African Journal of Biotechnology Vol. 9(34), pp. 5503-5510, 23 August, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Long term water integration in interconnected ramets of stoloniferous grass, buffalograss

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Accepted 8 July, 2010

Buffalograss (*Buchloë dactyloides*) is known for its drought resistant character. Natural resources are patchily distributed and resource sharing between interconnected ramets can enhance the performance of the whole genet. In order to examine whether there exists long term water integration between interconnected ramets of buffalograss, a greenhouse experiment was conducted. Interconnected ramet pairs of stoloniferous buffalograss were planted in two partitioned similar-sized containers and subjected to homogeneous (20 ml pot^{-1} d^{-1} or 100 - 150 ml pot^{-1} d^{-1}) or heterogeneous (20 ml pot^{-1} d^{-1} vs. 100-150 ml pot^{-1} d^{-1}) water supply; the whole experiment lasted for 91 days. In heterogeneous treatment, water translocation was equally effective in acropetal and basipetal directions. Elder ramet was more efficient in water use, but rooted ramet of elder ramet in moist condition experienced significant cost when it was connected to younger ramet in dry condition; whereas, no cost was found in any fragment of younger donor ramet. Ramet in dry condition produced more biomass than its connected ramet in moist condition and developed larger leaves. This "oversharing" phenomenon indicated that no net cost was involved in water integration, and water might not be the only resources transported within stolon xylem. Overall, long term water integration is an important strategy for buffalograss to cope with adverse natural drought conditions.

Key words: Water integration, interconnected ramets, heterogeneous treatment, *Buchloë dactyloides*, oversharing.

INTRODUCTION

Buffalograss (*Buchloë dactyloides*) is a warm season, stoloniferous C_4 plant, which is native to the Great Plains of North America (Frank et al., 2004). It was introduced to China in the 1940s and is widely use for water conservation in arid or semi-arid region of north China, like roadside where resources distribution is highly patchy. The drought resistant character of buffalograss has been related to its deep rooting system. It was reported that water absorbed by deep roots in moist condition can move through roots and leak into dry surface soil to maintain nutrients uptake (Huang, 1999).

For clonal plants like buffalograss that propagate vegetatively through stolon and spread over a larger area than any other non-clonal plants could, there exists a high possibility that one clone may encounter heterogeneous environments (Kleijn and Groenendael, 1999; Liao et al., 2003; Yu et al., 2004). The stolon not only functions as a storage organ, but also a tunnel for resources integration between interconnected ramets. Clonal integration is well-documented; three widely studied integrated resources refer to water, nutrients and carbohydrates. Compared to nutrient and carbohydrate, research with regards to water integration within clonal plant is comparatively limited (de Kroon et al., 1996; van Kleunen and Stuefer, 1999; Zhang et al., 2003a).

Translocation of water within interconnected clonal ramets was reported to be affected by xylem continuity, water gradient, leaf area (transpiration) and interior programmed sectoriality (Stuefer, 1996). In monocotyledon plant, vascular constraint is usually absent, and water can

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Abbreviations: EDR, elder daughter ramet; **YDR,** younger daughter ramet.

be extensively and intensively integrated within interconnected ramets, such as in *Psammochloa villosa* clones (Dong and Alaten, 1999; Liu et al., 2006). However, interior programmed sectoriality within clones refers to the offspring ramets that are weaned from mother ramet as soon as they get rooted, and this has been observed in *Calathea marantifolia* (Matlaga and da Sternberg, 2009) and *Calamagrostis epigejos* (Zhang et al., 2003b). Tracers, like dye and deuterium labelling, have been employed to demonstrate water translocation pattern among interconnected ramets (Alpert, 1990; de Kroon et al., 1996; Zhang et al., 2003a), however, these kinds of experiments can only last for short-term. Our purpose of carrying out this work was to examine whether there exists long-term water integration among rooted interconnected ramets. Similar approach has been applied in another monocotyledonous plant *C. epigejos* (Zhang et al., 2003b) and dicotyledonous plant *Hydrocotyle peduncularis* (Peterson and Chesson, 2002).

