ORIGINAL ARTICLE

Response of physiological integration in the clonal herb *Zoysia japonica* to heterogeneous water conditions

Yue Li[1] · Wantong Ning[1] · Sunan Xu[1] · Ning Yu[2] · Zhonglin Chen[1] · Lihong Zhang[1]

Abstract

Physiological integration may be an important ecological strategy for clonal plants living in heterogeneous environments. The physiological integration between *Zoysia japonica* parent and offspring ramets under different experimental water conditions was examined. We applied four treatments: (1) both the parents and offspring were well watered, (2) both experienced drought, (3) the parents were well watered and the offspring experienced drought, and (4) the parents experienced drought and the offspring were well watered. The growth, chlorophyll content, photosynthesis, chlorophyll fluorescence, and antioxidant enzymes of *Zoysia japonica* were measured. The chlorophyll contents, chlorophyll a/b ratio, and photosynthetic parameters (Pn, Gs) of offspring ramets were not reduced when parents under well-watered conditions and offspring ramets under drought conditions suggested that the physiological support provided by parents enabled their offspring to maintain photosynthetic efficiencies. The support provided to the offspring by their parents was also obvious in the antioxidant systems and proline accumulation of *Zoysia japonica* in heterogeneous water environments. Physiological integration was not detected when the parents experienced drought and the offspring were well watered. The parents provided physiological support to their offspring and did not expense significant benefits through physiological integration when parents were well watered and offspring experienced drought. When the opposite conditions were true, physiological integration did not exist.

Keywords Physiological integration · Drought stress · *Zoysia japonica* · Heterogeneous environments · Clonal herb

Introduction

In nature, resources such as water, light, and nutrients are usually patchily distributed in space, and these heterogeneous environments present challenges to plants that cannot actively move from resource-poor to resource-rich patches (Ying et al. 2018). To cope with these challenges, clonal plants produce a number of genetically identical ramets that can connect to each other by stolon or rhizome internodes over some distance for a period of time (Li et al. 2018).

Water, photosynthates, nutrients, and other substances can be translocated from source (leaves and storage tissues) to sink ramets (fruits and growing tissues) through the stolon and rhizome connections between them (Wei et al. 2018). This phenomenon, called physiological integration, has been previously described in many studies (Roiloa and Retuerto 2012; Wang et al. 2017). Physiological integration may be an important ecological strategy for clonal plants living in heterogeneous environments. The physiological responses of integrated ramets to a variety of environmental conditions, such as light (Wolfer and Straile 2012), salinity (Xiao et al. 2011), nutrients (He et al. 2010), defoliation (Xu et al. 2012), UV-B radiation (Li et al. 2011), and contaminants (Roiloa and Retuerto 2012), have previously been reported, and some studies have revealed the effects of variable water availability on morphological integration between ramets (Roiloa and Retuerto 2007, Wang et al. 2008, Zhang et al. 2008, Touchette et al. 2013). However, few reports have studied the effects of physiological integration between clonal ramets under heterogeneous water conditions (Zhang et al. 2012; Li et al. 2015).

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✉ Yue Li
  yuanlinliyue@163.com

✉ Lihong Zhang
  lihongzhang132@163.com

[1] School of Environmental Science, Liaoning University, Shenyang, China

[2] The Experiment Center of Environmental Monitoring of Liaoning Province, Shenyang, China

Water deficit is an important abiotic stress, which alters some physiological and biochemical processes in plants. It leads to reductions in growth and chlorophyll content, as well as changes in photosynthetic parameters (Zhong et al. 2019). For example, proline was shown to rapidly accumulate in water-deprived plants (Zahia et al. 2017). The content of malondialdehyde (MDA) in plants experiencing long-term drought stress and dangerous dehydration is considered to be an indicator of oxidative damage, and this parameter has been shown to increase in water-stressed grass leaves (Chen et al. 2012; Zhang et al. 2018). It has been well proved that water deficit has a dramatic effect on the plant antioxidant defense system. But changes in the activities of antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD)) under drought stress depend on flora and cultivars, as well as stress intensity and duration (Chen et al. 2012; Wang et al. 2019). When the dependent ramets grow under serious water deficit, translocation of water between interconnected ramets was weakened (Roiloa and Retuerto 2007; Zhang et al. 2008). Low soil–water conditions had significant effects on plant performance, which increased with the increase of water-stressed, integration may cause lower fitness than if ramets were physiologically independent. Stolon or rhizome connections between ramets allow water to be transported within the clone (Roiloa and Retuerto 2007). Drought stress was the main factor to increase greater ramet mortality in *Justicia americana* (Touchette et al. 2013), *Leymus chinensis* L. (Wang et al. 2008), tea [*Camellia sinensis* (L.) O. Kuntze] (Netto et al. 2010), bamboo (*Sasa palmata*) (Jing et al. 2020), and their ability to quickly transfer water to dehydrated tissues allows them to rapidly equilibrate. The above-mentioned physiological traits have been extensively reported in non-clonal plants and clonal plants under homogeneous water deficits; however, few studies of clonal plants in heterogeneous water environments have been conducted.

There is no report available on water integration of *Zoysia japonica* between parent ramets and offspring ramets in drought condition, considering their growth and physiological status. Therefore, in this paper we researched the effects of physiological integration between parent and offspring ramets in a clonal plant, *Zoysia japonica* (L.) Steud. (Poaceae, Zoysia), under heterogeneous water conditions. *Zoysia japonica* is the most popular lawn grass in East Asia (China, Japan, South Korea) and widely used for athletic playgrounds, golf courses, home lawns and parks (Kang et al. 2009; Chen et al. 2014). We assessed the integration effects on plant water potential, leaf and root biomass, chlorophyll contents, photosynthetic parameters, chlorophyll fluorescence parameters, antioxidant enzymes activities, and MDA and proline contents. We aimed to answer the following specific questions: (1) Does physiological integration improve the capacity of *Zoysia japonica* parent or offspring ramets to withstand water stress? (2) Are there differences between the physiological integration of parent ramets growing in well-watered and offspring ramets growing in water-deprived soils with that of parent ramets growing in water-deprived and offspring ramets growing in well-watered soils?

