

Growth of clonal modules on *Agropyron michnoi* in the Songnen Plain of Northeast China

Crecimiento de módulos clonales de *Agropyron michnoi* en la planicie Songnen del Noreste de China

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Abstract. Spatial expansion of clonal plants and growth of their modules are of concern in the field of plant ecology. After measuring a large number of samples, we analyzed the module components and the growth patterns of vegetatively propagated *Agropyron michnoi* clones in the Songnen Plain on Northeast China. The results showed that the plasticity of clonal growth was large; the coefficients of variation of both extensive areas and the quantitative characters of modules were more than 20%. The numbers of ramets, seedlings, and buds and the cumulative length of the rhizomes showed exponentially and linearly increasing patterns with increases of the area and the total number of modules. The biomass of each module, total number of modules and total biomass showed an allometric growth pattern, which was best described by power functions. For *A. michnoi*, there was a relatively stable investment to sexual reproduction; it showed a priority for allocating biomass to reproductive ramets, and also to rhizomes and buds formation.

Keywords: *Agropyron michnoi*; Clonal growth; Vegetative propagation; Number of modules; Biomass.

Resumen. La expansión de las plantas clonales y el crecimiento de sus módulos son de interés para la ecología vegetal. Después de medir una gran cantidad de muestras, analizamos los componentes a escala de módulo y los modelos de crecimiento de clones de *Agropyron michnoi* propagados vegetativamente en la Planicie de Songnen en el noreste de China. Los resultados mostraron que hubo una gran plasticidad del crecimiento clonal; los coeficientes de variación fueron mayores del 20% para áreas extensivas y las características cuantitativas de los módulos. El número de tallos, plántulas, y yemas y la longitud total de los rizomas mostraron modelos que se incrementaron exponencialmente y linealmente con incrementos del área y el número total de módulos. La biomasa de cada módulo, el número total de módulos y la biomasa total mostraron un modelo de crecimiento alométrico que fue descrito mejor utilizando funciones de potencia. Hubo una inversión relativamente estable hacia la reproducción sexual en *A. michnoi*; esta especie mostró prioridad en la partición de la biomasa hacia tallos reproductivos, y también hacia la formación de rizomas y yemas.

Palabras clave: *Agropyron michnoi*; Crecimiento clonal; Propagación vegetativa; Número de módulos; Biomasa.

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INTRODUCTION

During plant evolution, natural selection can retain a large number of plants which produce ramets with the same genetic structure. Plants which propagate vegetatively under natural conditions via production of buds on either rhizomes, stipes, root tubers, tubers, roots or other vegetative organs, are classified as clonal plants (Cook, 1983; Yu & Dong, 2001; Dong & Yu, 2007; Dong & et al., 2011). Growth from clonal organs makes a major contribution on higher plants, especially on plant communities where herbaceous species are dominant (Bazzaz & Harper, 1977; Charpentier, 2001). Sixty-six percent of plants in central Europe are clonal plants. In England, clonal plants are 68% of the most widely distributed perennial herbaceous species. In addition, 60% of common perennial herbaceous species in the Songnen Prairie of Northeast China are clonal plants (Yu & Dong, 2001; Dong & et al., 2011). Finally, 66.8% of wetland vegetation in China are clonal plants (Song & Dong, 2002).

Understanding of the quantitative variation and spatial expansion following establishment of the guerrilla style on clonal plants is a current hotspot in plant ecology (Shirreffs & Bell, 1984; Pitelka, 1984; Maillette, 1992; Oborny, 1994; Liu & Zhong, 1996; Chen et al. 1997; Yu & Dong, 1999; Shan et al., 2000; Oborny et al., 2001; Song & Dong, 2002; Iikka & Johannes, 2004; Tang et al., 2010). Clonal plants not only occupy a dominant position in the species composition of herbaceous plant communities, but also they can form single species dominant populations at a small scale through rhizome, root sprout, and creeping stem vegetative propagation of the guerrilla clonal plants (Harper, 1977; Dong et al., 2011). Clonal plants can play an important role in the restoration of degraded natural grasslands as they can expand quickly through rhizome reproduction.