Cost and benefit are always involved in clonal integration. For water integration, the cost and benefit are species-specific or variety-specific. Some species like *Carex hirta*, because of the effectiveness of water translocation, the final benefit paralleled the cost in integration, the clonal plants performed equally well in uniform and patchy water treatment. Whereas, for another *Carex* species, *Carex flacca*, water integration incurred significant cost for both donor and recipient ramets when mother ramets in moist conditions were connected with daughter ramets in dry conditions (de Kroon et al., 1996). While in some species, cost was only associated with elder donor ramets during water translo-cation, but not younger donor ramet, like *H. peduncularis* (Peterson and Chesson, 2002).

We conducted an experiment with interconnected mature ramets pairs experiencing homogeneous or heterogeneous water supply for 3 months. The objective of this study is to investigate how clonal integration affected the response of buffalograss to heterogeneous water supply. Three questions were raised up here: (1) Is there any existence of water integration between interconnected mature ramets pairs? If so, then how does integration affect the biomass of donor and recipient ramets? (2) What was transported through stolon, water alone or coupled with other resources? (3) What kind of morphological changes is involved?

MATERIALS AND METHODS

The species

The tested species were introduced from one wild variety in America in 1995, then propagated in the experimental field of the Temple of Heaven (39°53′N, 116°24′E, 63 m a.s.l.), Beijing, for 3 years. During this period, one plant was found to keep green for longer time in late autumn, and thus collected and named as ‘JingYin Yeniucao’. Subsequently, this special variety was vegetatively propagated in another experimental field of the Temple of Heaven from 1998 to 2005.

All experimental materials were derived from the same buffalograss plant, which was a clone of ‘JingYin Yeniucao’, to ensure that any difference found in the treatments were not due to genotypic effects. The experiment was conducted at the greenhouse of the Temple of Heaven, Beijing, China, from March 11 to September 7, 2005.

The experimental design

On March 11, 2005, similar-sized ramets (mother ramet) consisting of an initial stolon (primary stolon) 1.5 cm in length, with three leaves and attached adventitious roots were grown in containers of 16 cm in diameters and 13 cm in height, filled with an even mixture of washed horticultural sand and peat-based potting compost (volume 1:1). During the experiment, the primary stolon of each mother ramets was arranged in a line, two newly produced ramets on each primary stolon were allowed to root in two adjoined same sized containers (16 cm in diameters and 13 cm in height), filled with river sand. One of the ramets in each pair is referred to as elder daughter ramet (EDR), indicating its relative proximity to the mother ramet, and the other initial distal ramet as younger daughter ramet (YDR), while other ramets were kept unrooted. On July 6, 2005, the two newly produced ramets (EDR and YDR) and the stolon part connecting them were separated from the mother ramet by severing the stolon and the other side of the stolon was removed as well (Figure 1).

In a glasshouse experiment, similar-sized ramets EDR (proximal to parent ramet) and YDR (distal one) connected by uniform length of stolons were grown in two partitioned similar-sized containers. In homogeneous treatments, both ramets were given the same amount of water supply, high level (or low). In heterogeneous treatment, one ramet was supplied with high level of water and the other was kept in low level. The high and low level of water supply were 100 - 150 and 20 m tap water per container per day, respectively. The ramets in water treatment were given 30 ml 50% full-strength nutrient solution per container per week throughout the experiment to ensure the basic nutrient requirement of the plants (Yu and Dong, 2003). There were 6 replicates for each treatment. The plants were harvested after 91 days of treatments (from July 6 to September 7, 2005) and the following characters were measured: The above-ground biomass and root biomass of EDR and YDR, the primary stolon length, number, weight and node numbers of EDR and YDR. All characteristics were analyzed by means of one-way Analysis of Variance

In this experiment, ramet pairs (EDR and YDR) connected by stolon were subjected to homogeneous and heterogeneous treatments of water supply (LL, HH, HL and LH) (Table 1). All experimental units were randomly distributed in the glasshouse to eliminate the position effects. Before treatments, all ramets were size-standardized by removing all the leaves except three youngest and cutting roots to 3 cm in length. Therefore all experimental ramets were similar in morphology and weight. The high and low level of water supply were 100 - 150 and 20 ml tap water per container per day, respectively. The ramets in water treatment were given 30 ml 50% full-strength nutrient solution (4.373 g NH_4NO_3 L^{-1}, 2.063 g NaH_2PO_4 L^{-1} and 2.876 g KCl L^{-1}) per container per week throughout the experiment to ensure the basic nutrient requirement of the plants (Yu and Dong, 2003). There were 6 replicates for each treatment. Waterlogging did not occur because the pots were free draining.

