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Clonal Integration for the *L. chinensis* in a Heterogeneous Alkaline Stress Environment

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Abstract: The purpose of this study was to explore clonal integration of *Leymus chinensis* under heterogeneous alkaline conditions. The experiment consisted of three levels of substrate alkaline (8.5, 10, pH 11.5), two clonal integration treatments (rhizomes severed or not). Clonal integration enhanced the survival, growth of daughter ramets experiencing alkaline stress, especially for young ramets whereas the performance of mother ramets was reduced by clonal integration. Therefore, clonal integration did not affect performance of the whole clones.

Key words: Heterogeneous alkaline stress, clonal integration, adaptive strategy, *Leymus chinensis* grassland, China

INTRODUCTION

Leymus chinensis is perennial, rhizome clonal plant that has strongly adaptability and widely ecological plasticity. It can be adaptive to different menaces come from bad habitat conditions. It can also be widely grown in multi agrotype. It can be grown as dominant species, inferior species and co-existing species (Liu and Han, 2008). Because grow for a long time in bad environment, it has obvious changes in physiological and biochemical process. Hence, it can adapt to different menaces come from bad environment. The interramet vascular connections (e.g., rhizomes) of many clonal plants can last for several years, enabling the translocation of resources such as carbohydrates, water and nutrients between the connected ramets as clonal integration (Pitelka and Ashmun, 1985; De Kroon and Hutchings, 1995; Dong *et al*., 2010). Clonal integration influence survival, growth, physiology and morphology of ramets and thus provide benefits to the genet as a whole (Welham *et al*., 2002; Yu and Dong, 2003). Up to now, most studies of clonal plant ecology have been focused on their responses to resource heterogeneity (Pennings and Callaway, 2000; Van Kleunen and Fisher, 2001), especially of light (Huber and Hutchings, 1997; Stuefer and Huber, 1998), nutrients (Slade and Hutchings, 1987a, b; Alpert, 1996, 1999) and water (De Kroon *et al*., 1996) and salt (Kingsbury *et al*., 1984; Cramer *et al*., 1995). Efforts devoted to the responses of clonal plant to alkaline were surprisingly scarce.

Alkaline soils of extreme habitats are often contains high amounts of salts which can be easily washed out but without changes of the pH. The effects of Na_2CO_3 on the growth of plants were more severe than those of NaCl. Plants growing under alkaline conditions would become ABA deficient. ABA-deficient plants are extremely susceptible to various types of stress as described for a large range of ABA-deficient mutants (Taylor, 1991). Many results indicate that high pH direct effects on root extension and decrease the capacity of absorb of others nutrient element, it is the more serious stress for plants than including ion toxicity and Osmotic stress (Campbell and Nishio, 2000; Degenhardt *et al*., 2000; Diem *et al*., 2000; Shi and Zhao, 1997). To examine whether integration of clonal plants affects their responses to alkaline, researchers conducted a greenhouse experiment, researchers addressed the following questions: Does alkaline affect the growth of *L. chinensis* ramets? Does integration of clonal plants modify the alkaline effects and consequently influence the plants'ability to withstand alkaline?

MATERIALS AND METHODS

Collection and propagation: Plants seeds were collected from a natural population on grassland at songnen 44°45'N, 123°45'E) about 150 km West of haerbin, Heilongjiang province and propagated vegetatively for 2 months in a greenhouse at the University of China Agricultural before the experiment began.

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Experimental design: The experiments consisted of two treatments: alkaline (three levels), severing (two levels). On 5 April, 2012, 18 similar-sized ramet pairs of *L. chinensis* were picked out. Then, planted in a pair of cylindrical plastic containers (20 cm in diameter and 20 cm in height), separated in middle by plastic study. The containers was filled with the fine sand each side. Each ramet pair consisted of a mother and a tagged daughter ramet, interconnected by a rhizome. About 2 weeks later, alkaline treatments were applied, poured Na_2CO_3 solution in daughter ramet side, bring up to pH 8.5 (control), 10.0 (medium alkaline) and 11.5 (high alkaline). In order to reduce a potential high pH shock for the 10.0 and 11.5 alkaline treatments, alkaline was increased daily by 0.5 increments until the final concentrations were reached. The plants were watered with a 1/4 strength Hoagland's solution containing 10 mg $N\text{-}NO_3$ (0.71 mM). This nitrogen concentration was selected on the basis of earlier research (Alpert, 1999) to be limiting to growth but sufficient to support growthplants. Three replicate pots were allocated for each treatment.

Measurements: After 6 weeks, all plants were plants were harvested. The mother and the daughter ramets were separated. The number of tillers was counted and dry mass was determined after drying at 80°C for 48 h and weighed.

Data analysis: Researchers analyzed the experiment using ANOVAs run with SYSTAT 9.0. Investigate the effects of severing, alkaline and their interactions on *L. chinensis* mother and daughter ramets and whole clones separately.

RESULTS AND DISCUSSION

Alkaline had no effect on the survival of mother ramets. The youngest daughter ramets disconnected from the mother ramets did not survive in the high alkaline treatments.

