

## Water translocation between ramets of strawberry during soil drying and its effects on photosynthetic performance

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To explore the mechanisms underlying water regulation in clonal plants and its effects on carbon assimilation under water stress, we studied the responses of water status, gas exchange and abscisic acid (ABA) contents to water stress in leaves of pairs of strawberry ramets that consist of mother and daughter ramets. There was a greater decrease in photosynthetic rates ( $P_n$ ) and stomatal conductance ( $G_s$ ) in the disconnected mother ramets than the connected mother ramets upon exposure to water stress, indicating that water stress in mother ramets was alleviated by water translocation from the well-watered daughter ramets. Conversely, the connected mother ramets displayed enhanced symptoms of water stress when the connected daughter ramets were exposed to water deficit. The mother ramets had lower water potential ( $\psi_w$ ) due to their stronger osmotic adjustment than in well-watered daughter ramets; this resulted in water flow from the connected daughter ramets to mother ramets, thus alleviating water stress of mother ramets. During soil drying, there was a striking increase in ABA concentrations in leaves of the disconnected mother ramets, whereas leaf bulk ABA was much lower in the connected and water-stressed mother ramets than that in the drought-affected mother ramets in the disconnected group. In this study, though  $G_s$  was linearly correlated with leaf bulk ABA and  $\psi_w$ ,  $G_s$  in water-stressed mother ramets in disconnected group exhibited less sensitivity to the variation in leaf bulk ABA and  $\psi_w$  than that in connected and water-stressed mother ramets. Taken together, these results indicate that: (1) the flux of water translocation between the connected ramets is determined by a water potential gradient; (2) water translocation between connected ramets helps to keep sensitivity of  $G_s$  to ABA and  $\psi_w$  in drought-affected ramets, thereby benefit to effectively maintain the homeostasis of leaf water status and (3) the improvements in  $P_n$  in water-stressed ramets due to water translocation from well-watered ramets suggest the advantages of physiological integration in clonal plants in environments with heterogeneous water distribution.

*Abbreviations* – ABA, abscisic acid;  $G_s$ , stomatal conductance; LRWC, leaf relative water content;  $P_n$ , net CO<sub>2</sub> assimilation rate; SWCs, soil water contents;  $T_r$ , transpiration rate;  $\psi_w$ , leaf water potential;  $\psi_s$ , saturation osmotic potential;  $\Delta\Psi_s$ , osmotic adjustment ability.

## Introduction

Clonal plant species are widespread in the plant kingdom. Most clonal plants consist of independent unit, usually referred to as ramets, which are connected by stolons or rhizomes (Dong 1996, Roiloa and Retuerto 2006, Savini and Neri 2008). Water, nutrients and photosynthates are transported effectively from established ramets to developing ramets through stolons or rhizomes. Although the transport predominantly occurs in one direction, from the older to the younger parts of the clone (acropetal), reciprocal exchange of resources between sibling ramets growing in differing-quality patches has also been demonstrated (basipetal movement) (Alpert 1999, Jonathan 1991, Roiloa and Retuerto 2006).

Water is one of the most critical factors influencing plant growth and development. Generally, plant water status mainly relates to the regulation of root water absorption and water loss via stomatal (Yordanov et al. 2000). In clonal plants, owing to the physical and physiological connection, drought-affected ramets acquire water from connected and well-watered ramets (Cheng et al. 2003, Roiloa and Retuerto 2005). Because the gradient in water potential is the most important driving force for water movement in plant, water translocation among connected ramets should be determined by water potential (Lau and Young 1988, Price et al. 1992). However, observed differences in water potential between the ramets exposed to the heterogeneous water supply were not significant while large quantities of water were transported from the well-watered ramets to the drought-affected ones (Kroon et al. 1996). Therefore, further investigations are required to elucidate the driving force of the water translocation.

Plants lose water primarily through stomatal, which close upon water stress to reduce transpiration (Augé and Moore 2002, Jia et al. 2001, Yordanov et al. 2000, Zhang et al. 2006). It has been reported that abscisic acid (ABA) produced in water-stressed roots is transported to the leaves via the xylem and regulates stomatal movement (Davies et al. 2005, Dodd 2005, Starck 2006), thus affecting plant water status and water balance (Yordanov et al. 2000, Zhang et al. 2006). In clonal plants, physiological integration allows water to be transported from connected and well-watered ramets to drought-affected ramets, thereby alleviating water stress in drought-affected ramets under heterogeneous water environments (Alpert 1999, Cheng et al. 2003, Jonathan 1991, Zhang et al. 2009). Therefore, we wondered whether water translocation in clonal plants during soil drying would play an important role in the regulation of stomatal in water-stressed ramets and, if so, how?

Water regulation and utilization in clonal plants may be advantageous for growth in heterogeneous water environments. Plant water status under water stress has a great influence on photosynthesis, and this has been extensively studied in various plant species (Augé and Moore 2002, Lawlor 2002, Yordanov et al. 2000). So far, however, few studies have been conducted to study the effects of water stress on photosynthesis in clonal plants (Roiloa and Retuerto 2007, Zhang et al. 2009). Therefore, in this study, the effects of water regulation during soil drying in connected ramets on the performance of the photosynthetic apparatus were also carefully investigated.