Agropyron michnoi, a long-rhizome grass, belongs to the guerrilla clonal plants (Cook, 1983; Chen et al., 1997). It is a widely distributed grass in Northern China under natural conditions. It reproduces via vegetative reproduction and spatial expansion through rhizome and root sprouting. However, we know little about the potential of rhizome reproduction in *A. michnoi*, as well as module growth features for population expansion. Our objective was to analyze the clonal module components and growth pattern, and the variation in module number and biomass of *A. michnoi*. We measured the module numbers and the spatial extension of second-year vegetatively propagated *A. michnoi* clones. The study will provide insights into the growth characteristics of *A. michnoi*, and their application to the restoration of degraded grasslands.

MATERIALS AND METHODS

Natural conditions of the experimental site. The study was carried out in the Grassland Ecological Research Station of Northeast Normal University (44° 38' N, 123° 41' E), which is located at the southern area of the Songnen Plain, Changling

County Jilin Province. The study area is in a temperate zone with semi-humid, semi-arid climate. The average annual temperature is 4.6-6.4 °C. The annual accumulated, ≥ 10 °C temperature is 2545-3374 °C, with 136-163 days of frost-free period. Annual precipitation ranges from 313 to 581 mm, 60% of which occurs between June and September. The amount of annual potential evaporation is approximately 2 to 3 times that of precipitation (Li & Zheng, 1997).

At the beginning of May 2006, we established three plots, each with an area of 2m × 3m. *Agropyron michnoi* tillers were transplanted to the plots with a row and plant spacing of 35 cm. Transplantation was conducted after plots were irrigated to field water capacity. Plants were watered regularly after transplanting. Before establishment of the transplanted seedlings, weeds were regularly removed from the plots. Plants which developed diseases or were attacked by insects were treated accordingly. The experimental site had been used as a farm land before transplanting *A. michnoi*. The soil was classified as sandy soil.

Methods. At the beginning of October 2007, 10 clones of *A. michnoi* were randomly sampled in each plot. We firstly measured area parameters according to the natural clonal module's spatial extension. We then dug up plants from the border to the center. We tried our best to (1) keep the natural connections between the aboveground plant parts and the underground rhizomes, and (2) obtain complete clonal modules. After counting the number of ramets on each clonal module, and measuring the accumulated rhizome length, clonal modules were oven-dried at 80 °C to constant weight. They were then divided into four classes (1) the one-eared were reproductive branches; (2) those with no ears but with stalk elongation were vegetative branches; (3) the ones with leaves but without stalk elongation were seedlings; and (4) the ones with obvious hump but without leaves were buds.

Data processing. Area was calculated using parameters according to the rectangle-, triangle-, trapezoid-, or parallelogram-shape of the clonal module. The number of ramets was the sum of those reproductive and vegetative; the percentage of ramets with ears was the ratio of reproductive to the total number of ramets. We calculated biomass allocation among the reproductive or vegetative ramets, and rhizomes. For the analysis of biomass allocation, we treated seedlings and buds as vegetative shoots. To describe the relationships between (1) growth of all clonal modules and their spatial extension, and (2) module number and biomass, we used linear, exponential and power functions. Thereafter, we selected the ones with the highest correlation coefficient to characterize the quantitative model.

RESULTS AND DISCUSSION

Clonal module components and its growth plasticity. Late during the second year of transplantation, all *Agropyron michnoi*

which were formed by a single tiller consisted of modules including reproductive and vegetative ramets, seedling, buds and rhizomes. The module's growth, biomass allocation, and spatial extension area changed dramatically (Table 1). As shown in Table 1, the maximum and minimum values reflected the sample range; the average reflected the overall status, and the standard deviation and coefficient of variation reflected the absolute and relative variation degree of the sampled plants, respectively. After two seasons of growth, the coefficient of variation of *A. michnoi* clonal module numbers exceeded 20% (Table 1). This reflects the large plasticity of the module characters, although they were transplanted using the same distance among modules, and they were grown under the same conditions. Among them, the module number and coefficient of variation varied from 50.3 to 77.1%, while the percentage of modules with ears and the coefficient of variation for the number of vegetative ramets were low (21.5 and 22%). This reflects that *A. michnoi* clonal plants' investment to zoogamy is stable.