Biomass and tillering: Increased alkaline inhibited growth of mother, daughter ramets and whole clones. Severing decreased the biomass and tiller production of daughter ramets but increased those of mother ramets. Nevertheless, none of the measured traits (biomass, number of tillers) of daughter ramets differed between connected and severed treatments under control ($p = 0.83$, $p = 0.26$) and median alkaline ($p = 0.22$, $p = 0.45$) treatments. For the whole clones, both the biomass and number of tillers were not influenced by clonal integration (Fig. 1).

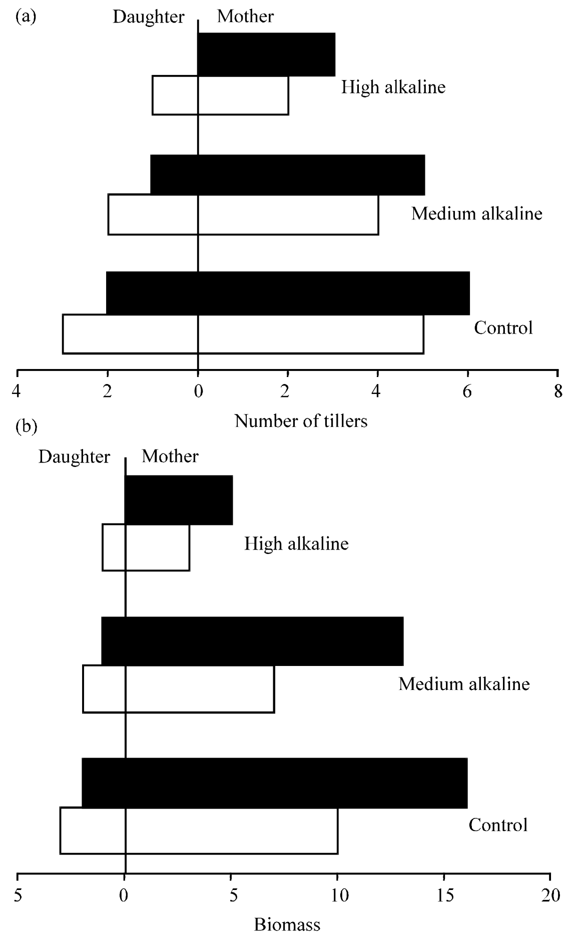

Fig. 1: Growth characters of *Leymus chinensis* under three heterogeneous alkaline level and two rhizome-severing treatments. Originally integrated ramet pairs: open bars; severed ramet pairs: black bars. a) Number of tillers and b) Biomass

Root biomass distribute: Clonal integration enhance root biomass distribute in control whereas severed ramet root biomass dramatically increase in medium alkaline stress but in high alkaline condition, root biomass decline again. (Fig. 2). This maybe is relatively more root biomass investment to rich resource for meet absorb water.

As expected, the growth of *L. chinensis* responded negatively to increased alkaline. The effects of integration Similar to earlier studies in which the same species was exposed to flooding (Xiao *et al*., 2010, 2011) under salt stress clonal integration enhanced the survival, growth of *S. alterniflora* a daughter ramets and reduced the performance of their mother ramets. Mother ramets of the aqustic plant *Alternanthera philoxeroides* showed similar responses to stolon severing (Wang *et al*., 2009). In *Spartina patens* daughter ramets connected to parents

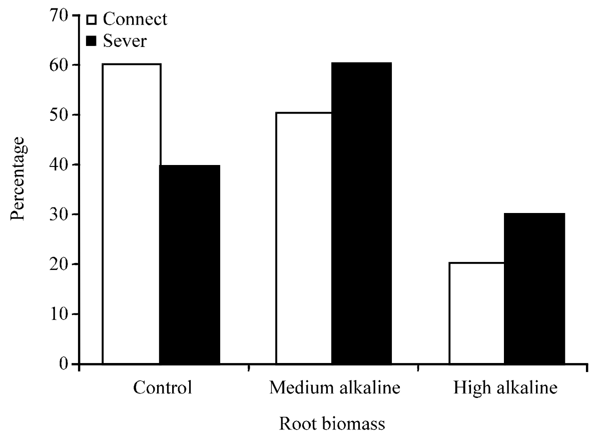

Fig. 2: Root biomass distribute of *Leymus chinensis* under three heterogeneous alkaline level and two rhizome-severing treatments. Originally integrated ramet pairs: open bars; severed ramet pairs: black bars)

had significantly greater leaf elongation rates than severed daughter ramets and leaf proline increased only in severed daughter ramets (Hester *et al*., 1994). This was interpreted by a reduced salt stress in connected daughter ramets, possibly via parental water translocation (Hester *et al*., 1994).

Clonal integration was considered to be disadvantageous in homogeneous environments and advantageous in highly heterogeneous ones (Alpert, 1999). However, performance of *L. chinensis* whole clones was not influenced by rhizome severing. Researchers assume that resources are transported from mother ramets to their clona offspring under moderate alkaline stress but a net benefit of integration at the clone level is not resulting under such conditions.

CONCLUSION

Clonal growth were inhibited by increased alkaline. Clonal integration enhanced the survival, growth of daughter ramets experiencing severe alkaline stress. This became evident especially with the young ramets whereas the performance of mother ramets was reduced by clonal integration. The metabolic basis of such physiological adjustments (e.g., photosynthate transport and water availability) among ramets under alkaline conditions needs to be addressed in future studies.

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