Response of Chinese sea buckthorn clonal growth and photosynthetic physiological mechanisms toward a soil moisture gradient

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Studies have reported on the regulation of clonal growth in Chinese sea buckthorn in response to environmental resource availability, but these studies have been limited to external mechanisms. In this report, we controlled irrigation to generate a soil moisture gradient in order to examine the photosynthetic physiological mechanisms regulating clonal growth in this species. The results indicated that as irrigation intensity increased, the soil water content increased vertically and tissue water content first increased and then decreased. Furthermore, Rubisco activase (RCA) and Mg-chelatase H subunit (CHLH) gene expression levels, photosynthetic capacity (net photosynthetic rate, transpiration rate, chlorophyll content, and stomatal conductance), and clonal growth (ramet growth, clonal proliferation, clonal propagation) all showed a quadratic parabolic change (i.e., first increasing and then decreasing). In addition, gene expression levels and tissue water content, photosynthetic capacity and gene expression levels, and clonal growth and photosynthetic capacity were all significantly positively correlated. When irrigation intensity (soil water content) is exceedingly low or high, the tissue water content is also low, RCA and CHLH gene expression levels are low, photosynthetic capacity is weak, clonal growth ability is inhibited, and clonal growth layout tends toward the “guerrilla type.” This type manifests as fewer and smaller clonal daughter ramets that are sparsely distributed with reduced clonal organ extension ability and branching intensity. When irrigation intensity (soil water content) is moderate, the tissue water content, gene expression levels, and photosynthetic capacity is high, clonal growth ability is completely uninhibited, and the clonal growth layout tends toward the “aggregated type.” This type is associated with numerous large clonal daughter ramets that are densely distributed with high clonal organ extension ability and branching intensity. Therefore, as irrigation intensity continuously changes from inordinately low to moderate to exceedingly high, Chinese sea buckthorn regulates clonal growth by photosynthetic capacity through photosynthetic gene expression. This results in a clonal growth layout continuum of “guerrilla-aggregated-guerrilla” that depends on irrigation intensity.

Keywords: Clonal Growth, Irrigation Intensity, Tissue Water Content, Photosynthetic Genes, RCA and CHLH Gene Expression, Hippophae rhamnoides ssp. sinensis, Mu Us Sandy Land

Introduction
Clonal growth refers to the process of asexual reproduction in plants to produce genetically identical, morphologically and physiologically independent or potentially independent individuals for spatial expansion under natural conditions, such as in stoloniferous or rhizomatous plants (Takahashi et al. 2011, Robledo-Arnuncio et al. 2014). Currently, research on clonal growth regulation is focused on external mechanisms, such as the response of clonal growth to environmental resource availability or heterogeneity and its ecological adaptation significance (He et al. 2009, Tang et al. 2010, Wang et al. 2012, Luo et al. 2013, Yan et al. 2013). However, clonal growth regulation in plants is a complex process, as internal changes are closely associated with clonal growth regulation. Following the removal of top growth in the shortleaf pine (Pinus echinata) and loblolly pine (Pinus taeda), 139 genes were differentially expressed in response to sprouting. The functions of these genes included substance metabolism, stress responses, growth and development, signal transduction, and hormonal regulation (Liu et al. 2011).

