Brassica* species and cultivars have also shown reduction in growth rate (Ashraf and Mehmood 1990; Gan et al. 2004; Kauser et al. 2006; Issararakisila et al. 2007) and dry matter production (Siddiqui et al. 2008; Hosseini and Hassibi 2011) under water deficit condition. The shoot/root ratio in *Brassica* normally declines under water and salt stress (Maggio et al. 2004; Badrudin et al. 2005; Jamil et al. 2005; Pace and Benincasa 2010). The reverse (increased shoot/root ratio) has also been reported, (Jamil et al. 2006), in conflict with most of the studies showing a marked reduction of this ratio in *Brassica* species grown at low osmotic potentials (Maggio et al. 2004; Kauser et al. 2006). In a study on *B. napus*, Sangtarash et al. (2009) held that under water stress decreased dry matter and photosynthetic rate are due to biochemical limitations.

Plant responses to water-deficient soil are based on hydraulic signaling and often include a decline in water uptake by roots, the turgor, and water potential of leaves and the osmotic adjustment (Clark et al. 2005). Roots are
the first part to be exposed to water stress; chemical signals produced from roots are transferred through the stem to leaves, which can reduce stomatal conductance (Schachtman and Goodger 2008). The reduced stomatal conductance is often the prime cause of reduced photosynthesis under water stress, as found with two *B. napus* cultivars (Cyclone and Dunkeld) (Kauser et al. 2006), leading to a reduced biomass accumulation in this investigation. Smaller leaf area is another cause for reduced yield via limited photosynthesis. However, the reduced rate of leaf expansion under stressful condition, as recorded for *B. carinata*, may be beneficial to plant under drought stress, as it reduces the area for transpiration (Mahajan and Tuteja 2005).

Since plant–water relations play a key role in the activation and/or modulation of antioxidant defense mechanism during drought conditions, leaf RWC is considered as a reliable indicator of the level of tissue hydration (Rojas-Serna et al. 2004). Water deficit often reduces RWC of plants (Husen 2010; Aref et al. 2013), as in the present study. Interestingly, however, rehydration for 5 days led to a quick and complete recovery, showing that *B. carinata* needs little time for RWC restoration on withdrawal of drought stress, which possibly helps in acquiring resistance.

Drought-induced reductions in chlorophyll contents are quite common in *Brassica* species and cultivars (Gibon et al. 2000; Kauser et al. 2006; Din et al. 2011). In the present investigation, the loss of chlorophyll content in *B. carinata* was significant only after 15-day exposure to drought stress, and a complete recovery was achieved on a 5-day rehydration. Although chlorophyll loss is a negative consequence of stress, it is also considered as an adaptive feature, which reduces light harvesting and hence the possibility of further damage to the photosynthetic machinery by activated oxygen radicals under excess of excitation energy (Munné-Bosch and Alegre 2000; Kranmer et al. 2002).

Stomatal conductance (*g*), net photosynthetic rate (*Pn*), and transpiration rate (*E*) decreased significantly under drought stress. This is in line with earlier studies on various *Brassica* species (Voleti and Uprety 1997; Kauser et al. 2006; Pan et al. 2011). Reductions in the photosynthetic activity were first triggered by stomatal closure, which limited the ambient CO₂ diffusion to the mesophyll and thus reduced the photosynthesis (Galmés et al. 2007). The phenomena of *g*, *Pn*, and *E* seemed to recover partially after 5 days of rehydration, as the process of recovery continued till the last day. *B. carinata* seems to require a longer rehydration period for complete recovery, possibly due to reduced stomatal and mesophyll conductance and to biochemical limitations (Galmés et al. 2007).

Chlorophyll fluorescence (*Fv/Fm*) is a useful tool for quantifying the effect of abiotic stress on photosynthesis (Tezara et al. 2005; Kauser et al. 2006; Husen 2010), particularly for estimating the degree of photo-inhibition (Tezara et al. 2005). In the present study, the *Fv/Fm* value ranged between 0.83 and 0.66 in the stressed plants. Decrease in *Fv/Fm* was significant only after 15 days of plant exposure to drought stress. Complete recovery of *Fv/Fm* ratio in *B. carinata*, after 5 days of rehydration, indicates that no photo damage was done to PS-II reaction centers. Since a decrease in *Fv/Fm* under drought stress is quite common in *B. napus* (Kauser et al. 2006), it may be interpreted as a photo-acclimation process (Abreu and Munné-Bosch 2008). It has been also reported that water stress-induced reduction in photosynthesis may be due to stomatal limitations, metabolic limitations, altered chlorophyll fluorescence, or combination of these factors (Athar and Ashraf 2005).

In conclusion, prolonged drought condition hampered the vegetative growth of *B. carinata* by affecting the dry
mass production, water status, chlorophyll contents, gaseous exchange, and photochemical efficiency in a dose-dependent manner. Quick recovery on rehydration indicated the ability of the species to cope up with environmental harshness and thrive under drought stress.

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