Material and methods

Plant culture and treatment

On 10 September 2017, 20 cuttings of *Zoysia japonica* clone were collected from 1-year-old turfgrass plots at the Ecological Research Center, Liaoning University, China. The cuttings were grown in plastic pots (diameter: 13 cm, depth: 14.5 cm) that were filled with a mixture of topsoil and coarse river sand (w/w, 1:1). The pots were then transferred to a greenhouse. From September 2017 to April 2018, the pots were weighed every 2 d and then watered to above 75% of field capacity. The pots were fertilized once every 2 weeks using a compound fertilizer (total organic N 6.0%, organic C 35%, P_2O_5 4%, Shenyang Zhongze New Fertilizer Co., Ltd. China) until the plants became dormant in November 2017.

At the beginning of April 2018, the plants began to sprout. On 1 May 2018, the pots were transferred to an experimental field in the Ecological Research Center under a semi-controlled environment, with daily maximum temperature of 32.6 ± 1.5 °C and minimum temperature of 22.9 ± 2.1 °C, and relative humidity of 65%.

When the stolon of a plant grew to the fifth nodule, the top of the stolon was planted in another pot. Each connected fragment was grown with the parent ramet in one pot and the offspring ramet in another, and the two pots (regarded as a pair) were close enough for the stolon to remain undamaged. The plants received the same daily management as above until the treatments began.

The treatments commenced on 8 August 2018. Twenty pairs of clone plants were randomized and divided into four groups. The plants under well-watered conditions were weighed every day and kept at 75% of field capacity. Drought (35% of field capacity) was induced by withholding water. Thus, the treatments were as follows: (1) the parent and offspring ramets were both well watered (W), (2) the parent and offspring ramets both experienced drought conditions (D), (3) the parent ramets were well watered and the offspring ramets experienced drought conditions (WD), and (4) the parent ramets experienced drought conditions and the offspring ramets were well watered (DW). Five replicate pairs of parent and offspring ramets were randomly assigned to each of the four treatments. The field capacity was measured in the morning of the experiment day, and on the fourth day, the field capacity decreased to 35%. From the fifth day, water in the soil was complemented appropriately

at 18:00 p.m. every day to keep the field capacity at 35%. All plant parts (leaves and roots) were harvested for further analysis on 25 August.

Plant water potentials

Root and leaf water potentials were measured using Dewpoint Water Potential System (WP4, USA) from 8:00 to 18:00. The root at a depth of 3–6 cm was selected for root water potential measurement, and the middle of the fully unfolded mature leaf on the top of plants was selected for leaf water potential measurement, at least five consistent measurements were taken from plants under different treatments, the last root and leaf water potentials data of each treatment were the mean of five measurements.

Biomass

At the end of the experiment, all plant parts (leaves and roots) of parent and offspring ramets were harvested separately, cleaned up, put into a heating oven at 105 °C for 15 min, dried to constant weight at 70 °C for 5 d, and then weighed to determine biomasses.

Chlorophyll content

The method of Banks (2018) was used to detect the chlorophyll (Chl) content of the plants. Chlorophyll was extracted from 0.1 g of the plants' leaves with 10 ml of 80% acetone, and the absorbance of the extraction was determined at 663 and 645 nm. The chlorophyll content was calculated using the following equations:

$$\text{Chl a} \left(\text{mg} \cdot \text{g}^{-1}\text{Fw}\right) = (13.95 \times A663 - 6.88 \times A645) \times 0.005/W,$$

$$\text{Chl b}(\text{mg} \cdot \text{g}^{-1}\text{Fw}) = (24.96 \times A645 - 7.32 \times A663) \times 0.005/W,$$

$$\text{Total Chl}(\text{mg} \cdot \text{g}^{-1}\text{Fw})$$
$$= \text{Chl a} + \text{Chl b}(18.08 \times A645$$
$$+ 6.63 \times A663) \times 0.005/W,$$

where A645 and A663 are the absorbances of the chlorophyll extract at 645 and 663 nm, respectively, and W is the fresh weight of leaves (g).

Photosynthetic and chlorophyll fluorescence parameters

The method of Zhong et al. (2019) was used to determine the photosynthetic and chlorophyll fluorescence parameters of the plants. From 10:00 to 12:00 on the sampling day, gas exchange was measured from individual leaves (one leaf per plant, three plants per replicate) using a portable photosynthesis system (Li-6400, Li-Cor Lincoln, NE, USA). The LI-6400 system with a 6400–40 Leaf Chamber Fluorometer was used to measure chlorophyll fluorescence. After dark adaptation of samples for 1 h, the minimal fluorescence yield (*Fo*) was determined under low modulated light of 1.0 μmol·m^{-2}·s^{-1}, and the maximal fluorescence yield (*Fm*) in darkness was measured with a 0.8 s saturating pulse at 8000 μmol·m^{-2}·s^{-1}. The maximal photochemical efficiency (Fv/Fm)：Fv/Fm = (Fm − Fo)/Fm (Genty et al. 1989).