Individual morphological variation among populations may originate from genetic changes (Rana & Shirokt 2012). It is evident from this that studying the relationship between internal changes and clonal growth might aid in further understanding the internal mechanisms of regulation, particularly the material and energy basis by which photosynthetic intensity determines clonal growth (Wang et al. 2016). At the same time, plant photosynthesis is regulated by genes. An example is ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39 – Rubisco), which is a key enzyme that participates in the first rate-limiting step of car-
bon assimilation (Jurczuk et al. 2015). The activity status of Rubisco is regulated and controlled by Rubisco activase (RCA). In other words, Rubisco must undergo activation by the RCA gene to elicit its carboxylase and oxygenase activity, thereby increasing photosynthetic efficiency (Parry et al. 2013, Carmo-Silva et al. 2015). Conversely, chlorophylls are major pigments by which photosynthetic plants absorb and transfer light energy, and their biosynthetic route is completed by magnesium chelatases. Among magnesium chelatases, Mg-chelatase H subunit (CHLH) is a functional gene that regulates chlorophyll synthesis (Papenbrock et al. 2000, Pontier et al. 2007, Ren et al. 2011). Therefore, RCA and CHLH play important roles in photosynthesis. This solicits an assessment of the causal relationship between RCA and CHLH gene expression, photosynthetic physiological characteristics, and clonal growth. The Chinese sea buckthorn (Hippophae rhamnoides ssp. sinensis) is an important versatile woody species used for afforestation in arid and semiarid regions in Northern China. It is a classic clonal plant that propagates via suckers, exhibiting extremely strong lateral root horizontal extension and branching abilities, and generating large amounts of sucker plants during propagation. This not only provides vegetation cover for areas that are difficult to afforest but also assists in the expansion of the forest edge and renewal of forest gaps, thereby maintaining population stability and clonal persistence (Li et al. 2001, 2004, 2010a, He et al. 2006, Guo et al. 2015). After the parental plant has died, its clonal daughter ramets can continue to undergo asexual propagation (Li et al. 2001, Takahashi et al. 2011, Robledo-Arnuncio et al. 2014, Guo et al. 2015). Therefore, its clonal attributes have conferred the Chinese sea buckthorn with the potential for forest formation from a single tree and long-living. However, large areas of artificial forests have undergone premature senescence (Li et al. 2005, Hui et al. 2009). The cause of this was determined to be drought stress, which weakens the clonal growth abilities of Chinese sea buckthorn (Li et al. 2010b, He et al. 2012, Zhang et al. 2016, Zeng et al. 2016, Cao et al. 2016). However, studies on the relationship between soil moisture and clonal growth have only explained the clonal growth regulatory mechanisms from an ecological perspective (Li et al. 2010b, He et al. 2012, Zeng et al. 2016, Cao et al. 2016), and the photosynthetic physiological regulatory mechanisms for clonal growth are not well understood. In contrast, research on the photosynthetic physiology of sea buckthorn has focused on the response laws of photosynthetic physiology characteristics to water levels (Li et al. 2002), and there is a lack of analysis on the causal relationship between photosynthetic physiological characteristics and plant growth. In a preliminary research (Cao et al. 2016), the relationship between clonal growth and irrigation intensity was studied with 2-year-old sea buckthorn, and it was found that the optimal irrigation intensity was close to 6 times the local annual precipitation, which is the highest irrigation intensity employed in that experiment. Therefore, the result could not fully describe the response of clonal growth to the irrigation from deficit to surplus. In our study, the irrigation intensity was adjusted to 3, 6, and 9 times the local annual precipitation, and the experiment was carried out for 3 years. Through regulating soil water content and tissue water content by irrigation, we investigated the response of photosynthetic gene expression, photosynthetic physiological characteristics, and clonal growth ability towards a moisture gradient. The causal relationships between photosynthetic physiology and clonal growth were investigated as well. The aim was to reveal the response laws of Chinese sea buckthorn clonal growth to irrigation intensity and its photosynthetic physiology regulatory mechanisms.

Materials and methods

Study site and plant material

The study site was located at Dingbian County in Shaanxi province (north-central China) at the southern edge of the Mu Us Sandy Land. The geographical coordinates of the site are 107°15′ ~ 108°22′ E, 36°49′ ~ 37°53′ N. The site has a mid-temperate arid and semiarid continental monsoon climate and experiences droughts and water shortages. The site has four distinct seasons, sufficient light, and frequent sandstorms. The annual mean temperature is 7.9 °C, mean annual precipitation is 316 mm and is mostly concentrated in July to September, the annual evaporation amount is 2490 mm, and the annual mean relative humidity is 53.1%. The geomorphological characteristics of the site include undulating dunes and continuous sand belts, and the area has an altitude of 1303-1418 m a.s.l. The soil consists mainly of aeolian sandy soil and saline-alkaline soil, which are nutrient-deficient and have poor water and nutrient retention abilities. The zonal vegetation is semi-desert grassland which is floristically composed of psammophytes, xerophytes, salt- and alkali-tolerant plants, and mesophytic meadows. The experiment was conducted at the Research and Development Base of Dingbian Forestry Station, and the soil is artificially-leveled aeolian sandy soil. The Chinese sea buckthorn naturally grows on banks of rivers and lakes and in valleys in the research area, where the soils furnish good water conditions. The identical one-year-old seeded seedlings were used in this experiment. The experiment began in 2011, and after three years of continuous observation, the final survey and measurement were conducted at the end of the experiment in 2013.