Our results showed that the investment of *A. michnoi* to sexual reproduction was relatively stable; however, vegetative growth has a much greater ecological plasticity. Those findings are in agreement with growth characteristics observed in other grasses [*Carex duriuscula* (Wang et al., 2001), *Phragmites*

australis (Wu et al., 2014)] and major crops [*Oryza sativa* (Wang et al., 2012)] in the Songnen grassland. These species have to balance between vegetative and reproductive growth to adapt to the varying environmental conditions. Growth of vegetative modules is very sensitive to environmental conditions, and it often adopts very flexible strategies. However, the investment on reproductive modules is very stable and inheritable, which ensures population regeneration. Under changing environmental conditions, populations are most likely to ensure allocation to reproduction while sacrificing vegetative modules at the same time.

Correlations between module traits and the area of spatial extension. The numbers of ramets, seedlings and buds, and the accumulated length of rhizomes increased exponentially with the spatial extension area on the measured *A. michnoi* clonal modules ($p < 0.01$) (Fig. 1).

The phenotypic plasticity shown by the clonal characters on *A. michnoi* suggests that module growth and spatial extension share a common regulation. The ramet and bud numbers shared the same b value (0.0006) in the exponential functions; at the same time, the seedling number and accumulated rhizome length have a b value of 0.0007. These results indicate that the speed at which the module number increased was consistent with the spatial extension.

Correlation between various module traits and the total number of modules per clone. The numbers of ramets, seedlings and buds, and the cumulative length of rhizome increased linearly with the spatial extension area on *A. michnoi* (Fig. 2). The observed linear correlations were significant ($p < 0.01$).

The observed linear correlations demonstrate that the number of total modules increased as the growing speed of each module also increased. For any given increase in the number of total modules per clone, the cumulative length of rhizomes increased by a factor of 0.8612 cm, the bud number increased by 0.477, the branch number increased by 0.2853, and the seedling number increased by 0.2371. These results suggest that clonal *A. michnoi* forms rhizomes and buds first at the end of the growth season, to ensure their clonal growth in the next year.

Correlations between Module Biomass and Biomass Allocation versus Total Clone Biomass. Module biomass of reproductive and vegetative ramets, and that for rhizomes increased ($p < 0.01$) as total clone biomass also increased (Fig. 3, A1, B1, C1). While biomass allocation to reproductive ramets increased ($p < 0.01$) as total clone biomass increased, allocation of biomass to vegetative ramets and rhizomes decreased ($p < 0.01$) with increases in the total clone biomass (Fig. 3, A2, B2, C2).

Correlations between Module Biomass and Biomass Allocation versus the Total Number of Modules per clone. As the total number of modules per clone increased, the module biomass

Table 1. Statistical characterization of module components and the degree of variation in *Agropyron michnoi* clones (n=30).

Tabla 1. Descripción estadística de componentes de los módulos y su grado de variación en clones de *A. michnoi* (n=30).

Statistical characterization	Max	Min	Mean	SD	CV (%)
Area (cm ²)	2290.2	50.3	1097.6	680.8	62.0
Ramets (N)	279	37	122.3	70.7	57.8
Reproductive ramets (RR,N)	230	21	85.1	61.4	72.2
Vegetative ramets (VR,N)	110	10	37.2	18.7	50.3
Seedlings (N)	222	12	84.7	58.6	69.2
Buds (N)	499	24	172.8	116.2	67.2
Total modules (N)	964	130	379.9	238.4	62.8
Length of rhizomes (cm)	966.3	139.3	429.7	247.7	57.6
Total biomass (g)	102.3	10	41.6	26.3	63.2
Biomass of RR (g)	75.9	4	25.8	19.9	77.1
Biomass of VR (g)	42.1	4.4	13.4	7.4	55.2
Biomass of rhizomes (g)	5.1	0.6	2.3	1.3	56.5
Earring rate (%)	89.2	43.4	64.8	14.0	21.5
Biomass allocation of BRR (%)	77.6	36	57.3	12.6	22
Biomass allocation of BVR (%)	58.3	15.6	36.3	12.3	33.9
Biomass allocation of rhizomes (%)	16.4	2.5	6.4	3.3	51.6