Extraction and assay of antioxidant enzymes

Fresh leaves (0.5 g) were ground in a buffer solution (0.1 M phosphate buffer, pH 6.8) using a mortar and pestle on ice. The supernatant was obtained from the homogenate, which was centrifuged at 12,000×*g* for 15 min at 4 °C and was used as the crude extract solution for determining SOD, CAT, and POD. The methods of Nahar et al. (2018) were used to measure SOD, POD, and CAT activities. SOD activity was determined as follows: the reaction mixture was prepared by mixing 2 ml of 130 mM methionine, 2 ml of 100 mM EDTA, 2 ml of 750 mM nitro blue tetrazolium, and 20 ml of 10 mM riboflavin. Subsequently, 0.1 ml crude enzyme extract was mixed with 10 ml reaction mixture. The absorbance was measured at 560 nm using a spectrophotometer. SOD enzyme was inhibited at 50% of the photochemical reduction of NBT. The POD activity was estimated as follows: 0.1 ml of enzyme extract was added to a tube containing 2.0 ml of sodium acetate–acetic acid buffer, 30% H_2O_2, and guaiacol. The absorbance was measured at 460 nm per minute using a spectrophotometer, and 0.001 unit change in absorbance equals one unit of enzyme activity. CAT activity was determined as follows: 50 μl enzyme extract was transferred into a tube containing 5 ml CAT reaction solution of 100 mM phosphate buffer and 10 mM H_2O_2. The absorbance was measured at 240 nm using a spectrophotometer and was read every 30 s. CAT activity was expressed as 0.01 units per minute.

Proline and MDA contents

The method described by Bates (1973) was used to estimate the proline content. The method of Tsikas (2017) was used to determine the MDA content of the plants. For the MDA content assay, the plants were homogenized with 5% (w/v) trichloroacetic acid (TCA). The supernatant was reacted with 0.5% (w/v) thiobarbituric acid (TBA) in a boiling water bath for 20 min. The MDA content was measured spectrophotometrically at 532 nm and checked for nonspecific turbidity at 600 nm. The MDA content was determined using the following formula:

$$MDA\ (nmol \cdot g^{-1}FW)\ =\ \left[(A532 - A600) \times V \times 1000/\varepsilon\right] \times W,$$

where A600 and A532 are the absorbances at 600 and 532 nm, respectively, ε is the specific extinction coefficient (155 mM·cm^{-1}), V is the volume of crushing medium, and W is the fresh weight (g).

Statistical analysis

The data represent the mean values ± standard deviation (SD) ($n = 5$). The photosynthetic and chlorophyll fluorescence parameters were determined using the Photosynthetic Assist software. A one-way analysis of variance (ANOVA) and LSD multiple 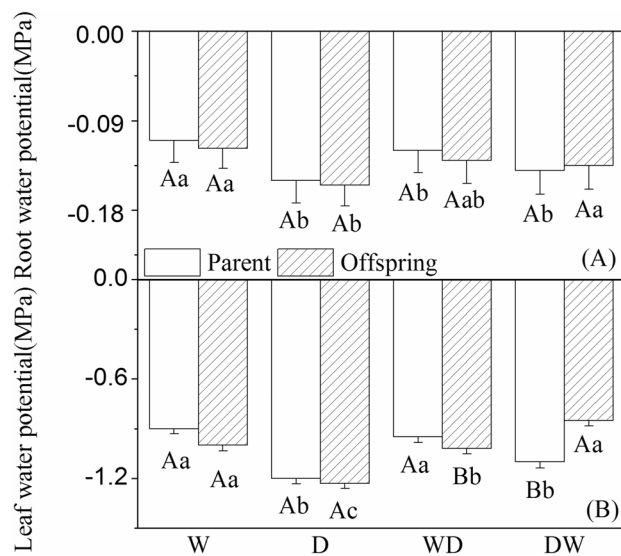comparison test ($p < 0.05$) were used to investigate the experimental data. All the statistical analyses were conducted using the SPSS package.

Results

Changes in root and leaf water potentials

The root and leaf water potentials of parent and offspring in the D treatment were significantly lower than the respective values in W (Fig. 1). The root water potential of the offspring in the WD was lower than that in W, and higher than in D, but did not express significant level (Fig. 1A). The leaf water potential of the offspring in the WD treatments was dramatically lower than that in W, but observably higher than that in D (Fig. 1B). The root and leaf water potentials of the offspring in the DW treatment were not different from those in W.

If the parent ramets grew under sufficient water condition, the root and leaf water potentials of the offspring ramets under heterogeneous drought stress were less affected by drought stress. By contrast, if offspring ramets grew under sufficient water condition, the root and leaf water potentials of the parent ramets were affected deeply by drought stress. The parents provided physiological support to their offspring when parents were well watered and offspring experienced drought.

Biomass

The leaf and root biomass of the parent ramets in the W and WD treatments was significantly higher than those in the D and DW treatments (Table 1). Drought stress induced a remarkable reduction in leaf and root biomass of offspring ramets. The leaf and root biomass of the offspring ramets in the DW treatments was dramatically higher than those in WD. The root biomass for offspring ramets under drought stress decreased and the difference with respect to well-watered offspring ramets was statistically significant. The

Fig. 1 The root and leaf water potentials of *Zoysia japonica* parent and offspring ramets in different treatments. The results from the statistical analysis are presented as mean ± standard deviation (SD) ($n = 5$). Capital letters indicate the significant difference between parent and offspring ramets under the same treatment, and small letters indicate the significant difference between different treatments ($p < 0.05$, least significant difference (LSD) multiple test)

Table 1 The leaf and root biomass (g) of *Zoysia japonica* parent and offspring ramets in different treatments