Accordingly, the following questions were addressed in the present study: (1) the driving force of water translocation in clonal plants and (2) the influence of water translocation on stomatal behavior and photosynthetic activity.

## Materials and methods

### Plant material

On May 21, 2008, strawberry seedlings were collected in the field. Couples of Camarosa ramets (*Fragaria × ananassa*), joined by their own stolons (generally, second and third ramets in a runner chain with four leaves), were transplanted into two pots (28 cm in diameter and 27 cm in height) containing a mixture of turfy soil and sand (4:1, v/v) under natural sunlight, with a maximum irradiance of  $1527 \pm 34 \mu\text{mol m}^{-2} \text{s}^{-1}$  and day/night temperature of 35/22°C. Humidity was about 40–60%. The stolons were kept connected until the daughter ramets had the same size as the mother ramets (approximately 20 cm in height). To minimize potential drought and nutrient stresses, water and fertilizer were supplied sufficiently throughout the study.

### Experiment design

In the present experiments, the connected ramets pairs (the older ramet being referred as mother and the other as daughter) were divided into two groups, in which the stolons between ramets were either cut (disconnected group; 40 couples of ramets pairs) or left intact (connected group; 80 couples of ramets pairs). In order to study the responses of photosynthesis and stomatal conductance to varying water stress, three watering treatments were applied (Table 1): at first, all daughter ramets were continually maintained under optimal irrigation while mother ramets were subjected to drought by omitting irrigation; 4 days later, the connected treatment was divided into two sub-groups;

**Table 1.** Experimental treatments. Ramets were either connected via stolons (Treatments II and III) or the stolons were cut (Treatment I). Water supply was controlled separately for daughters and mothers.

		Mother ramet	Daughter ramet
Unconnected group	Treatment I	Dry (10 days)	Wet (10 days)
Connected group	Treatment II	Dry (10 days)	Wet (10 days)
	Treatment III	Dry (10 days)	Wet (4 days) to Dry (6 days)

one sub-group (40 couples of ramets pairs) consisted of daughter ramets that were continually well irrigated, whereas the other sub-group (40 couples of ramets pairs) consisted of daughter ramets that began to suffer water shortage gradually upon withholding water. During the experiments, a waterproof shed was utilized on rainy days to avoid possible effects of rainwater on the water content in pots.

All measurements were conducted every other day in July and August. The first fully expanded leaves were used for all measurements.

#### Measurement of soil water content and leaf relative water content

Soil water contents (SWCs) were determined gravimetrically every other day throughout the whole drying period. After the soil fresh weight ( $F_w$ ) in pot was determined, the soil was oven dried at 105°C for 24 h to determine the dry weight ( $D_w$ ). The SWC was calculated as  $SWC = 100 \times (F_w - D_w)/D_w\%$ .

Leaf relative water content (LRWC) was determined as  $LRWC = 100 \times (F_w - D_w)/(T_w - D_w)\%$ , where  $F_w$  is the fresh mass of leaves,  $T_w$  the turgid mass after 4 h floating on distilled water at 25°C (irradiance 15  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and  $D_w$  the dry mass after oven drying for 24 h at 80°C.

#### Measurement of water potential and saturation osmotic potential

Leaf water potential ( $\psi_w$ ) was measured at 8:00 a.m. with a HR-33-TR Dew Point Microvoltmeter (Wescor) after leaf disks (diameter: 6 mm) had been equilibrated in the chamber (Wescor C52 chamber) for 2 h (Bethke and Drew 1992).

Saturation osmotic potential ( $\psi_s$ ) was determined in the similar way as the measurement of  $\psi_w$  after the cell sap was collected according to Flagella et al. (1996) with minor modification. First the leaf samples were floated on distilled water for 12 h at 25°C, and wiped with filter paper to remove excess liquid. Then the sample was packed in vacuum bags and frozen for 12 h at -20°C. After that, the frozen samples were allowed to thaw for

30 min. The cell sap from thawed leaves was extracted by a glass syringe, and then the extract (5  $\mu\text{l}$ ) was put onto the filter paper disks (diameter: 6 mm) in the sample chamber (Wescor C52 chamber).