Experimental design and setup

A univariate regression design was employed, and different irrigation intensities were applied by adjusting the irrigation period with a fixed flow rate to simulate a natural precipitation gradient. Based on preliminary experimental results (Li et al. 2010b, Cao et al. 2016), three irrigation gradients were set up in this study, which were three, six, and nine times the mean annual precipitation of the study site. Flood irrigation from a well was carried out for 280, 560, or 840 sec for each irrigation gradient with a runoff of 6 L sec⁻¹ on the dates of 5th, 10th, 15th, 20th, 25th, and 30th from May to August in 2011-2013, and a non-irrigated group was used as a control (Tab. 1). A randomized arrangement was used for the field layout, with a plot area of 3 × 10 m. Triplicates were set up, resulting in a total of 12 plots. The ridges between the plots had a width of 0.5 m and a height of 0.3 m. Asphalts and thick plastic films were buried up to a depth of 1 m in the middle of the ridges for separation to prevent water seepage. Thirty seedlings (one-year-old) were planted in every plot, with a distance of 1.0 × 1.5 m between the seedlings. The two sides of every replicate contained guard rows.

Experiment surveys and measurements

All the measurements were conducted in 2013, the third year of the experiment. Clonal growth parameters including ramet growth ability, clonal proliferation ability, and clonal propagation ability were measured at the end of the growth phase in September (Li et al. 2001, 2004, 2010b, He et al. 2006, 2012, Guo et al. 2015, Cao et al. 2016). For ramet (parent and daughter ramet) growth ability, the amount of growth in tree height, base diameter, and crown width were measured for every tree. For clonal proliferation ability, the

| Treatment No. | Moisture gradient (fold) | AvIR (kg y⁻¹) | EqPrec (mm) | Irrigation Dates (May-August, 2011-2013) |
|---------------|--------------------------|--------------|-------------|---------------------------------------|
| 1             | 0                        | -            | 300         | Not irrigated                         |
| 2             | 3                        | 40.500       | 900         | 5th, 10th, 15th, 20th, 25th, 30th      |
| 3             | 6                        | 81.000       | 1800        | 5th, 10th, 15th, 20th, 25th, 30th      |
| 4             | 9                        | 121.500      | 2700        | 5th, 10th, 15th, 20th, 25th, 30th      |
number of individuals was measured, i.e., the number of individual clonal (sprout) daughter ramets in every experimental plot was tallied. The “tracking and digging” method was used for measuring clonal propagation ability, i.e., starting from a primary lateral root (from the parent plant) of an average standard tree, we followed and excavated the connecting secondary lateral roots (originating from the primary lateral roots) and tertiary lateral roots (originating from the secondary lateral roots) and measured the thickness, length, and quantity of the primary lateral roots and the total number of lateral roots. Soil moisture content and moisture content of leaves, chlorophyll content, and photosynthetic physiological indexes were repeatedly measured for three cycles, i.e., August 16th-19th, 21st-24th, and 26th-29th. The measurements were made from 8:00 to 20:00 every day. Net photosynthetic rate, transpiration rate, stomatal conductance were measured from 8:00 to 20:00 every day. Net photosynthetic rate, transpiration rate, stomatal conductance were measured from 8:00 to 20:00 every day. Net photosynthetic rate, transpiration rate, stomatal conductance were measured from 8:00 to 20:00 every day. Net photosynthetic rate, transpiration rate, stomatal conductance were measured from 8:00 to 20:00 every day. Net photosynthetic rate, transpiration rate, stomatal conductance were measured from 8:00 to 20:00 every day.

Results

Responses of soil and tissue water content to irrigation intensity

From Fig. 1, it is evident that soil water content (y) and irrigation intensity (x) were highly significantly positively correlated, while tissue water content (y) exhibited a quadratic parabolic change of the downward opening with increasing irrigation intensity and soil water content (x). The inflection point for the regression formula represents the optimal irrigation intensity (or soil water content) when the tissue water content is the highest and values greater or lower than the optimal irrigation intensity (or soil water content) will result in a reduction in tissue water content. This shows that as irrigation intensity increases, the soil water content increases vertically, while tissue water content initially increases before decreasing.

Responses of photosynthetic gene expression levels to moisture gradient

As reported in Tab. 2, as irrigation intensity (x) increases, RCA and CHLH gene expression levels (y) exhibit a quadratic parabolic change of the downward opening. The inflection point of the formula represents the optimal irrigation intensity when expression levels are the greatest, and values greater or lower than the optimal irrigation intensity will cause gene expression levels to decrease (similar to Fig. 1b). At the same time, the variation trends in gene expression levels with increasing soil water content are consistent with the variation trends when irrigation intensity increases. However, gene expression levels and tissue water content (x) showed a significant positive correlation (similar to Fig. 1a). This indicates that the expression levels of RCA and CHLH increase prior to decreasing as irrigation intensity (or soil water content) and tissue water content increase. Therefore, irrigation intensity (soil water content) to irrigation intensity, as well as of (c) tissue water content to soil water content (for more details, see Experiment Surveys and Measurements section).