	Parent ramets		Offspring ramets	
	Leaf biomass	Root biomass	Leaf biomass	Root biomass
W	0.5210 ± 0.042Aa	0.0812 ± 0.0055Aa	0.2560 ± 0.019Ba	0.0493 ± 0.017Ba
D	0.4265 ± 0.048Ab	0.0450 ± 0.0036Ac	0.2014 ± 0.042Bc	0.0450 ± 0.013Bc
WD	0.5032 ± 0.09Aa	0.0796 ± 0.0062Aa	0.2358 ± 0.025Bb	0.0463 ± 0.011Bb
DW	0.4700 ± 0.092Ac	0.0732 ± 0.0013Ab	0.2510 ± 0.054Ba	0.0471 ± 0.014Bb

The results from the statistical analysis were presented as mean ± standard deviation (SD) ($n = 5$). Capital letters indicate the significant difference between parent and offspring ramets under the same treatment, and small letters indicate the significant difference between different treatments ($p < 0.05$, least significant difference (LSD) multiple test)

root biomass of the offspring ramets in the D, WD, and DW treatments was observably different from those in W.

If the parent ramets grew with ample water, the leaf and root biomass of the offspring ramets under heterogeneous drought condition was less affected by drought stress. On the contrary, if offspring ramets grew with ample water, the leaf and root biomass of the parent ramets was greatly affected by drought stress. Physiological integration occurred when the parent ramets were under sufficient water condition.

Chlorophyll content

The chlorophyll contents (a, a + b) of the parent ramets in the D treatment and those (a, b) in the DW treatments were significantly lower than those in W (Fig. 2A–C). The Chl contents (a, b, a + b) and Chl a/b ratios (Fig. 2D) of the offspring ramets in the D, WD, and DW treatments were not dramatically different from those in W. Chla, Chla + b, and Chla/b of parent ramets were definitely reduced in the D treatment, whereas stress had no effect on offspring ramets. In the WD treatments, there was no observable difference in the Chl contents of parent and offspring ramets. Chla, Chlb, and Chla + b of parent ramets were observably decreased in the DW treatments, and analysis of offspring ramets indicated no obvious differences.

When the parent and offspring ramets were in dry conditions, the parent ramets transferred water to the offspring ramets. The moisture transfer reduced the damage of drought to the offspring ramets and ensured the normal chlorophyll contents in the leaves of the offspring ramets. When the parent and offspring ramets of *Zoysia japonica* were in heterogeneous water condition, physiological integration existed between the parent and offspring ramets when the parent ramets were in sufficient water condition. This integration ensured the normal chlorophyll contents of the offspring ramets. When offspring experienced drought and parents were well watered, the offspring ramets physiologically integrated to the parent ramets and the chlorophyll contents of the offspring ramets decreased.

Photosynthetic parameters

The net photosynthetic rate (Pn) and stomatal conductance (Gs) of the parent and offspring ramets in the D were significantly lower than those in W, but there was no dramatic difference between the respective values in the WD and W treatments (Fig. 3A, B). The Pn and Gs values of the offspring in the DW treatment were not considerably different from those in W. Gs in WD treatments increased remarkably its concentration in the parent ramets than offspring ramets. The transpiration rate (E) values of offspring in the WD treatments were not noticeably different with W treatment (Fig. 3C). The water use efficiency (WUE) of the offspring ramets in the D and WD treatments were not significantly different from the values in W (Fig. 3D).
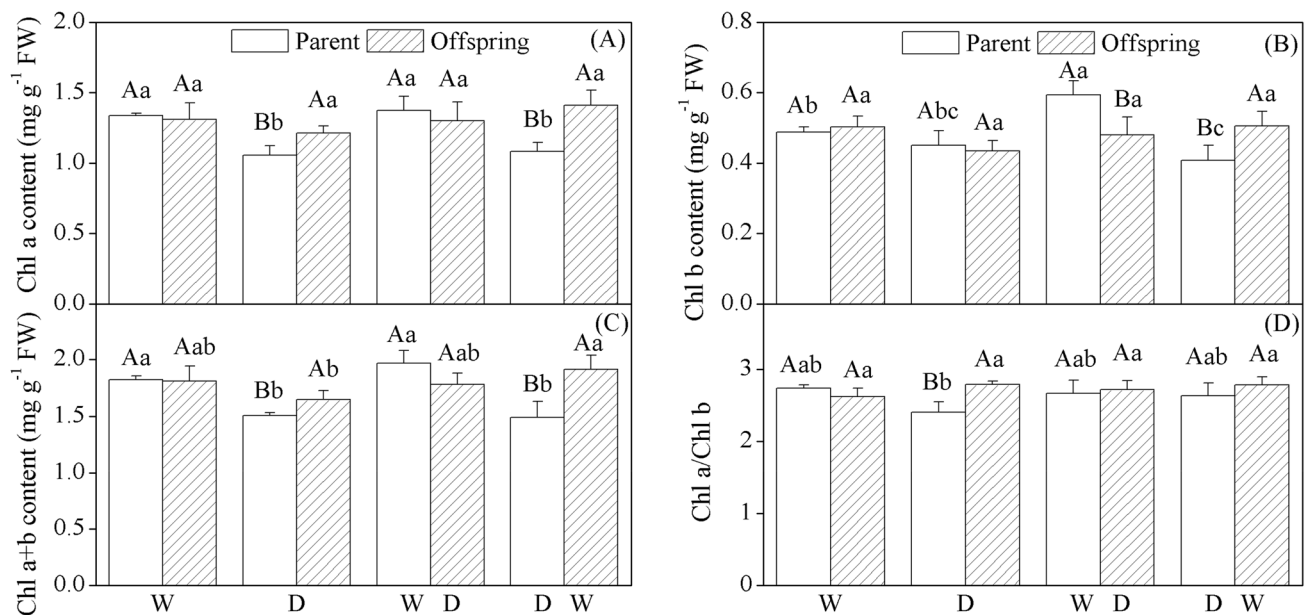