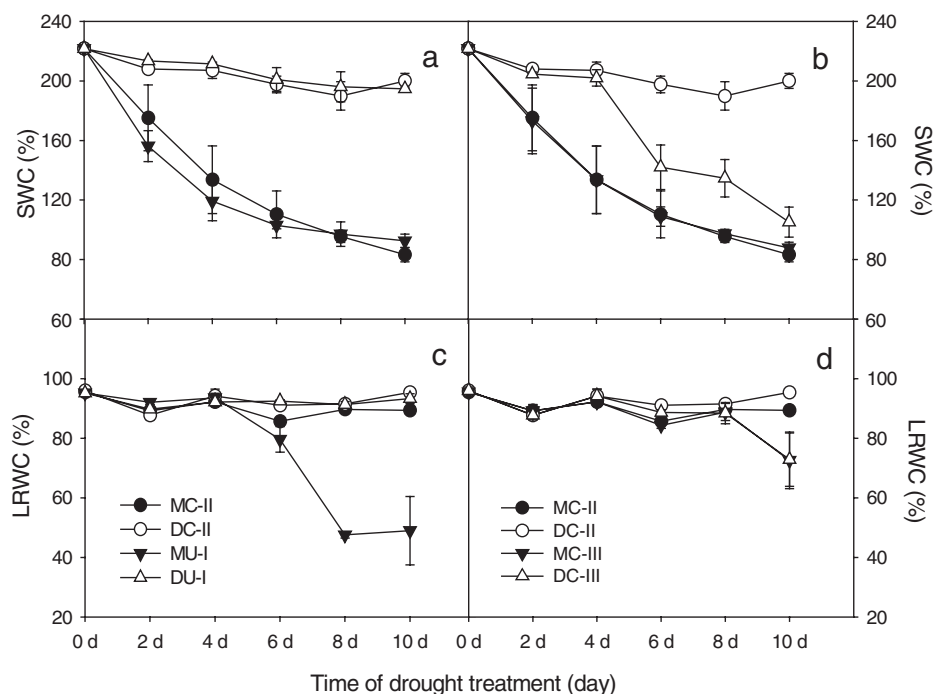
Osmotic adjustment ability ( $\Delta\Psi_s$ ) was calculated according to the formula  $\Delta\Psi_s = \Psi_u^{100} - \Psi_s^{100}$  (Flagella et al. 1996), where  $\Psi_u^{100}$  is the osmotic potential at saturation in unstressed leaf and  $\Psi_s^{100}$  the osmotic potential at saturation in stressed leaf.

#### Gas exchange analysis

Gas exchange was measured at a leaf chamber temperature of 25–30°C and ambient CO<sub>2</sub> concentration (350  $\mu\text{mol mol}^{-1}$ ) under an irradiance of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (saturated light intensity) with the first fully expanded leaves, using a gas exchange system (Li-6400, Li-Cor, Lincoln, NE) equipped with a light source (6200-02B LED, Li-Cor). All measurements were performed before 10:00 a.m., so photoinhibition of photosynthetic apparatus was avoided throughout the experiments. Net CO<sub>2</sub> assimilation rate ( $P_n$ ), stomatal conductance ( $G_s$ ) and transpiration rate ( $T_r$ ) were recorded when CO<sub>2</sub> uptake was steady.

#### ABA analysis

The method for extraction and purification of ABA was modified from that described by He (1993). Leaf samples of 2 g were harvested following measurements of gas exchange and ground in a mortar (at 0°C) in 10 ml 80% (v/v) methanol extraction medium containing 1 mM butylated hydroxytoluene as an antioxidant. The extract was incubated at 4°C for 4 h and centrifuged at 4000 rpm for 15 min, also at 4°C. The supernatant was passed through a C18 SepPark column (Waters Assoc., Bedford, MA), which had been pre-washed with 10 ml 100% and 5 ml 80% methanol, respectively. The column was then washed and eluted with 10 ml 100% methanol and 10 ml ether, respectively. The hormone fraction eluted with methanol and ether from the column was dried under N<sub>2</sub>. It was then dissolved in 2 ml phosphate buffer saline (PBS) containing 0.1% (v/v) Tween 20 and 0.1% (w/v) gelatin (pH 7.5) for analysis by an enzyme-linked immunosorbent assay (ELISA). The mouse monoclonal antigens and antibodies against ABA, and immunoglobulin G-horseradish peroxidase (IgG-HRP) used in the ELISA were produced at the Phytohormones Research Institute, China Agricultural University, China (He 1993). The method for quantification of ABA by ELISA was described by Yang et al. (2001). In order to exclude the influence of the difference in leaf water



**Fig. 1.** Effects of water stress on SWC and LRWC in strawberry. Data are means  $\pm$  SE ( $n = 5$ ). MU-I, mother ramets in disconnected group in Treatment I; DU-I, daughter ramets in disconnected group in Treatment I; MC-II, mother ramets in connected group in Treatment II; DC-II, daughter ramets in connected group in Treatment II; MC-III, mother ramets in connected group in Treatment III; DC-III, daughter ramets in connected group in Treatment III.

status on ABA content, all ABA contents were expressed on a dry leaf weight basis.

Each experiment was performed at least five times with five ramets independently.