For Chinese sea buckthorn, three fully-expanded leaves with different orientations were selected from average standard plants in every plot. Twenty-four hours after irrigation, 5 g of young leaves were sampled from the top of average standard plants for the gene expression analyses, then rapidly placed in liquid nitrogen and transported back to the laboratory for storage at -80 °C. In the laboratory, the UNIQ-10 Pillar TRizol® total RNA extraction kit (SK1321/SK1322 – Thermo Fisher Scientific Inc., Waltham, MA, USA) was used to extract total RNA. Following that, cDNA synthesis was carried out before real-time PCR was used to measure the relative amounts of the RCA and CHLH genes in the cDNA samples.

Data analysis

All data were analyzed by regression analysis and correlation analysis using the statistical software SPSS® ver. 17.0 (IBM, Armonk, NY, USA).

Tab. 2 - Results of the regression analysis between gene expression parameters, irrigation intensity, and tissue water content. (R): correlation coefficient; (IP): equation inflection point.

| Predictor (x)               | Regression variable (y) | Regression equation | R    | p-value | IP   |
|-----------------------------|-------------------------|---------------------|------|---------|------|
| Irrigation intensity        | RCA                     | \( y = 0.162x^2 + 1.607x + 0.458 \) | 0.785 | 0.013   | 4.960 |
| Tissue water content        | RCA                     | \( y = 0.344x - 17.872 \) | 0.688 | 0.013   | -    |
|                            | CHLH                    | \( y = 1.446x - 73.884 \) | 0.822 | 0.001   | -    |
Fig. 1b). Additionally, the variation in these indicators with increasing soil water content is consistent with the variation trends when irrigation intensity increases. However, these indicators and tissue water content (x) were highly significantly positively correlated (similar to Fig. 1a). These results show that these indicators increase before decreasing with irrigation intensity (or soil water content) and increase with tissue water content. Therefore, irrigation intensity or soil water content has indirect effects on the changes in photosynthetic physiological characteristics, while tissue water content has a direct effect.

As indicated in Tab. 4, net photosynthetic rate, transpiration rate, chlorophyll content, and stomatal conductance increase with increasing RCA and CHLH gene expression levels. When the aforementioned response laws were combined, it was evident that the

### Tab. 3 - Results of the regression analysis between photosynthesis physiological parameters, irrigation intensity, and tissue water content. (R): correlation coefficient; (IP): equation inflection point.

| Predictor (x) | Regression variable (y) | Regression equation | R   | p-value | IP   |
|--------------|-------------------------|---------------------|-----|---------|------|
| Irrigation intensity | Net photosynthetic rate | $y = -0.306x^2 + 3.583x + 16.790$ | 0.872 | <0.001 | 5.855 |
|                | Transpiration rate       | $y = -0.192x^2 + 2.311x + 5.024$ | 0.983 | <0.001 | 6.018 |
|                | Chlorophyll content      | $y = -0.012x^2 + 0.139x + 1.336$ | 0.810 | 0.008  | 5.792 |
|                | Stomatal conductance     | $y = -0.007x^2 + 0.076x + 0.356$ | 0.813 | 0.008  | 5.429 |
| Tissue water content | Net photosynthetic rate | $y = 1.004x - 36.421$ | 0.878 | <0.001 | -     |
|                | Transpiration rate       | $y = 0.570x - 24.507$  | 0.841 | 0.001  | -     |
|                | Chlorophyll content      | $y = 0.040x - 0.830$   | 0.889 | <0.001 | -     |
|                | Stomatal conductance     | $y = 0.019x - 0.677$   | 0.804 | 0.002  | -     |

### Tab. 4 - Results of the regression analysis between photosynthetic physiology parameters and gene expression parameters. (R): correlation coefficient.