Fig. 2 The chlorophyll (Chl) contents and Chl a/b of *Zoysia japonica* parent and offspring ramets under different treatments. The results from the statistical analysis are presented as mean ± standard deviation (SD) (n = 5). Capital letters indicate the significant difference between the parent and offspring ramets under the same treatment, and small letters indicate the significant difference between different treatments (p < 0.05, least significant difference (LSD) multiple test)
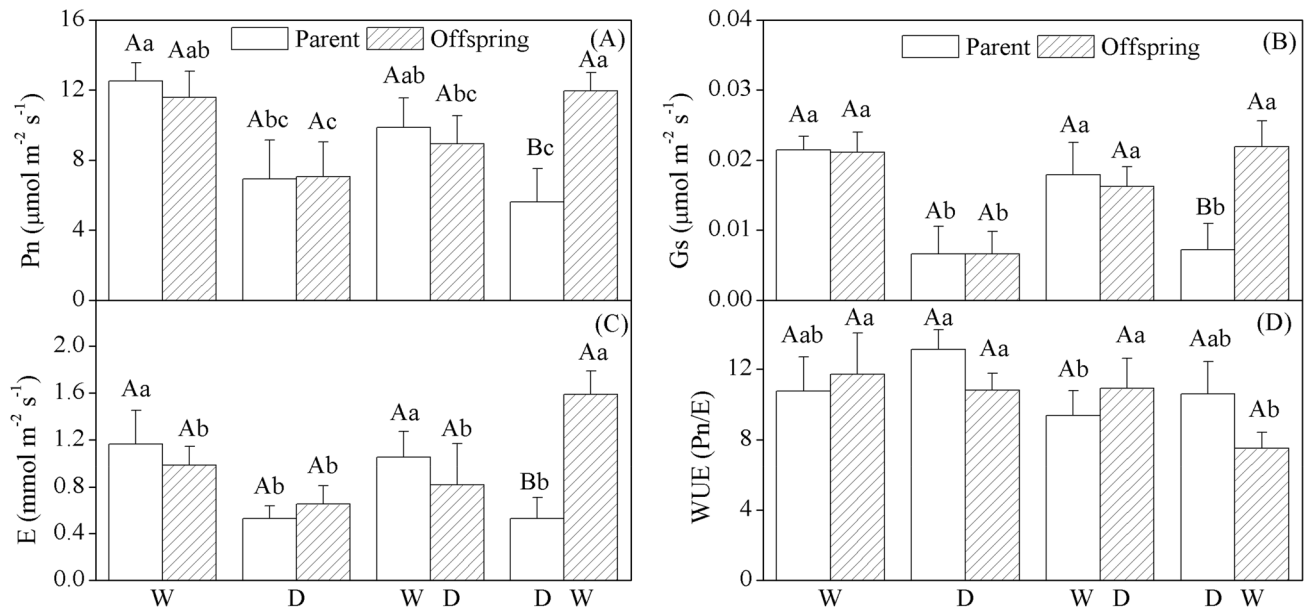
Fig. 3 The net photosynthetic rate (*Pn*), stomatal conductance (*Gs*), transpiration rate (*E*), and water use efficiency (*WUE*) of *Zoysia japonica* parent and offspring ramets under different treatments. The results from the statistical analysis are presented as mean ± standard deviation (SD) (*n* = 5). Capital letters indicate the significant difference between parent and offspring ramets under the same treatment, and small letters indicate the significant difference between different treatments ($p < 0.05$, least significant difference (LSD) multiple test)

When the parent and offspring ramets of *Zoysia japonica* were in heterogeneous water condition, physiological integration happened between the parent and offspring ramets when the offspring ramets were in dry conditions. This integration ensured normal *P*n and *G*s values of the offspring ramets. When parents experienced drought and offspring were well watered, physiological integration did not exist and photosynthetic values of the parent ramets decreased.

Chlorophyll fluorescence

The minimal fluorescence (*F*o), maximal fluorescence (*F*m), and variable fluorescence (*F*v) values of the parent and offspring ramets in the D treatments were significantly lower than the respective values in W (Fig. 4A–C). The *F*m value of the offspring ramets in the WD treatments decreased markedly than that in D (Fig. 4B). The *F*o, *F*m, and *F*v values of the offspring ramets in the DW treatments were not significantly different from the values in W. The *F*v/*F*m values of the parent and offspring ramets in the D treatment were much lower than the corresponding values of the ramets in W (Fig. 5A). The *F*v/*F*o values of the parent ramets in the D treatments dropped greatly than that in W, while the value of the offspring showed difference from W, but did not reach a significant level (Fig. 5B). The *F*v/*F*m and *F*v/*F*o values of the offspring ramets in the WD treatments reduced observably than those in W, while the *F*v/*F*m and *F*v/*F*o values were not considerably different from the

values in W (Fig. 5A, B). Under heterogeneous water condition, *F*o, *F*m, *F*v, *F*v/*F*m, and *F*v/*F*o values of the parent and offspring ramets had little change. There was no discernible integration between the parent and offspring ramets.