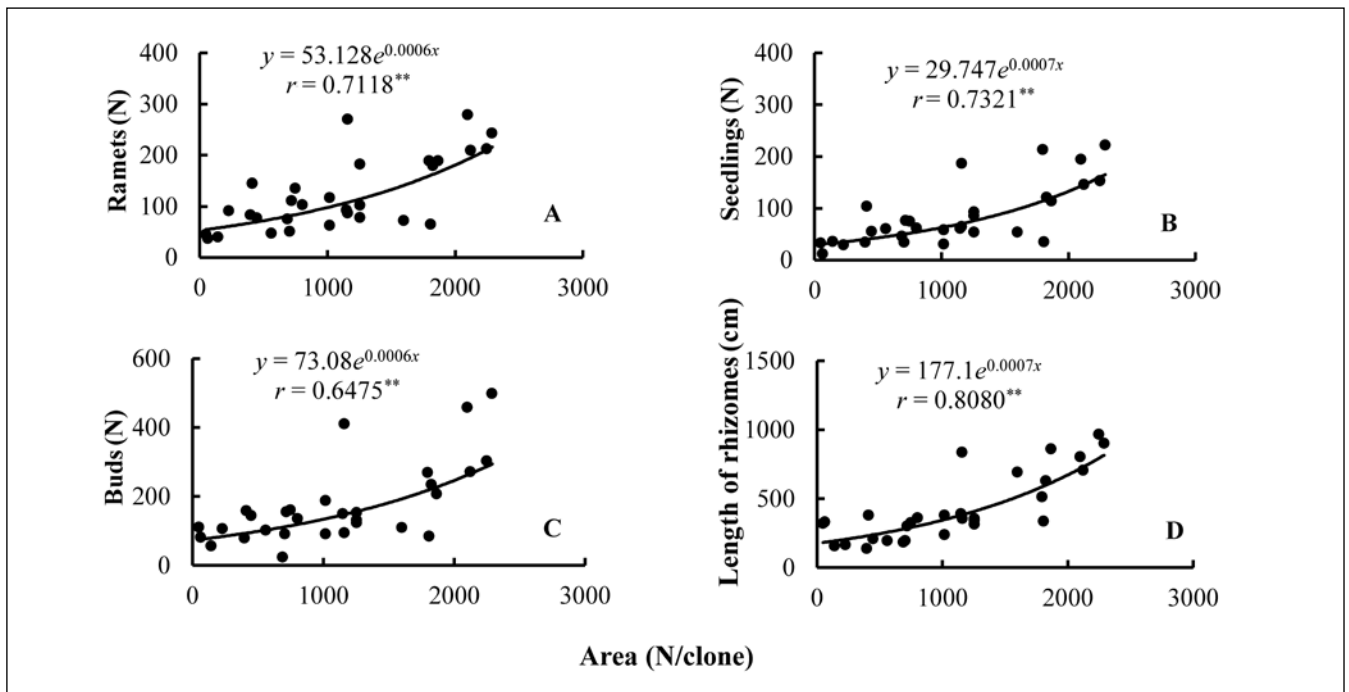


Fig. 1. Correlations between the numbers (N) of ramets (A), seedlings (B), buds (C), and cumulative length (cm) of rhizomes (D) versus clonal area of *A. michnoi* clones (n=30).

Fig. 1. Correlaciones entre el número (N) de tallos de reproducción asexual (A), plántulas (B), yemas (C), y longitud (cm) total de rizomas (D) versus el área clonal de clones de *A. michnoi* (n=30)