## Results

### Changes in soil and leaf relative water content

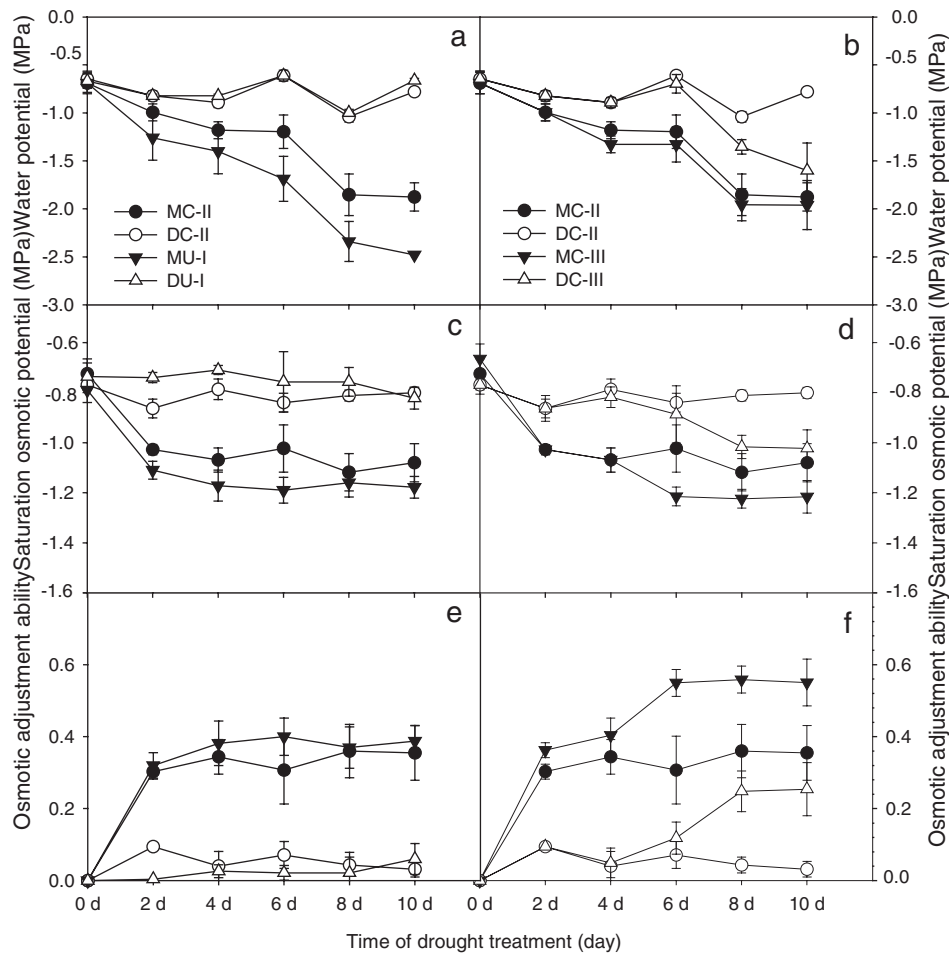
As shown in Fig. 1, the SWC declined rapidly after withholding water from pots, whereas the values of SWC in well-watered pots remained high and relatively constant (Treatments I and II) (Fig. 1A). Four days later, previously well-watered daughter ramets in connected group were subjected to water shortage; their SWC also declined rapidly (Treatment III) (Fig. 1B).

To check the changes in plant water status after withholding water, LRWC was determined. The LRWC of the mother ramets in the disconnected group (Treatment I) decreased markedly (to about 48%). In contrast, the LRWC of the mother ramets connected with well-watered daughter ramets (Treatment II) decreased marginally and was about 5% lower than that in control plants (Treatment I) after 10 days of drying (Fig. 1C). In Treatment III, the soil water deficit that started on Day 4, imposed on previously well-irrigated daughter

ramets, not only caused a considerable decrease in the LRWC of daughter ramets by Day 10 but also a further decrease in the LRWC of the connected mother ramets (Fig. 1D, where the two points overlap). All the above data indicated that the water stress experienced by mother ramets was influenced by the water status of the connected daughter ramets.

### Changes in leaf water potential and osmotic adjustment ability

As shown in Fig. 2, the water potential ( $\psi_w$ ) and the saturation osmotic potential ( $\Psi_s$ ) in well-watered daughter ramets in Treatments I and II remained high and relatively constant throughout the duration of drought treatment. However, in mother ramets (not watered),  $\psi_w$  and  $\Psi_s$  significantly declined for both connected and disconnected groups (Treatments I and II) with progressive soil drying and tissue dehydration. The values of  $\psi_w$  and  $\Psi_s$  in water-stressed mother ramets in the connected group (Treatment II) were higher than those in the water-stressed mother ramets in the disconnected group (Treatment I) (Fig. 2A, C). In Treatment III, soil drying of previously well-watered daughter ramets not only resulted in their own rapid decrease in  $\psi_w$  and  $\Psi_s$  but also a further decline in these