Antioxidant enzymes

The CAT activities of the parent and offspring ramets in the D treatment were significantly higher than the respective values in W. The CAT activity of the offspring ramets in WD treatments was remarkably higher than that in W, while it did not express noticeable difference in the DW treatments (Fig. 6A). The POD activities of the parent and offspring ramets in D were noticeably lower than those in W, and the activities of the offspring ramets in WD and DW treatments also dramatically lower than those in W (Fig. 6B). The SOD activities of the offspring ramets in the D and WD treatments were higher than those in W, and the activities of the offspring ramets in the DW treatments were lower than those in W, but did not express significant difference (Fig. 6C). There was a sharp drop of POD activity in parents and offspring ramets under drought conditions (D treatment), while SOD and CAT activity increased. CAT activity did not increase with parents' DW treatment, as was found in D treatment, and POD activity of parents was definitely lower in WD treatments than in the W treatment. When the parent and offspring ramets of *Zoysia japonica* were in heterogeneous water condition, SOD, POD, and CAT of the parent and
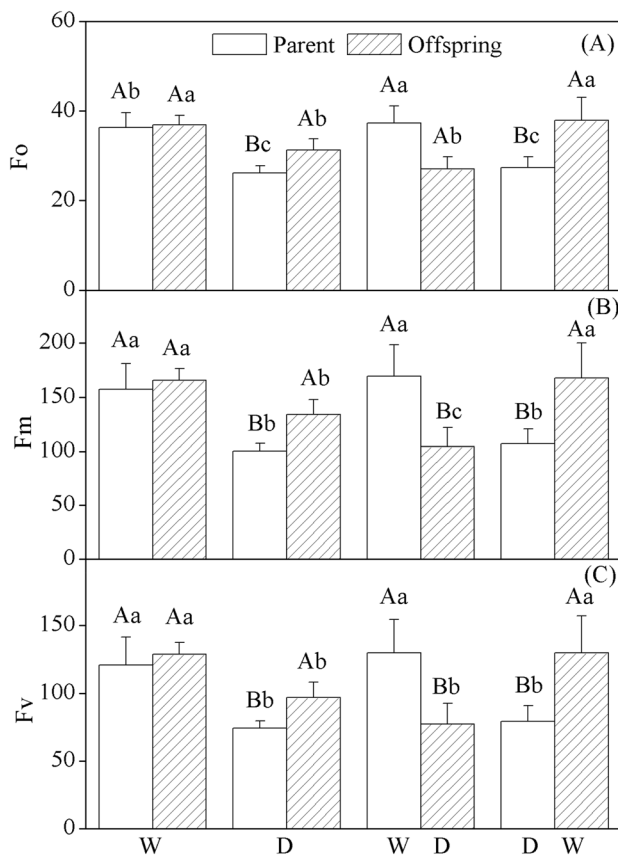
Fig. 4 The minimal fluorescence (Fo), maximal fluorescence (Fm), and variable fluorescence (Fv) of *Zoysia japonica* parent and offspring ramets under different treatments. The results from the statistical analysis are presented as mean ± standard deviation (SD) ($n = 5$). Capital letters indicate the significant difference between parent and offspring ramets under the same treatment, and small letters indicate the significant difference between different treatments ($p < 0.05$, least significant difference (LSD) multiple test)

Fig. 5 The variable fluorescence/maximal fluorescence (Fv/Fm) and variable fluorescence/minimal fluorescence (Fv/Fo) of *Zoysia japonica* parent and offspring ramets under different treatments. The results from the statistical analysis are presented as mean ± standard deviation (SD) ($n = 5$). Capital letters indicate the significant difference between the parent and offspring ramets under the same treatment, and small letters indicate the significant difference between different treatments ($p < 0.05$, least significant difference (LSD) multiple test)

offspring ramets were little influenced by drought. Physiological integration existed between the parent and offspring ramets when the parent ramets were in sufficient water condition. When SOD, POD, and CAT of the parent and offspring ramets were greatly affected by drought, the offspring provided physiologically little or no support to the parent when the offspring ramets were in sufficient water condition.

Proline and MDA contents

The proline contents of the parent and offspring ramets in the D treatment were significantly higher than those in W, but there was no remarkable difference between these values in the WD and W treatments (Fig. 7A). Proline accumulation of the parent ramets under drought stress can contribute to proline content of the offspring ramets in response to the drought effect.
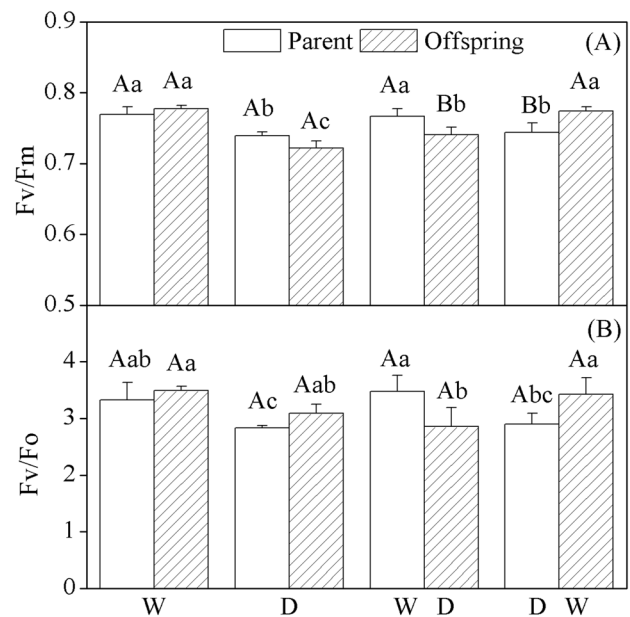
The MDA contents of the parent and offspring ramets in D were significantly higher than the corresponding values of ramets in W. The MDA content of the offspring ramets in WD treatment was higher than that in W, and the value of MDA in DW treatment was different from that of W (Fig. 7B). The MDA content had an increase in the D treatment than in the other three treatments. MDA content in WD treatments considerably decreased its concentration in the parent ramets.