Measurement and analysis

The plants were harvested after 91 days of treatments and the following characters were measured: The above-ground biomass and root biomass of both EDR and YDR, the primary stolon number, length, weight and node numbers of both EDR and YDR, leaf length and width of EDR and YDR. All the biomass (dry weight) was determined (0.0001 g) after drying the plant portions at 75°C for 48 h

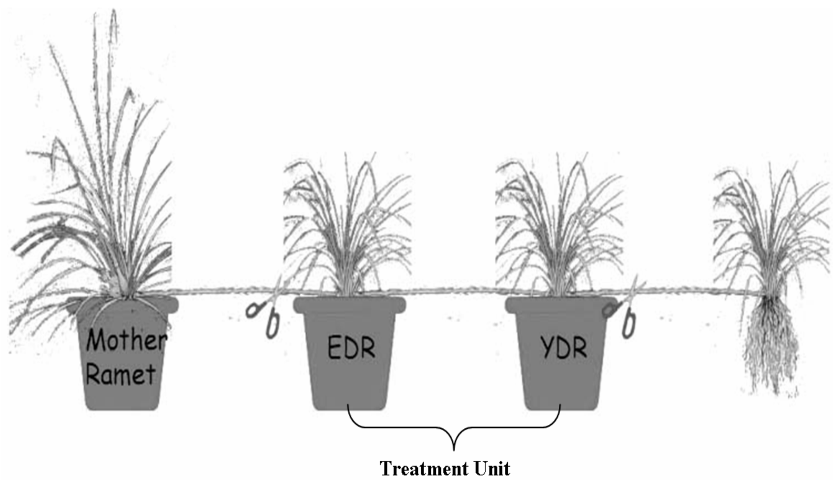

Figure 1. Diagrammatic presentation of a monopodial buffalograss stolon segment consisting of mother ramet, elder daughter ramet (EDR) and younger daughter ramet (YDR) at the start of this experiment.

Table 1. The pattern of water application to ramet pairs.

Treatment	Elder daughter ramet (EDR)	Younger daughter ramet (YDR)
LL	Low water	Low water
LH	Low water	High water
HL	High water	Low water
HH	High water	High water

Characteristics were analyzed by means of one-way analyses of variance.

RESULTS

Water integration

Because both ramets initiated new stolons and produced offspring ramets during the experiment, above-ground biomass included two components: Mass of the original rooted ramet and mass of its new stolons and offspring ramets. Below-ground biomass referred to the sum of root mass and tillering nodes.

No programmed sectoriality was found between buffalo-grass interconnected ramets after 3 months experiment. Ramets in dry pots gained significant benefits from interconnected wet ramets, whereas, no cost was caused for wet donor ramets and younger donor ramet even accumulated more biomass than when it was under uniform moist treatments. Both ramets under hetero-geneous dry treatments developed significance more above-ground biomass than the corresponding ramets did under homogeneous dry treatments, 71.6 and 144.5% for EDR and YDR separately. However, under-ground biomass of both ramet were only slightly enhanced (Figure 2a and b). Ramets in moist conditions did not experience any cost when connected to ramets in dry condition. In contrast, YDR in moist patches developed 26.4% more above-ground biomass when it was asso-ciated with EDR in dry patches (Figure 2a). It was clear that there existed extensive water integration between interconnected EDR and YDR ramets.

The highest biomass of EDR was not developed under heterogeneous wet treatment or uniform wet treatment, but under heterogeneous dry treatment (Figure 2a and b). One possible reason was that the optimal water supply for EDR was neither high nor low. Another possible explanation would be that what was transferred from YDR to EDR under LH treatment was not only water; there might be other resources as well, like nutrients, which was usually dissolved in water and extracted by roots together. No significant difference was found between EDR above-ground biomass under uniform high and low water treatment. The reason might be that the water supply of EDR under homogeneous high water treatment was in excess of EDR's water requirement.

Difference between EDR and YDR

Under homogeneous water treatment, EDR produced more significant primary stolons than YDR (Figure 2e), albeit, rooted ramet biomass of EDR and YDR were similar (Figure 2c). However, because primary stolons and new ramets accounted for the largest portion of above-ground biomass, the final above-ground biomass of EDR was much higher than that of YDR. Above-ground biomass of EDR did not differ between homogeneous high and low water supply, whereas, YDR developed more than doubled above-ground biomass with enhanced water supply (Figure 2a). Compared to EDR, YDR invested a larger portion of its biomass to roots and resulted in higher root to shoot ratio in all treatments (Figure 3C). All these suggested that elder ramets were more efficient in water use and surplus water supply did not really gain extra biomass for EDR.

