Pattern of Nitrogen Integration and Its Ecological Implications in Clonal Plant *Zoysia japonica*

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Key words: nitrogen, clonal integration, *Zoysia japonica*, isotope

Introduction Clonal integration between ramets of a clonal plant was regarded beneficial for ramet establishment, sustaining stressed ramets, and for resource uptake over large or heterogeneous areas. However, clonal integration may have potential costs, thus, clonal integration may not necessarily exist among physically connected ramets. In natural conditions, *Zoysia japonica* usually forms long-chained and long-lived stolons in various habitats of the grasslands through the way of vigorous clonal growth. We therefore hypothesized that at least one of the underlying mechanisms lie in that the capacity of clonal integration in *Z. japonica* clones must be strong and extensive. Exemplifying nitrogen as an important nutrient element, we carried out an isotopic experiment trying to test the nitrogen integration pattern in *Z. japonica* clones, through which try to explain some of the clonal behavioral performances of *Z. japonica*.

Material and methods The experiment was conducted in the East China Normal University, Shanghai, China. Soil type is clay sand loam. Inorganic N is 209.6 mg/kg. Total soil N is 0.23%. *Zoysia japonica* Steud. as an C_4 perennial herb extensively distributes in China and surrounding countries. The basic morphological unit of *Z. japonica* plant is so-called multiple-node or compound internode, a repeatable sequence of two shortly compressed and one elongated internode. So-called A-and B-tiller successively grow at the bases of two compressed internodes at opposite sides of the multiple-node. The experimental clones were cultivated in the experimental field. Each clone grew from a standardized individual ramets (single multiple-node with two opposite tillers), which cut from a single large clone of *Z. japonica* propagated in the greenhouse before the experiment. A special treatment was given to the middle ramet of each clone: before each middle ramet anchored their roots, plastic cups filled with soil were put under the multiple-node and buried in the field soil, preparing for receiving the ramet roots. At the end of the growth season, two days before harvesting the clones, the plastic cups containing the roots and soil were carefully dig out, and the inside soil were removed, and the inside roots as well as the plastic cups were carefully washed with distilled water and deionized water, then the washed roots were put back into the plastic cups preparing for the isotopic experiment using the ^{15}N-labelled nutrient solution. The nutrient solution used to feed the middle ramet roots was compounded according to the modified ingredients of the Hoagland solution, in which KNO_3 was changed into $^{15}NH_4Cl$ ($\delta^{15}N$ 10.39%) and $K^{15}NO_3$ ($\delta^{15}N$ 10.32%) respectively. Before being fed into the root system of the middle ramets, the nutrient solution containing $^{15}NO^{3-}$ and $^{15}NH_4^+$ respectively, was diluted, resulting in the final concentration 2.5mm/L. Two days latter, each clone was carefully harvested, washed and air-dried. The isotopic experiment replicated tree times with three *Z. japonica* clones similar in architecture. In the experimental period, the mean soil water content was 27.20%. Seven samples of ramets, including leaves, roots and stolons, were taken on each side of the feeding ramets respectively, along the primary, whereas in the case of secondary stolons, less samples were taken due to their short length. Samples were oven-dried and ground into fine powder (approx. 20μm). $\delta^{15}N$ in the samples were detected using a mass spectrometer (DELTAplusXP type), through which the translocation patterns of nitrogen in two forms in *Z. japonica* clones were analyzed. Three statistical indexes, kurtosis, skewness and gradient were calculated.

Result $^{15}NO_3^-$ and $^{15}NH_4^+$ showed acropetal and basipetal translocation patterns in the primary stolons of *Z. japonica* respectively, and more ^{15}N was translocated acropetally, and less basipetally. $\delta^{15}N$ of A-tillers or A-branches were usually higher than those of B-tillers or B-branches, and those in stolons were at middle levels and relatively stable. $\delta^{15}N$ of roots usually showed higher values at the feeding ramets, then degreased quickly. In case of $^{15}NH_4^+$, the gradients of the basipetal and acropetal translocation patterns of ^{15}N in ramets along the primary stolons were greater than the case of $^{15}NO_3^-$. When feeding the middle ramets of *Z. japonica* stolons with same concentration of $^{15}NO_3^-$ and $^{15}NH_4^+$ respectively, $\delta^{15}N$ values of the feeding ramets in the case of $^{15}NH_4^+$, was also greater than in the case of $^{15}NO_3^-$. In any cases, the gradients of the distribution patterns of $\delta^{15}N$ values of A-tillers along the primary stolons were greater than those of B-tillers. The range of $^{15}NO_3^-$ and $^{15}NH_4^+$ translocation in both basipetal and acropetal directions along the *Z. japonica* stolons respectively, was 7 to 15 multiple-nodes. Acropetal translocation of ^{15}N tended to be farther than basipetal, and those of $^{15}NO_3^-$ tended to be farther than those of $^{15}NH_4^+$, especially in the acropetal direction. Acropetal translocation patterns of ^{15}N in the secondary stolons were detected after $^{15}NO_3^-$ and $^{15}NH_4^+$ being fed from the root systems of the middle ramets of the primary stolons, respectively. The acropital translocation patterns of $^{15}NH_4^+$ and $^{15}NO_3^-$ in the secondary stolons were basically similar in form to, but different in quantity from that in the primary stolons, the former was less than latter. In secondary stolons, in the case of $^{15}NH_4^+$, $\delta^{15}N$ of different organs were greater than those $^{15}NO_3^-$, and those in secondary A-stolon were greater than those in secondary B-stolon in both cases of $^{15}NO_3^-$ and $^{15}NH_4^+$. $\delta^{15}N$ of ramets on the secondary A or B-stolons directly connected with the ^{15}N feeding ramets, were usually greater than those of the ramets on the corresponding secondary A or B-stolons grew from the multiple-nodes preceding or after the feeding sites, and $\delta^{15}N$ of ramets on the acropetal secondary A or B-stolons respectively were usually greater than those basipetal.

Conclusions The results at least partially supported the hypothesis that clonal integration in *Z. japonica* clones was strong and extensive, which also at least partially explained the natural performance of *Z. japonica* clone in terms of such as long-chained and long-lived stolons in various habitats and strong capacity of clonal propagation. The extensive clonal integration pattern in *Z. japonica* clone may have some obvious benefits. However, the relevant costs may also be obvious in terms of such as maintenance, inhibiting branching, and facilitation of pathogen infection within the clone, which needs to be revealed in detail through a series of other experiments.