Discussion

Performance of ramets under homogeneous water conditions

In our study, drought stress significantly decreased the plant water potentials, Pn, Gs, Fo, Fm, Fv, Fv/Fm, and POD of *Zoysia japonica* parent and offspring ramets in homogeneous environments, and considerably increased the CAT, proline, and MDA contents. This is consistent with the findings from previous studies (Zahia et al. 2017; Edziri et al. 2018; Zhong et al. 2019). Antioxidant enzymes of the parent and offspring ramets were little influenced by drought. Physiological integration existed between the parent and
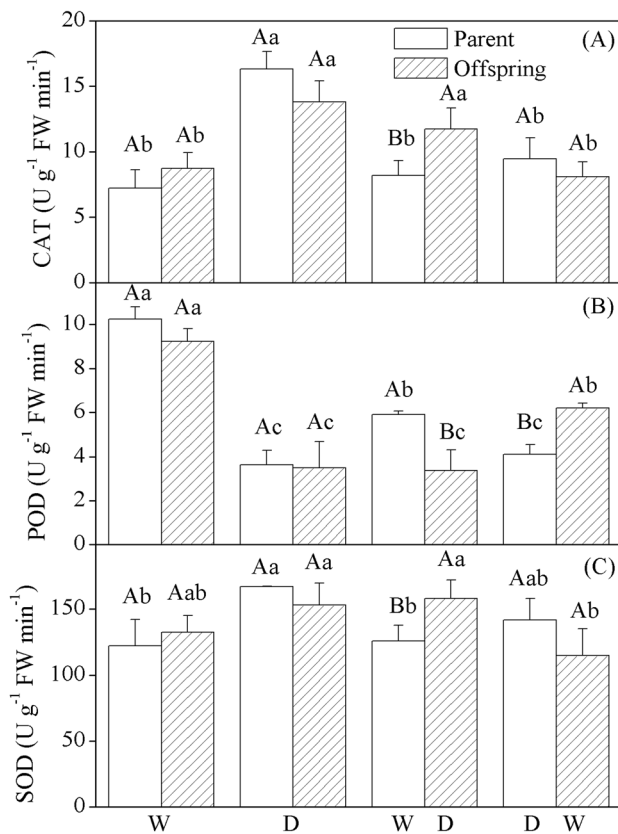
Fig. 6 The superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities of *Zoysia japonica* parent and offspring ramets under different treatments. The results from the statistical analysis are presented as mean ± standard deviation (SD) ($n=5$). Capital letters indicate the significant difference between the parent and offspring ramets under the same treatment, and small letters indicate the significant difference between different treatments ($p < 0.05$, least significant difference (LSD) multiple test)

Fig. 7 The proline and malondialdehyde (MDA) contents of *Zoysia japonica* parent and offspring ramets under different treatments. The results from the statistical analysis are presented as mean ± standard deviation (SD) ($n=5$). Capital letters indicate the significant difference between parent and offspring ramets under the same treatment, and small letters indicate the significant difference between different treatments ($p < 0.05$, least significant difference (LSD) multiple test)

offspring ramets when the parent ramets were in sufficient water condition.

The results of our study showed that physiological integration between parent and offspring ramets existed under homogeneous water deficits, and cost–benefit between the parent and offspring was clearly beneficial for survival of *Zoysia japonica* offspring ramets. The results were consistent with other studies showing that offspring ramets are maintained by the translocation of resources from their parents, such as *Fragaria orientalis* (Zhang et al. 2008), *Justicia Americana* (Touchette et al. 2013), *Thymus vulgaris* (Ashrafia et al. 2018), and *Ipomoea aquatic* (Lin et al. 2018).

Performance of ramets under heterogeneous water deficit

The root and leaf water potentials of the offspring ramets under well-watered and offspring ramets under drought were lower than that in the ramets both well-watered, and
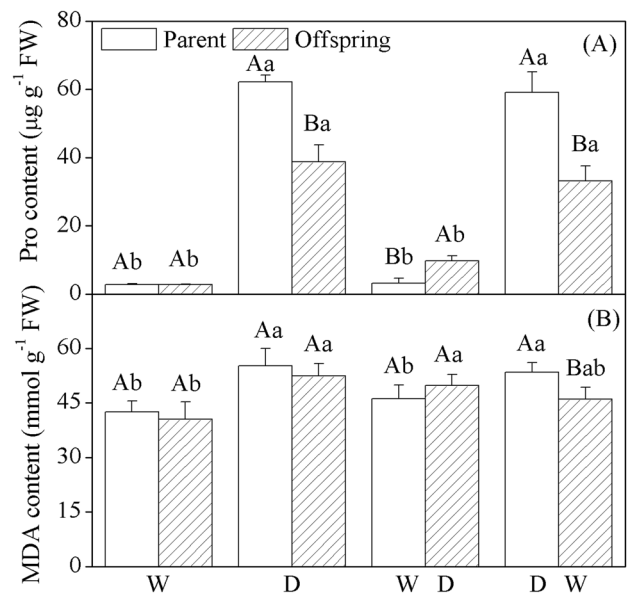
higher than ramets both experienced drought conditions. This result supported that ramets of clonal plants share resources through integration, which allows stoloniferous plants to internally transport water from areas of high supply to areas of high demand (Kouassi et al. 2014; Sonja et al. 2017; Edziri et al. 2018; Zhong et al. 2019).