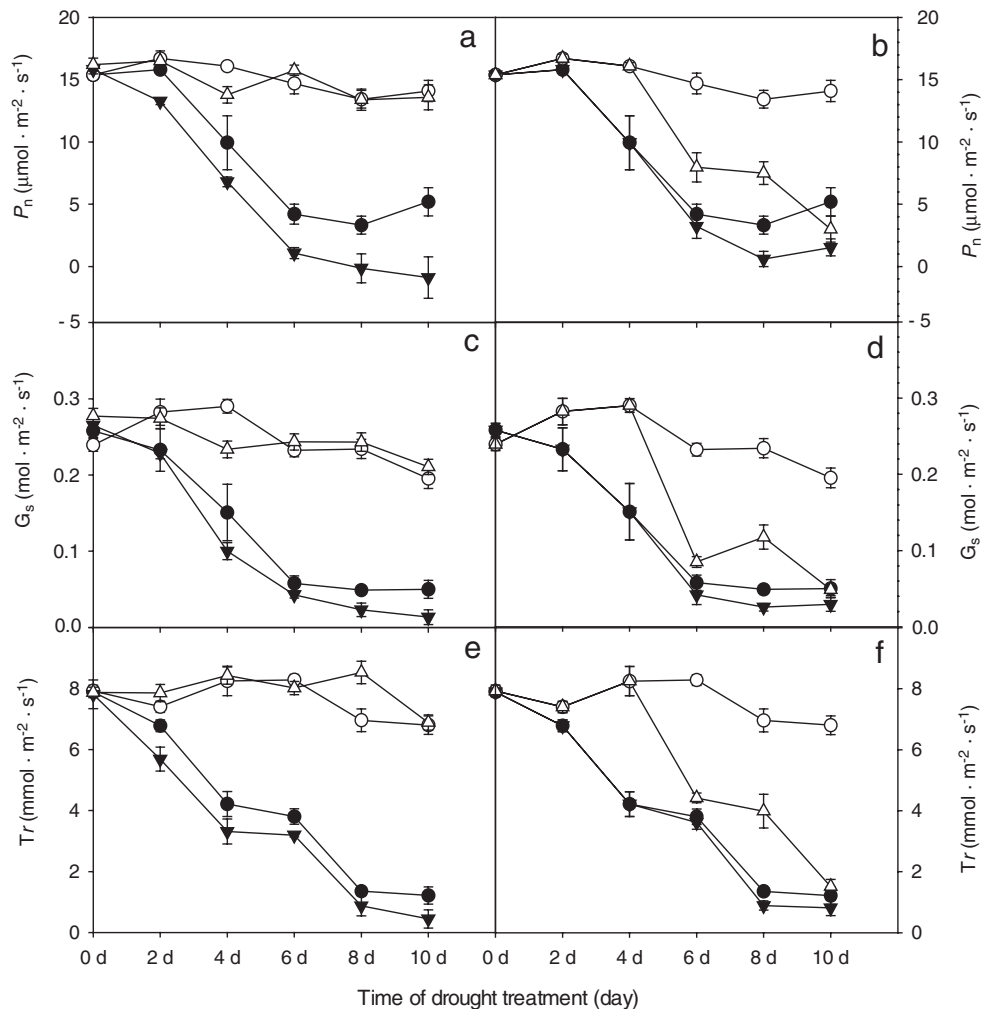
**Fig. 2.** Effects of water stress on water potential ( $\psi_w$ ), saturation osmotic potential ( $\Psi_s$ ) and osmotic adjustment ability ( $\Delta\Psi_s$ ) of leaves in strawberry. Values are means  $\pm$  SE ( $n = 5$ ).

two parameters for the connected and water-stressed mother ramets (Fig. 2B, D).

To estimate the osmotic adjustment ability ( $\Delta\Psi_s$ ), we calculated  $\Delta\Psi_s$  according to the osmotic potential at saturation in an unstressed leaf ( $\Psi_u^{100}$ ) and the osmotic potential at saturation in a stressed leaf ( $\Psi_s$ ). As shown in Fig. 2E, the well-irrigated daughter ramets had a low  $\Delta\Psi_s$  both in connected and disconnected groups (Treatments I and II); however, soil water deficit of the mother ramets caused a large increase in  $\Delta\Psi_s$  in parallel with the decreases in  $\Psi_w$  and  $\Psi_s$ . In Treatment III,  $\Delta\Psi_s$  in both mother and daughter ramets was substantially enhanced upon withholding water (Fig. 2F), and the values of  $\Delta\Psi_s$  were higher than those in Treatment II, respectively, implying that soil water deficit of daughter ramets not only strengthened their own  $\Delta\Psi_s$  but also in the connected and water-stressed mother ramets.

### Changes in photosynthetic rate, stomatal conductance and transpiration rate

Fig. 3 illustrates the time course of changes in photosynthetic rates ( $P_n$ ), stomatal conductance ( $G_s$ ) and transpiration rate ( $T_r$ ) during soil drying.  $P_n$ ,  $G_s$  and  $T_r$  in well-watered daughter ramets of Treatments I and II remained unchanged throughout the experimental period. Soil water deficit significantly decreased  $P_n$ ,  $G_s$  and  $T_r$  in mother ramets of Treatments I and II; the extent of this decrease was in parallel with the degree of soil water deficit such that greater water deficit led to more significant inhibition of  $P_n$ ,  $G_s$  and  $T_r$  (Fig. 3A, C, E). Compared with Treatment I, the values of  $P_n$ ,  $G_s$  and  $T_r$  in mother ramets in the connected group (Treatment II) were higher than those in mother ramets in the disconnected group (Treatment I) (Fig. 3A, C, E), which implied that well-watered daughter ramets in Treatment II released the water stress in the mother ramets because of water translocation via intact stolon.



**Fig. 3.** Effects of water stress on photosynthetic rate ( $P_n$ ), stomata conductance ( $G_s$ ) and transpiration rate ( $T_r$ ) in strawberry. Values are means  $\pm$  SE ( $n = 6$ ).