To further elucidate the difference between EDR and YDR ramets, we measured leaf area in homogeneous and heterogeneous water treatment. In uniform water treatment, leaf width showed positive response to water supply, whereas, leaf length did not respond to water supply while leaf area was positively correlated to water supply. In heterogeneous water treatments, leaf width of EDR and YDR was equalized because of the connection, the magnitude were as much as leaf width of ramets in moist conditions (Table 2). However, ramets in dry conditions produced slightly longer leaves than interco-nnec-ted ramets in moist conditions (Table 2), which suggested that ramets in dry conditions had slightly larger leaf area than interconnect wet ramets.

Pattern of biomass distribution

Compared to rooted ramets, new stolons and offspring ramets were more responsive to heterogeneity, and primary stolon biomass accounted for more than 80% of above-ground biomass. Rooted ramets of EDR in moist conditions experienced 26% biomass cost when it was connected to YDR in dry condition (Figure 2c), whereas, there was no cost for new stolons and offspring ramets (Figure 2e). The benefit that EDR in dry conditions gained from connection to YDR in moist conditions was enhanced proliferation of primary stolons and offspring ramets (Figure 2e). Primary stolon number and length of EDR in dry condition increased by 42.4 and 36.6%, separately (Figure 3A and B), but rooted ramet production of EDR did not benefit from integration. Compared to EDR, all fragments of YDR tend to develop more biomass in heterogeneous treatment (Figure 2c, d and f). Primary stolon internode length of both ramets did not show significant response to uneven water supply or different amount of water supply (Figure 3D). Thus, it was concluded that heterogeneity had a stronger effect on vegetative production; rooted ramet of elder ramet experienced significant cost when acting as a donor ramet, and gained no profit when serving as a recipient ramet. However, all fragments of younger ramet benefited tremendously from connection in heterogeneous water treatment.

Integration within buffalograss clones reduced plastic response of ramets to localized microhabitat. Compared to homogeneous treatment, ramets under heterogeneous treatment developed more root mass in wet pots and slightly less root mass in dry pot (Figure 2d). Under heterogeneous treatment, connection reduced root to shoot ratios of ramets in dry pots, 38.3 and 54.8% for EDR and YDR, separately, but enhanced root to shoot ratios of donor ramets in wet patches, 17.5 and 23.1% for EDR and YDR, respectively (Figure 3C).

Direction of water flow

As the eldest and most basipetal ramet, rooted ramets of EDR in moist conditions experienced significant cost due to its connection to YDR in dry conditions and rooted ramet of EDR in dry conditions did not have any profit from connection with YDR in moist condition (Figure 2c). On the contrary, rooted ramets of YDR which was more acropetal, benefited in both dry and moist conditions in heterogeneous treatments. This indicated that water movement in monocotyledonous buffalograss was still prone to acropetal, even if directional constraint in monocotyledon is not obvious.

DISCUSSION

This study provided evidences that physiological integration enhanced the whole genet fitness of buffalo-grass subjected to uneven water supply. It has been extensively studied that physiological integration amelio-rated ramets growth in stressful conditions via support from interconnected ramets in unstressed conditions (Alpert, 1999a,b; Peterson and Chesson, 2002; Yu et al., 2004). Our results were consistent with previous reports, ramets in dry conditions gained significant benefit when connected to ramets in moist conditions. However, the effect of integration on unstressed ramets was uncertain, it might cause cost for unstressed ramets, especially when elder ramets in moist conditions were connected to younger ramets in dry conditions. Never-theless, younger ramets in moist *H. peduncularis* (Peterson and Chesson, 2002) and *C. flacca* (de Kroon et al., 1996). In this experiment, only rooted ramets of elder ramet in moist conditions experienced significant cost from connection to younger ramet in dry conditions, but stolons and offspring ramets conditions did not experience any costs when connected to older ramets growing in dry conditions, as observed in production were not affected, instead, both of them proliferated better in heterogeneous treatment. For younger ramet, all fragments (rooted ramets, roots, and new stolons and offspring ramets) of younger ramet growing in moist conditions tend to produce more biomass in heterogeneous than in homogeneous treatment. The mechanism of this response is unknown, but it might have been due to the asymmetry between elder and younger ramets on account of apical dominance. Younger ramets was known to have higher transpiration rate (de Kroon et