In this study, the Chl contents (a, b, a + b), Chl a/b, *P*n, *G*s, *E*, and *WUE* of parent under well-watered and offspring ramets under drought conditions were not significantly different from the corresponding values of parent and offspring ramets in homogeneous well-watered environments; however, the Chl contents (a, b, a + b), *P*n, *G*s, and *E* of offspring ramets under heterogeneous drought conditions were higher than those under homogeneous drought conditions. These results suggested that the physiological support provided by parents under well-watered conditions allowed the offspring ramets under drought conditions to maintain photosynthesis. These results are consistent with many studies that have reported that the negative effects of heterogeneous environments were ameliorated by the integration of ramets (Roiloa and Retuerto 2006; Zhang et al. 2008; Touchette et al. 2013; Ashrafia et al. 2018; Lin et al. 2018)**.** However, our data showed that the parents did not incur significant costs owing to clone integration. Roiloa and Retuerto (2006) studied the effects of integration in *Fragaria vesca* growing in a heterogeneous soil environment and concluded that the parents did not incur significant integration costs in terms of survival or

biomass. This finding is noteworthy because some previous studies have reported that the benefits gained by plants in stressful environments are at an expense to the parent ramets (Roiloa and Retuerto 2006, 2012; Touchette et al. 2013).

Chlorophyll fluorescence is a useful physiological test to detect disturbance in the leaf metabolism and seedlings growth because it is a non-invasive, non-destructive, and quick process (Husen 2009; Banks 2018). Our results showed that Fo, Fm, and Fv were positively correlated with photosynthetic pigment contents. This is in accordance with the findings of previous studies (Sonobe and Wang 2018). Moreover, the Fv/Fm values of the offspring ramets under heterogeneous drought conditions were higher than those under homogeneous drought conditions. The possible explanation for this response may be the integration between parent ramets under well-watered conditions and offspring ramets under drought conditions.

In addition to the effects on photosynthesis and chlorophyll fluorescence, the support provided to offspring ramets by their parents was also obvious in the antioxidant systems of *Zoysia japonica* under heterogeneous water environments. The CAT activities in the offspring ramets under heterogeneous drought conditions were lower than those under homogeneous drought conditions. These results are similar to the observations reported by Roiloa and Retuerto (2006), which showed that the parent ramets of clonal plants growing in suitable microsites may have provided support to offspring ramets in stressed microsites. When SOD, POD, and CAT of the parent and offspring ramets were greatly affected by drought, the offspring provided physiologically little or no support to the parent when the offspring ramets were in sufficient water condition.

Proline accumulation under stress is viewed as an adaptive response (Chen et al. 2014). It is necessary to add its functions contributing to an increase in plant resistance under stress factors (Zahia et al. 2017; Cíntia et al. 2018; La et al. 2019). As one of the strategies, *Zoysia japonica* has evolved to tackle drought stress as a reservoir of C and N protects enzymes, scavenges free radicals, and regulates cytosolic pH and NAD(P)$^+$/NAD(P)H ratio. This, in particular, can help to reduce damage of the photosynthetic apparatus under drought stress (Netto et al. 2010). The increase of the photosynthetic pigment contents, photosynthetic parameters, and water potential in *Zoysia japonica* showed that the proline participated in protecting the photosynthetic apparatus from the consequences of water deficiency. There was positive correlation between drought extent and proline accumulation rate. The proline contents of the parent and offspring ramets in drought condition were dramatically higher than those in the other three treatments; therefore, osmotic regulation was the important physiological mechanism for *Zoysia japonica* to adapt to drought adversity. However, further studies should be needed on the association between the

fairly low proline contents in the offspring ramets and the influence of parent ramets on offspring ramets. We found that the proline accumulation in offspring ramets under heterogeneous drought conditions was markedly lower than that under homogeneous drought condition. The reason for this may be that, through physiological integration, the parent ramets under well-watered conditions decreased the water stress of their offspring under heterogeneous drought conditions, and this caused the proline content of offspring ramets to remain stable. Proline accumulation of the parent ramets under drought stress integrated physiologically proline content of the offspring ramets to respond to the drought effect.

The level of free radical production was monitored by the lipid peroxidation in *Zoysia japonica*. MDA was an indicator of free radical production, a main cytotoxic product of lipid peroxidation and a main cytotoxic product of lipid peroxidation (Netto et al. 2010; Cíntia et al. 2018). The level of MDA content was found to increase observably in the drought condition, and there was a significant and positive correlation between parent ramets than offspring ramets. It showed that the negative effect of Gs decrease of *Zoysia japonica* resistance to drought was associated with oxidative stress development, which increased with a decrease in carbon dioxide content, while electron transfer during photosynthesis continued (Lawlor and Tezara 2009). MDA content in the leaves was higher under high photosynthesis. The reduction of MDA content during well-watered condition of *Zoysia japonica* indicated that the formation and removal of free radicals may be in equilibrium and this equilibrium can be achieved at a much faster rate under heterogeneous water deficit (Netto et al. 2010).

This brought us back to the original two questions in the introduction of the paper. (1) Physiological integration can improve the capacity of *Zoysia japonica* parent or offspring ramets to withstand water stress. Physiological integration did not influence growth characters, photosynthesis, antioxidant systems, and proline accumulation of parent ramets in homogeneous water conditions. Nevertheless, under drought conditions, the biomass and photosynthesis of offspring ramets would be promoted, including Chl contents, antioxidant systems, and proline accumulation under a homogeneous water supply. (2) There are significant differences between the physiological integration of parent and offspring ramets growing in drought conditions. Physiological integration was not detected when the parents experienced drought and the offspring were well watered. The parents provided physiological support to their offspring through physiological integration when parents were well-watered and offspring experienced drought.

YL performed the data analyses and wrote the manuscript; SX and ZC helped perform the analysis with constructive discussions.

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