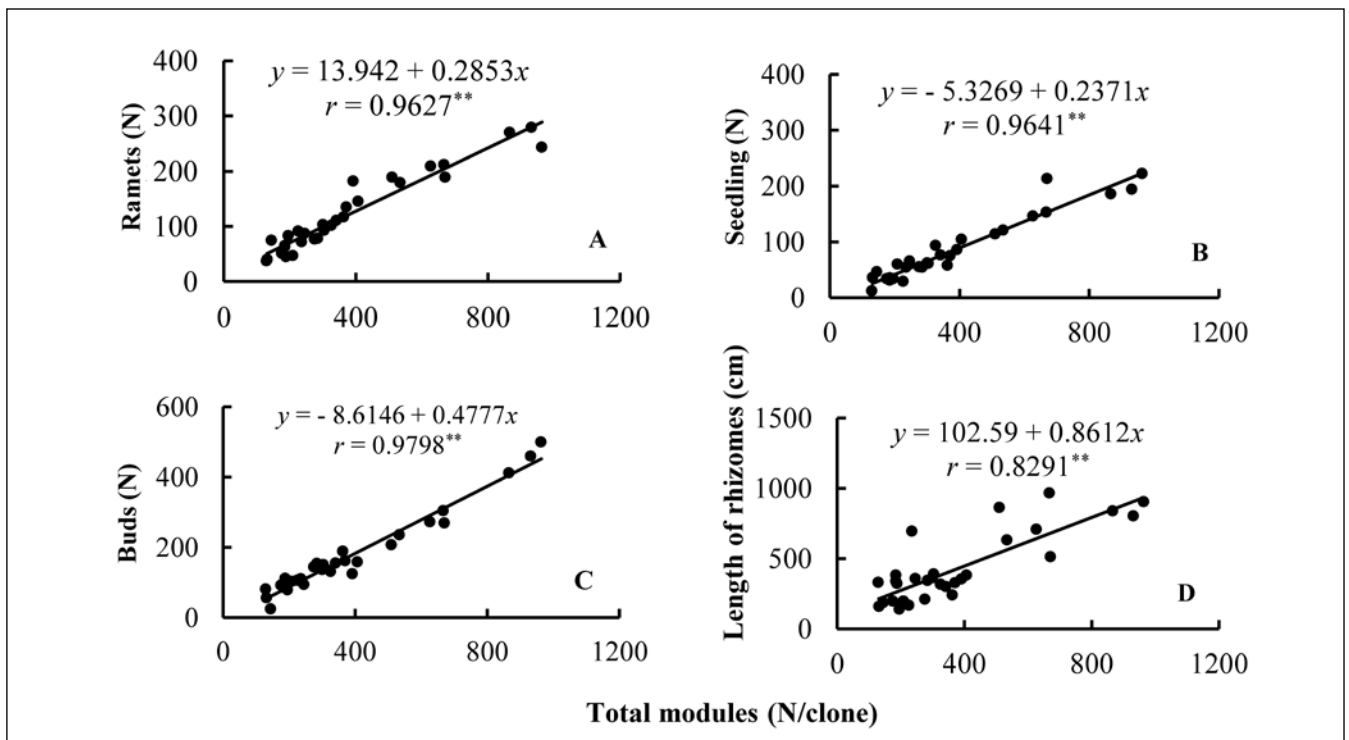


Fig. 2. Correlations between the numbers (N) of ramets (A), seedlings (B), buds (C), and cumulative length (cm) of rhizomes (D) versus the total number of modules in *A. michnoi* clones (n=30).

Fig. 2. Correlaciones entre el número (N) de tallos provenientes de reproducción asexual (A), plántulas (B), yemas (C) y longitud (cm) total de rizomas (D) versus el número de módulos total en clones de *A. michnoi* (n=30).