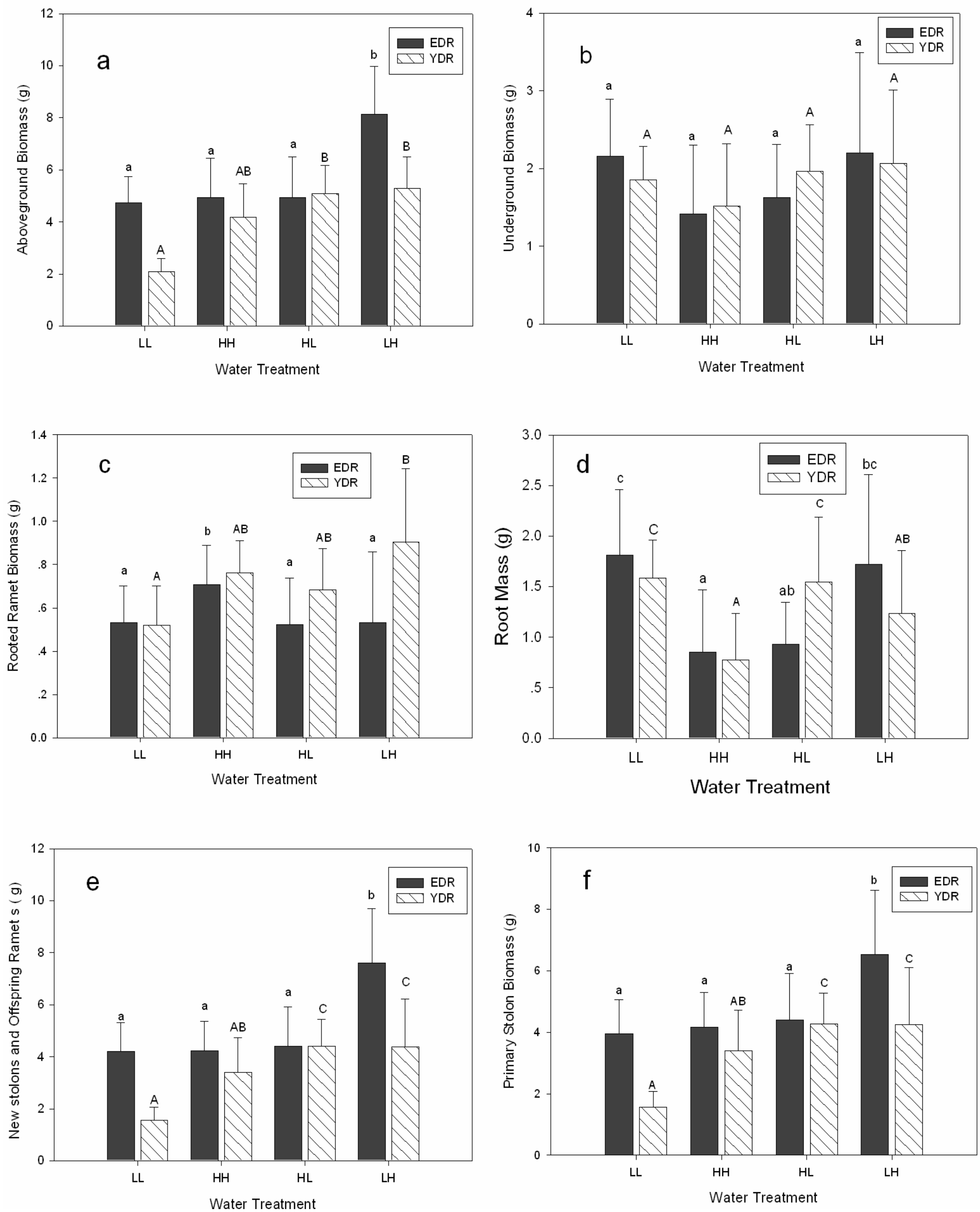

Figure 2. (a) Aboveground biomass, (b) underground biomass, (c) rooted ramet biomass, (d) root mass, (e) new stolons and offspring ramets and (f) primary stolon biomass of buffalograss EDR and YDR subjected to homogeneous and heterogeneous water treatments. Vertical lines show SE. Treatment code is as presented in Table 1.