| Predictor (x) | Regression variable (y) | Regression equation | R   | p-value | IP   |
|--------------|-------------------------|---------------------|-----|---------|------|
| RCA          | Net photosynthetic rate | $y = 1.876x + 18.440$ | 0.820 | 0.001  | -     |
|              | Transpiration rate       | $y = 0.922x + 7.014$  | 0.680 | 0.015  | -     |
|              | Chlorophyll content      | $y = 0.068x + 1.396$  | 0.744 | 0.006  | -     |
|              | Stomatal conductance     | $y = 0.035x + 0.387$  | 0.730 | 0.007  | -     |
| CHLH         | Net photosynthetic rate | $y = 0.563x + 16.462$ | 0.867 | <0.001 | -     |
|              | Transpiration rate       | $y = 0.319x + 5.532$  | 0.828 | 0.001  | -     |
|              | Chlorophyll content      | $y = 0.021x + 1.315$  | 0.820 | 0.001  | -     |
|              | Stomatal conductance     | $y = 0.011x + 0.351$  | 0.769 | 0.003  | -     |

### Tab. 5 - Regression relationships between clonal growth parameters, irrigation intensity, and tissue water content. (R): correlation coefficient; (IP): equation inflection point.

| Predictor (x) | Regression variable (y) | Regression equation | R   | p-value | IP   |
|--------------|-------------------------|---------------------|-----|---------|------|
| Tree height  | $y = -0.029x^2 + 0.390x + 1.279$ | 0.913 | <0.001 | 6.724 |
| Base diameter| $y = -0.593x^2 + 7.647x + 21.352$ | 0.952 | <0.001 | 6.448 |
| Crown width  | $y = -0.015x^2 + 0.201x + 1.211$ | 0.915 | <0.001 | 6.700 |
| Number of daughter ramets | $y = -14.306x^2 + 167.317x + 47.450$ | 0.952 | <0.001 | 5.847 |
| Primary lateral root thickness | $y = -0.121x^2 + 1.799x + 3.065$ | 0.929 | <0.001 | 7.434 |
| Primary lateral root length | $y = -1.377x^2 + 19.835x + 39.922$ | 0.972 | <0.001 | 7.202 |
| Number of primary lateral roots | $y = -0.472x^2 + 5.839x + 14.183$ | 0.970 | <0.001 | 6.185 |
| Total number of lateral roots | $y = -39.639x^2 + 530.628x + 837.883$ | 0.922 | <0.001 | 6.693 |
| Tree height  | $y = 0.111x - 4.502$ | 0.793 | 0.002  | -     |
| Base diameter| $y = 2.143x - 90.303$ | 0.851 | <0.001  | -     |
| Crown width  | $y = 0.059x - 1.856$ | 0.805 | 0.002  | -     |
| Number of daughter ramets | $y = -42.423x - 2171.986$ | 0.869 | <0.001  | -     |
| Primary lateral root thickness | $y = 0.621x - 29.547$ | 0.866 | <0.001  | -     |
| Primary lateral root length | $y = 6.352x - 291.788$ | 0.870 | <0.001  | -     |
| Number of primary lateral roots | $y = 1.588x - 68.839$ | 0.887 | <0.001  | -     |
| Total number of primary lateral roots | $y = 170.729x - 8171.593$ | 0.903 | <0.001  | -     |
irrigation intensity determines soil water content, the soil water content in turn determines tissue water content, the tissue water content in turn determines the expression levels of photosynthetic genes, and the expression levels of photosynthetic genes regulate photosynthetic physiological characteristics.

**Response of clonal growth to moisture gradient and photosynthetic characteristics**

As irrigation intensity (x) increases, tree height and base diameter; crown width; daughter ramet number; thickness, length, and quantity of primary lateral roots; and total number of lateral roots (y) exhibit a quadratic parabolic relationship (Tab. 5). The inflection point of the formula represents the optimal irrigation intensity when these parameters are the highest, and values greater or lower than the optimal irrigation intensity will cause these indicators to decrease (similar to Fig. 1b). The variation in these indicators with increasing soil water content is consistent with the variation when irrigation intensity increases. However, these indicators (y) and tissue water content (x) demonstrated an extremely significant positive correlation (similar to Fig. 1a). These results indicate that ramet growth, clonal proliferation, and clonal propagation ability increase before decreasing with irrigation intensity (or soil water content) and increase with tissue water content. Therefore, irrigation intensity (or soil water content) has indirect regulatory effects on clonal growth, while tissue water content has direct regulatory effects on clonal growth.

It is evident in Tab. 6 that tree height and base diameter; crown width; number of daughter ramets; thickness, length, and quantity of primary lateral roots; and total number of lateral roots were significantly positively correlated with photosynthetic physiological indicators. These results demonstrated that the growth, clonal proliferation, and clonal propagation abilities of Chinese sea buckthorn increase with increasing net photosynthetic rate, transpiration rate, and stomatalconductance.