In Treatment III, a sharp decline in  $P_n$ ,  $G_s$  and  $T_r$  in the daughter ramets was observed as soil water deficit increased; similarly, exposure of daughter ramets to soil water deficit also caused a further depression in  $P_n$ ,  $G_s$  and  $T_r$  in the water-stressed mother ramets (Fig. 3B, D, F), revealing that water translocation from daughters to drought-affected mothers was lessened.

### Changes in leaf ABA content

As shown in Fig. 4, leaf ABA contents in well-watered daughter ramets were kept at a relatively low level in both connected and disconnected groups. During soil drying, there was a striking increase in ABA accumulation in leaves of the disconnected mother ramets (Fig. 4A). By contrast, leaf bulk ABA in the water-stressed mother ramets of the connected group (Treatment II) was

much lower than that in the drought-affected mother ramets in the disconnected group (Treatment I) (Fig. 4A). In Treatment III, water stress resulted in synchronous elevation in leaf ABA contents in both mother and daughter ramets; further, leaf ABA contents in the mother ramets in Treatment III were significantly higher than those in the daughter ramets (Fig. 4B).

### Discussion

#### The driving force of water translocation between connected ramets

Kroon et al. (1996) considered that the quantity of water translocation was unrelated to the difference in water potential between connected ramets because they observed little difference in water potential. Our results do not support this conclusion. In the present

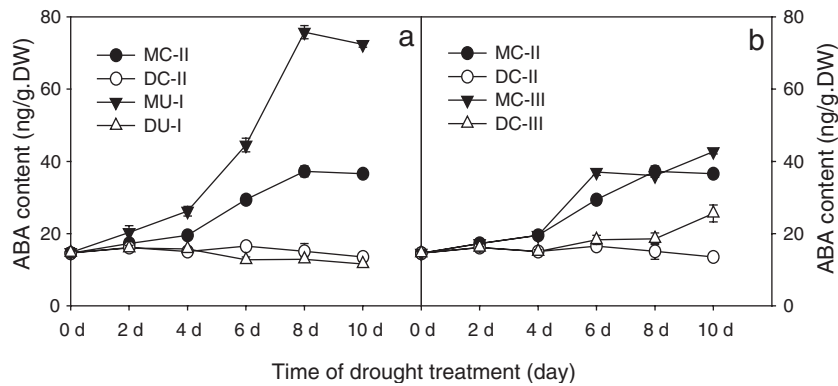


Fig. 4. Effects of water stress on ABA accumulation in leaves of strawberry. Values are means  $\pm$  SE (n = 6).

study, the well-watered daughter ramets in Treatment II released the water stress in the mother ramets because of water translocation via intact stolon, as shown by the improvement in leaf water status,  $P_n$ ,  $G_s$  and  $T_r$  in water-stressed mother ramets compared with those in disconnected group (Fig. 3). During this treatment, the LRWC of water-stressed mother ramets was almost equal to that of daughter ramets owing to the water supply from well-watered daughter ramets (Fig. 1C, D); this occurred even though an increase in water potential gradient between mother and daughter ramets was observed (Fig. 2A, B). In Treatment III, withholding water in daughter ramets not only caused a rapid decrease in LRWC and photosynthetic performance in the daughter ramets themselves but also a further decline in these parameters in the connected mother ramets (Figs 1 and 3), suggesting that water translocation from daughter to drought-stressed mother was lessened. More importantly, the gradient in water potential between mother and daughter ramets was also obviously reduced in Treatment III when compared with Treatment II (Fig. 2B). Accordingly, the flux of water translocation between connected ramets is related to the difference in water potential gradient. However, Kroon et al. (1996) thought that water translocation was not related to the water potential difference between connected ramets. Obviously, our result is inconsistent with the observation of Kroon et al. (1996). Possibly, with a pressure chamber, the choice of *Carex* as plant material might make observation of the balancing pressure a little difficult. In our study, water potential in strawberry leaves could be easily and conveniently obtained using Dew Point Microvoltmeter. In addition, the discrepancy between the investigation by Kroon et al and ours may also result from a large difference in vapor pressure deficit (VPD) and plant age. To reveal the detailed causes of the discrepancy between the two studies, further investigation is required. According to

our results obtained from strawberry, we believe that a water potential gradient was obviously established between connected ramets under a heterogeneous water environment and that water translocation between connected ramets should be determined by the difference in water potential. Of course, if the differences in leaf water potential between interconnected ramets exposed to heterogeneous treatments were really not significant in *Carex* plants as reported by Kroon et al. (1996), then the leaf area and evaporation may well explain the water translocation.

Water stress can induce the accumulation of osmotic compatible substances, increasing their osmotic adjustment ability, which in turn effectively prevents cells from dehydration and enhances drought resistance (Lawlor 2002, Ludlow 1987, Yordanov et al. 2000). Theoretically, leaf water potential depends on changes in leaf osmotic potential and pressure potential (Leaf water potential = Leaf osmotic potential + Pressure potential). In this study, we demonstrated that the changes in leaf water potential were mainly a result of osmotic adjustment, revealed by the decrease in the saturation osmotic potential and an increase in the osmotic adjustment ability (Fig. 2). By strengthening  $\Delta\Psi_s$ , the water-stressed mother ramets would maintain low water potential, facilitating water flow from connected and well-watered daughter ramets (Fig. 2). In contrast, high  $\Delta\Psi_s$  and low water potential in the water-stressed daughter ramets would minimize water outflow when water supply is in deficit in the whole clonal system. This may account for our observation of why water translocation from daughter to mother ramets was reduced in Treatment III.