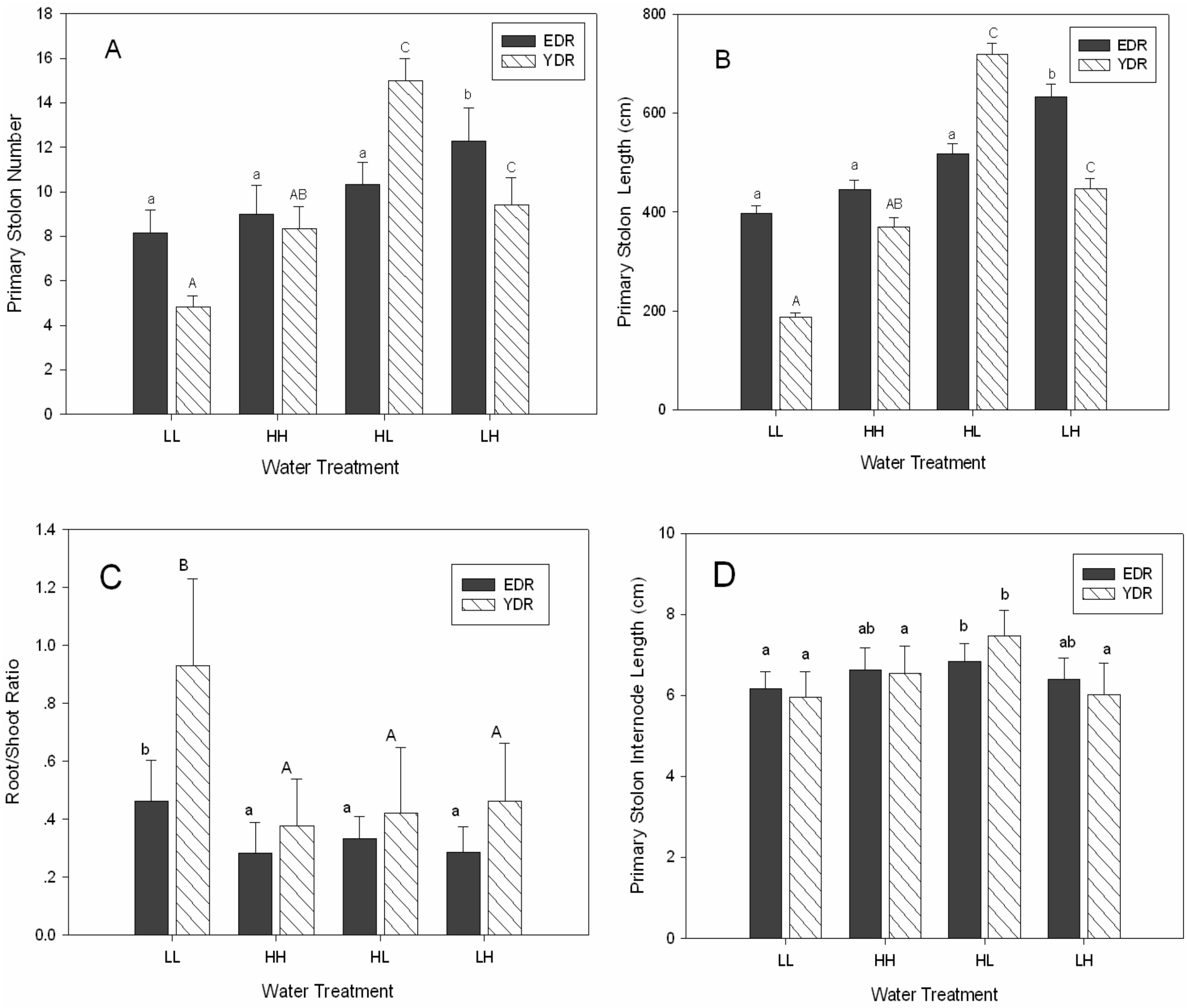

Figure 3. (A) Primary stolon number, (B) primary stolon length, (C) root/shoot ratio and (D) primary stolon internode length of buffalograss EDR and YDR subjected to homogeneous and heterogeneous water treatments. Vertical lines show SE. Treatment code is as presented in Table 1.

Table 2. Leaf length and width of interconnected buffalograss Elder Daughter Ramet (EDR) and Younger Daughter Ramet (YDR) under homogeneous and heterogeneous water treatments.