**Discussion**

**Responses of soil and tissue water content to irrigation intensity**

Soil and plant tissue water content gradients can be affected by regulating irrigation intensity. We observed that soil water content and irrigation intensity were highly significantly positively correlated, while tissue water content showed a quadratic parabolic change of the downward opening with increasing irrigation intensity and soil water content. This was similar to the results obtained by Li (2011). From the relationship between the three factors, it is evident that as irrigation intensity increases, the soil water content increases vertically and constitutes a passive response process. As irrigation intensity or soil water content increased, tissue water content increased initially before decreasing. This suggests that tissue water content does not only increase when irrigation is increased and an optimal irrigation intensity is present, implying that there are certain internal regulation processes. In addition, irrigation intensity, soil water content, and tissue water content formed a complementary process, i.e., irrigation intensity determines soil water content and soil water content in turn determines tissue water content.

**Responses of RCA and CHLH to the moisture gradient**

Previous studies have found that gene expression levels in plants are altered by the levels of environmental factors, such as temperature, moisture, and light. In photosynthetic plants, the regulatory mechanisms of RCA towards Rubisco activity are widespread (Parry et al. 2013, Carmo-Silva et al. 2015): Rubisco activity in herbs was found to decrease significantly during long-term drought stress. After three days of watering, the Rubisco activation state recovered to good levels (Xu et al. 2013). Rubisco, RCA activity, and RCA gene expression have been observed to decrease during severe drought stress in many plants (Parry et al. 2002, Lawlor & Tezara 2009). A study on *Arabidopsis thaliana* found that CHLH gene expression is regulated by the circadian rhythm, with high expression levels under light conditions and undetectable levels under dark conditions (Matsumoto et al. 2004). From these studies, it is evident that certain external conditions will induce changes in the expression levels of RCA and CHLH. Our study found that as irrigation intensity increases, the expression levels of RCA and CHLH exhibit a quadratic parabolic change, and gene expression levels and tissue water content showed a significant positive correlation. The irrigation intensity when the tissue water content and RCA and CHLH gene expression levels are the highest represents the optimal irrigation intensity. When irrigation intensity is lower than its optimum, tissue water content, and RCA and CHLH gene expression levels increase with increasing irrigation intensity. When the irrigation intensity is higher than its optimum, tissue water content, and RCA and CHLH gene expression levels decrease with increasing irrigation intensity. This variation law conforms to a tolerance law (Shelford 1913), i.e., plants have some limit or range of tolerance to any ecological factors. RCA and CHLH gene expression levels increased with increasing tissue water content. The insufficient or excessive water content will lead to reduced tissue water content, while reduced tissue water content inhibits the expression of RCA and CHLH. Appropriate irrigation will cause tissue water content to reach its maximum value. At this point, the tissue water content will promote the expression of RCA and CHLH. From this we can see that irrigation intensity modifies soil water content, the soil water content in turn determines tissue water content, and tissue water content affects the expression levels of RCA and CHLH.

**Responses of photosynthetic characteristics to RCA or CHLH**

Rubisco is a key enzyme in photosynthetic carbon assimilation, and its activity directly affects the rate of photosynthesis. Therefore, reduced Rubisco activity is often a non-stomatal factor causing reduced photosynthetic rates (Salvucci & Crafts-Brandner 2004), while a stable Rubisco catalytic activation state requires activation by the RCA gene (Parry et al. 2013, Carmo-Silva et al. 2015). Weng et al. (2002) found that
reduced photosynthetic capacity during flag leaf senescence after the heading stage in rice is intimately associated with the decreased expression of Rubisco-activating enzymes (RCA). Their study further showed that initial Rubisco activity was highly significantly positively correlated with stomatal conductance and photosynthetic capacity. The activity of the Rubisco-activating enzyme (RCA) is significantly positively correlated with transpiration rate and photosynthetic rate (Weng et al. 2001). This study showed that net photosynthetic rate, transpiration rate, chlorophyll content, and stomatal conductance were significantly positively correlated with the expression levels of RCA and CHLH, i.e., net photosynthetic rate, transpiration rate, chlorophyll content, and stomatal conductance increase with increasing RCA and CHLH expression levels.

Acknowledgments
The following abbreviations have been used throughout the paper: RCA - Rubisco activase; CHLH - Mg-chelatase H subunit.

Author Contribution
S CB and KH N contributed equally to this paper (co-first authors).

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Responses of sea buckthorn to irrigation

Tab. S1 – The Chinese sea buckthorn clonal growth dataset.

Fig. S1 - Site conditions and plant material

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