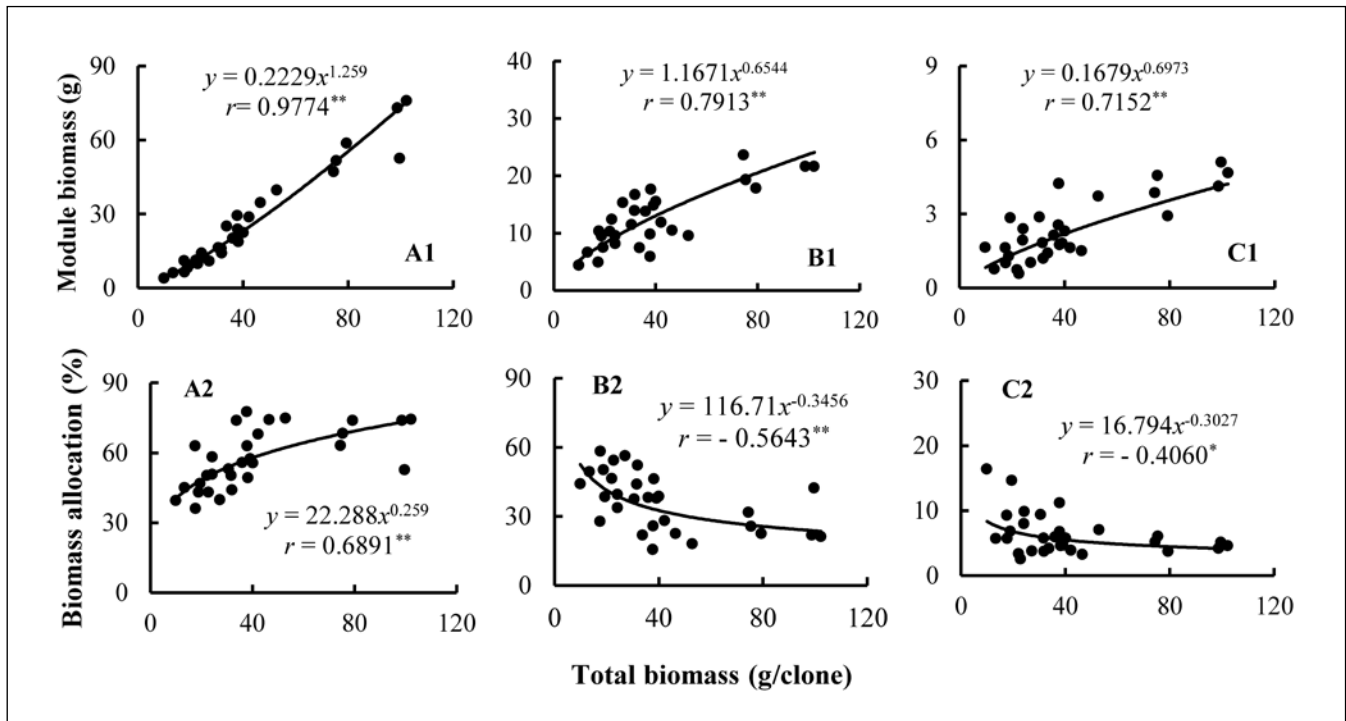


Fig. 3. Correlations between biomass and biomass allocation of reproductive ramets (A1; A2), vegetative ramets (B1; B2), and rhizomes (C1; C2) versus total biomass of *A. michnoi* clones (n=30).

Fig. 3. Correlaciones entre la biomasa y la partición a tallos asexuales reproductivos (A1; A2), tallos asexuales vegetativos (B1; B2), y rizomas (C1; C2) versus la biomasa total de clones de *A. michnoi* (n=30).