Parameters	Water treatment			
	LL[†]	HH	HL	LH
Leaf length of EDR	17.17[a‡]	17.17[a]	16.83[a]	17.86[a]
Leaf length of YDR	16.67[a]	17.67[ab]	20.83[b]	16.43[a]
Leaf width of EDR	1.67[a]	2.08[b]	2.00[b]	2.00[b]
Leaf width of YDR	1.67[a]	2.00[b]	2.00[b]	1.93[b]

[†] Water treatment code as in Table 1; [‡] Means followed by the same letter in each row are not significantly different (P = 0.05).

al., 1996) and younger ramet in dry pot developed significant larger leaf area when connected to elder ramet in wet pot (Table 2). All these factors may facilitate younger ramet to sequester more water and other resources from interconnected older ramet, which subsequently led to the over-depletion of rooted ramets biomass of elder ramet.

In heterogeneous treatments, ramets in dry conditions developed as much as or even more biomass than well-watered ramets. More and longer primary stolons were generated from stressed sections in heterogeneous treatments (Figure 3A and B). This phenomenon is called "oversharing" (Alpert, 1999a). Similar result has been observed in *Fragaria chiloensis* growing on sand dunes for carbon-based resources: shaded ramets have greater vegetative reproduction and more total biomass than the connected unshaded ramets (Alpert, 1999a). The cause of this response and whether it is beneficial remain obscure. There are several possible explanations for this. Firstly, the high water supply we provided here was not optimal for this species. Unlike nutrients, extra water supply does not necessarily result in enhanced biomass, but integration between interconnected ramets may facilitate the water supply to a near optimal point for ramets in dry pots (Peterson and Chesson, 2002). Secondly, apart from water, ramets in dry pots might sequester other resources from interconnected wet ramets, since other resources like nutrients, are usually dissolved in water and extracted by plants together. Thirdly, donor ramets need extra cost to extract the water from soil, while recipient ramets do not.

It is obvious that there existed extensive water integration within buffalograss clones. The large vascular bundle in stolon connecting two ramets is like an open tunnel with two possible ways to motivate water flow. One is the gradient of water potential that mainly works at night when plant's stomates are closed. Hydraulic lift facilitates water flow from ramet in moist pots to ramet in dry pots via vascular system (de Kroon et al., 1996; Peterson and Chesson, 2002). Another factor driving water flow is transpiration which is highly correlated to the difference in leaf area (de Kroon et al., 1996). In homogeneous treatment, plants tend to develop larger leaves with higher water supply. However, in heterogeneous treatments, ramets in dry pots had larger leaf area than their connected ramets in wet pots. Combining larger leaf area and more stolon and offspring ramets of ramets in dry conditions, greater transpiration was predicted, and more water was extracted from ramet in wet pot to ramet in dry pot. This could be another reason why ramet in dry pot overyielded than their interconnected ramet in wet pot.

Results of this experiment have important implications on our understanding of buffalograss characteristics. First, elder ramets is more efficient in water use than younger ramets, this might be associated with the drought resistant character of buffalograss; second, younger ramets benefited more from spatial variation in water availability than elder ramets. Thus, we can conclude that the drought resistant character of buffalo-grass is not only attributed to its deep extensive root system and its ability to extract water from deep soil profile and deliver it to surface soil (Huang, 1999), but also to the extensive water integration of its interconnected ramets. However, it was unexpected that more vegetative reproduction was concentrated in low-water patches. Further research is needed to evaluate whether this allocation pattern is more advantageous for the long-term benefit of the whole genet and its ecological significance need to be addressed.

Conclusions

Extensive water integration is an important strategy for buffalograss to survive in natural environment. Ramets in dry conditions can be supported by interconnected ramet in wet conditions. Both ramets exhibited better performance with uneven water supply because of water integration and almost no cost was involved in this process apart from rooted ramet biomass of elder ramet.

ACKNOWLEDGEMENTS

This study was supported by Key Projects in the National Science and Technology Pillar Program in the Eleventh Five-year Plan Period (Grant No. 2006BAD16B09-2). We gratefully acknowledge constructive comments from Josef F. Stuefer (Radboud University, Nijmegen), Dan Undersander (University of Wisconsin) and Martin A. Petrovic (Cornell University) on an earlier version of this manuscript.

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