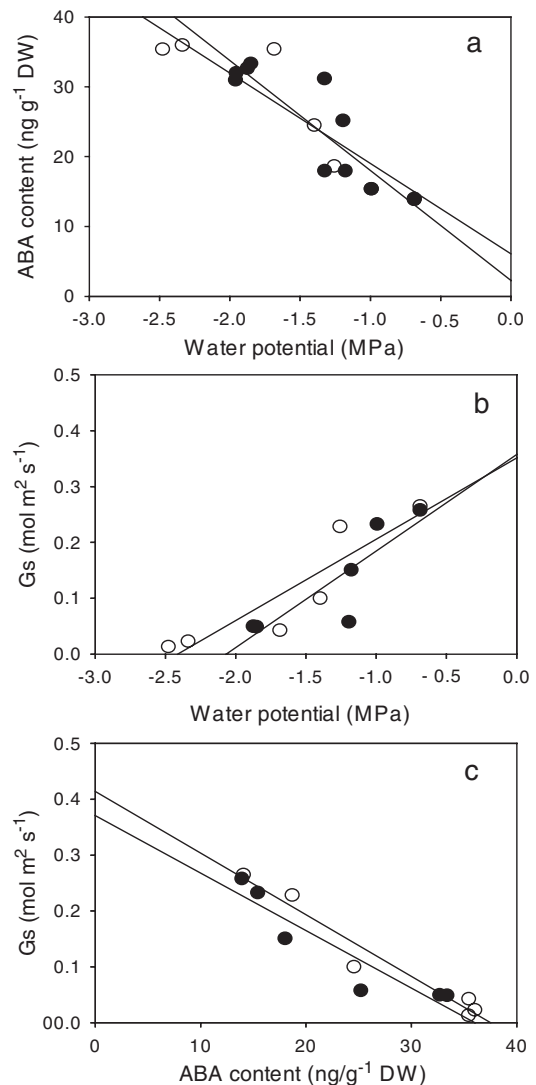
#### Effects of water translocation on stomatal regulation

For clonal plants, besides water translocation between ramets, stomatal regulation is another important factor

influencing water utilization under water-deficit conditions. Yet, whether water translocation plays an important role in the regulation of stomatal has not been clearly revealed. Our data demonstrated that water translocation from well-watered ramets (donor ramets) could effectively alleviate the decrease in  $G_s$  in water-stressed ramets (recipient ramets), whereas  $G_s$  in donor ramets was not affected (Fig. 3C). In contrast, water translocation between the connected ramets would exacerbate the decline in  $G_s$  in donor ramets when the whole clonal system was exposed to water deficit gradually (Fig. 3D).

Stomatal conductance is restricted when leaf water potential declines due to soil drying (Davies et al. 2005, Dodd 2005, Jia et al. 2001). In this study, the linear relationship between  $G_s$  and  $\psi_w$  supports this conclusion (Fig. 5B). With respect to the mechanisms of stomatal movement, various plant species have been extensively investigated. Most previous studies demonstrated that a decrease in stomatal conductance is often related to an increase in ABA concentration in the leaves or the xylem flux (Jia et al. 2001, Yordanov et al. 2000, Zhang et al. 2006). Our data also demonstrated that leaf bulk ABA was negatively correlated with water potential both in connected and disconnected ramets during soil drying (Fig. 5A), indicating that leaf ABA content was significantly increased as  $\psi_w$  declined. More importantly, the mother ramets exhibited a rapid increase in leaf ABA content and a significant decrease in stomatal conductance during soil drying both in connected or disconnected group (Figs 3 and 4). Further analysis revealed that  $G_s$  and leaf bulk ABA contents show a significant linear relationship (Fig. 5C), revealing that ABA plays an important role in the control of leaf stomatal closure under water stress in clonal plants. However, we observed that the values of  $G_s$  in the mother ramets in connected groups were lower than that in disconnected mother ramets at a given leaf bulk ABA content or  $\psi_w$  (Fig. 5B, C), demonstrating that physiological integration in clonal plants can enhance the sensitivity of  $G_s$  to ABA and  $\psi_w$  under water stress. This may be of great significance in maintaining high water use efficiency in clonal plant in spatially heterogeneous environments.