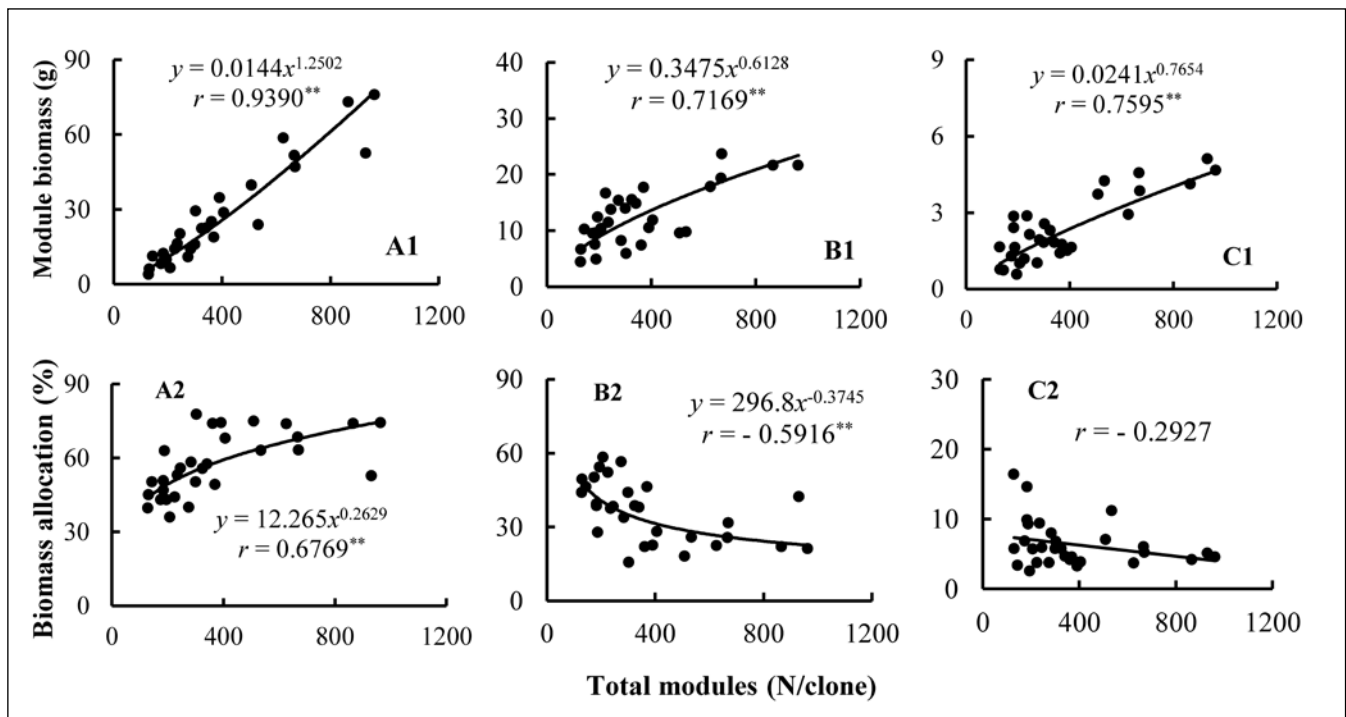


Fig. 4. Relationships between module biomass or biomass allocation to reproductive (A1; A2) or vegetative ramets (B1; B2), and rhizomes (C1; C2) versus the total number of modules per clone on *A. michnoi* clones (n=30).

Fig. 4. Relaciones entre la biomasa de módulos o la partición de biomasa a tallos reproductivos (A1; A2) o vegetativos (B1; B2) y rizomas (C1; C2) versus el número total de módulos por clon en *A. michnoi* (n=30).

also increased ($p < 0.01$) on reproductive and vegetative ramets and rhizomes (Fig. 4, A1, B1, C1). While biomass allocation to reproductive ramets increased ($p < 0.01$), it decreased ($p < 0.01$) to vegetative ramets and rhizomes as the total number of modules increased on clones of *A. michnoi* (Fig. 4, A2, B2, C2).

Therefore, we found that the module number and biomass allocation to different modules, and the increase of total creature number, were highly allometric. Reproductive and vegetative ramets were the main products of the current growth season. However, rhizomes were the cumulative product of the two study growing seasons. The module number and biomass allocation data were those corresponding to the end of the second year growing season. However, they could still reflect that increases in biomass allocation to reproductive ramets are associated with reduced allocation to vegetative ramets and rhizomes. *Agropyron michnoi* might tend to increase biomass allocation to reproductive ramets to ensure sexual reproduction.

CONCLUSIONS

In the Songnen Plain, the clonal spatial extension of *A. michnoi* and other growth characters have a large plasticity. At the end of the second year growing season the average clonal extension area was $1097.6 \pm 680.8 \text{ cm}^2$; each clone had 122.3 ± 70.7 ramets, 84.7 ± 58.6 seedlings and 172.8 ± 116.2 buds. For each clone, the total module number was 379.9 ± 238.4 , cumulative length of rhizomes was $429.7 \pm 247.7 \text{ cm}$, total biomass was $41.6 \pm 26.3 \text{ g}$, earring ratio was $64.8 \pm 14.0\%$, and biomass allocation to reproductive ramets was $57.3 \pm 12.6\%$. Among them, the plasticity of earring rate and allocation to reproductive ramets were relatively lower. The investment to sexual production was more stable.

The growth of *A. michnoi* modules was consistent. The numbers of ramets, seedlings and buds, and the accumulated length of rhizomes increased exponentially with the increase of the spatial extension. The module number and spatial extension rate were highly consistent. At the end of the growing season, production of rhizomes and buds are the priorities of the studied clonal plant.

The module number, biomass of each module and total biomass of *A. michnoi* were allometrically correlated and were best described by power functions. Increases in biomass allocation to reproductive ramets caused a decrease in biomass allocation to vegetative ramets and rhizomes; *A. michnoi* tended to allocate more biomass to reproductive ramets.

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