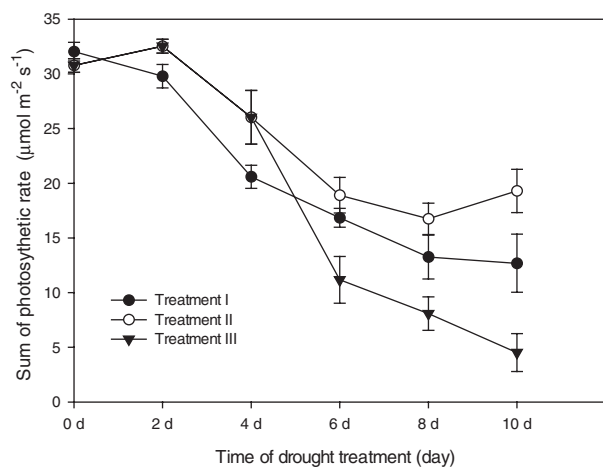
In addition, our result revealed a large difference in leaf bulk ABA and  $G_s$  between the drought-affected mother ramets and the well-watered daughters in Treatment II (Figs 3 and 4). Was ABA produced in water-stressed ramets delivered through stolons to connected and well-watered ramets, thereby affecting their stomatal conductance? As we know, ABA produced from the water-stressed root is transported to the leaf mainly with upward xylem flow along a water potential gradient. Actually, the water potential in water-stressed ramets was



**Fig. 5.** Linear correlations between leaf bulk ABA contents and water potential ( $\psi_w$ ) (A); stomatal conductance ( $G_s$ ) and  $\psi_w$  (B); and  $G_s$  and ABA (C), both in connected (●) and disconnected (○) groups. All data are from Figs 2–4.

lower than that in connected and well-watered ramet (Fig. 2). Possibly, ABA could not be transported between connected ramets against the water potential gradient. However, many other factors also have important influence on leaf bulk ABA, including degradation of leaf ABA, etc. (Jiang and Hartung 2008, Yordanov et al. 2000, Zhang et al. 2006). Thus, leaf bulk ABA content may not afford direct proof for the ABA flow between the ramets pairs. To clarify this question, further studies are still required. Yet, one conclusion can be reached in this study that the stomatal conductance in well-watered ramets is not affected by the production of ABA in the drought-stressed neighboring ramets.





**Fig. 6.** Sum of photosynthetic rates in a pair of ramets system in different treatments. Data were derived from Fig. 3.

### Effects of water regulation on photosynthetic capacity in clonal plant

Water stress has a great influence on plant photosynthetic capacity (Lawlor 2002, Yordanov et al. 2000). In the present study,  $P_n$ ,  $G_s$  and  $T_r$  decreased rapidly in parallel with the decrease in SWC (Figs 1 and 3), indicating that the soil water status of a ramet itself is a core factor responsible for the decline of  $P_n$ ,  $G_s$  and  $T_r$  in water-stressed ramets for either connected (Treatment II) or disconnected groups (Treatment I). However,  $P_n$  of mother ramets in the connected group (Treatment II) was higher than that in the disconnected group (Treatment I) when daughter ramets had ample water supply (Fig. 3). For a connected group (Treatment II), a well-watered ramet not only improved the  $P_n$  of a water-stressed ramet through water supply but also enhanced the combined photosynthetic capacity and photosynthesis efficiency for a pair of connected ramets compared with a pair of disconnected ones (Fig. 6). From these results, it is easy to see that physiological integration in strawberry enhances the photosynthesis and drought tolerance of water-stressed ramets, enabling the clones to maintain their performance in less favorable environments. However, water-stressed ramets disconnected from well-watered ramets performed like a non-clonal plant, and their photosynthetic capacity was strikingly suppressed due to absence of physiological integration (Fig. 3), thus exhibited less drought tolerance. Therefore, the maintenance of physiological integration among ramets of clonal plants can increase their survival and growth when connected ramets are located in soil with large differences in water availability.

Although physiological integration may be beneficial to the whole clone, it may also involve costs, such as the

maintenance of stolons, rhizomes and dependent ramets (Cheng et al. 2003, Dong 1996). Particularly when the dependent ramets are growing under extremely stressed conditions, physiological integration may result in lower fitness than if ramets were physiologically independent (Salzman and Parker 1985). In our investigation, when both the connected ramets are subjected to water stress, such as Treatment III, with the progressive water stress in daughter ramets, not only the  $P_n$  of the daughter ramets exhibited a large decrease but also the  $P_n$  of the connected and water-stressed mother ramets was decreased further (Fig. 3B). Clearly, these results demonstrated that the net benefits of physiological integration directly depend on water acquisition by donor ramets, and that water translocation may be reduced when donor ramets cannot acquire water in excess of their own demands. Because of the deficit of water resources in the whole clone system, the relationship between the connected ramets is no longer that of rescuer and rescuee, but competitors. The competition between them for water not only resulted in a strikingly decreased  $P_n$  in each ramet but also a considerable decrease in the sum of photosynthetic rate of the pair of ramets (Fig. 6). Consequently, the growth and survival capacity of the ramets would be unavoidably decreased due to strong water competition between ramets.

In summary, we conclude that: (1) the flux of water translocation between the connected ramets is determined by water potential gradient; (2) water translocation between connected ramets helps to maintain a high sensitivity of  $G_s$  to ABA and  $\psi_w$  in drought-affected ramets, thereby effectively maintaining the homeostasis of leaf water status; and (3) the improvements in  $P_n$  in water-stressed ramets due to water regulation from well-watered ramets suggest the growth advantages of physiological integration in clonal plants in environments with heterogeneous water distribution.

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