African Journal of Agricultural Research Vol. 6(21), pp. 5005-5009, 5 October, 2011
Available online at http://www.academicjournals.org/AJAR
ISSN 1991-637X ©2011 Academic Journals

Full Length Research Paper

Apical meristem differentiation in reproductive and vegetative shoots in rhizomatous *Leymus chinensis*

Zhang Zhaojun[1], Gao Jicai[2], Zhang Yuting[1], Jin Chengji[3], Peng Xuemei[1], Lin Jixiang[1], Wang Junfeng[1] and Mu Chunsheng[1]*

[1]Key Laboratory of Vegetation Ecology of the Ministry of Education, Institute of Grassland Science, Northeast Normal University, Changchun, 130024, China.
[2]Jinlin Province Center of Lightning Protection and Disaster Mitigation, Changchun, 130062, China.
[3]Grassland Management Station of Korean Autonomous Prefecture of Yanbian, Yanji, 133001, China.

Accepted 19 September, 2011

To explore the difference in apical meristem (SAM) differentiation between reproductive shoots and vegetative shoots, as well as the effects of low winter temperature on spiking of *Leymus chinensis (L. chinensis*) using paraffin-embedded slices, we compared the changes in SAM differentiation of grasses after regreening but before spiking date, of vegetative shoots after spiking, and of grass that grew continuously in a greenhouse throughout winter. For the grass after regreening but before spiking data, SAM differentiation was observed in the early stage, elongation stage, single ridge stage, double ridge stage and subsequent stages simultaneously. For the vegetative shoots that were measured after spiking, SAM differentiation was observed only in the early stage, elongation stage and single ridge stage but not in the double ridge stage or subsequent stages. For grass that grew in a greenhouse through the winter, new leaves developed continuously, increased in stem length and had no spiking, and SAM differentiation was observed only in the early stage, elongation stage and single ridge stage. Only grass experiencing SAM differentiation in the double ridge stage can head. The study also determined that low winter temperature played an extremely important role in the spiking of *L. chinensis*.

Key words: *Leymus chinensis*, reproductive shoots and vegetative shoots, shoot apical meristem differentiation, spike formation, low winter temperature.

INTRODUCTION

Poaceae (*Leymus chinensis*) is a rhizomatous species of perennial grass, distributed in the eastern part of the Eurasian steppe, Russia Transbaikal, Northeastern Mongolia, Northeastern China and North Korea (Kuo, 1987). It is characterized by salt, cold and drought tolerance and plays an important role in soil and water conservation as well as ecological engineering. *L. chinensis* has a large number of leaves, high nutritional values and good palatability, and thus is a high-quality forage grass (Zhou and Yang, 2006; Yan et al., 2006). *L. chinensis* primarily reproduces vegetatively in natural habitats. Its ability to reproduce sexually is poor with a spiking rate of 20 to 40%, a seed rate of 30 to 50% and a germination percentage of 20 to 30% (Ma et al., 1984; Yi et al., 2001; Li et al., 2003). Of the factors affecting the seed yields of *L. chinensis*, the quantities of spikes per unit area is the most critical factor, while the quantities of seed per ear and the thousand-seed weight are of lesser influence (Wang et al., 2010).

Previous researches have shown that the number of spikes per unit area is restricted by both genetic factors and external influences such as weather conditions and cultivation practices. Climatic conditions in the previous autumn (August to October) and the spring of following growing season (April to May) (Yang et al., 2001), long-term continuous mowing and continuous grazing regimes (Wang, 2001) and the years of growth and the nutrient conditions of grassland (Chen, 1983) have all been demonstrated to influence resulting numbers of spikes per unit area. Poaceae spikes originate from the differentiation and development of the shoot apical

*Corresponding author. E-mail: mucs821@gmail.com. Tel: +86-431-85098113. Fax: +86-431-85695065.

meristem (SAM). When grasses reach a certain stage and are under specific environmental conditions, SAM transitions from vegetative growth to reproductive growth and primordial spike differentiation begins. The understanding the process of the SAM-based primordial spike differentiation and spikelet formation, particularly as it is influenced by environmental conditions, will aid in ecological typing and identifying breeding characteristics associated with high yield.

On the Songnen Plain of northeastern China, *L. chinensis* regreens in mid-early April, spikes at the end of May, and seed matures at the mid-late July. Then, *L. chinensis* progresses into the vegetative growth after grain filling. The aboveground parts of *L. chinensis* can be divided into reproductive shoots and vegetative shoots (non-spiked shoots) (Mu et al., 2004). The spiking rate of *L. chinensis* is low, so *L. chinensis* is dominated by vegetative reproduction. Differences in *L. chinensis* SAM differentiation, between reproductive shoots and vegetative shoots, have not been previously reported. In this study, using a paraffin-embedded section method, a comparative histomorphology study was performed to investigate the difference in SAM differentiation for two stages of *L. chinensis:* after regreening but before spiking, and the non-spiked shoots while the other reproductive shoot had already spiked.

In addition, to investigate the effects of temperatures on SAM differentiation, we also examined grass growing continuously in a greenhouse throughout the winter without the experience of cold temperature.The purpose of this study was to explore differences in SAM differentiation between reproductive shoots and vegetative shoots and to analyze the effects of environmental conditions on SAM differentiation and reproductive shoots formation of *L. chinensis*.

MATERIALS AND METHODS

Site description

The study was conducted on an artificial *L. chinensis* grassland located at the Grassland Research Station of University, Changling County, Jilin Province of China (123°44'E, 44°44'N, altitude is 137.8~144.8 m). The soil of the *L. chinense* meadow steppe was salinized with a pH value of 7.5~9.0. The study area is characterized by a temperate semi-arid continental monsoon climate with an annual average temperature is 4.9℃, an annual accumulated temperature (≥ 10℃) of 2545.9~3374.9℃, a mean annual precipitation of 300~500 mm (occurring mainly during June to August) and a mean annual evaporation of 1500~2000 mm. The frost-free period is about 140 days from the end of April to early October.

Sampling and preparation

For examining the differentiation status of SAM in main stem, three replicates, of 20 grass shoots each, were randomly sampled during the 'recruit to heading' stage (April to June) in each successive sampling time in both 2004 and 2005, and by 15 days intervals. The differentiation status of SAM in vegetative shoots was also studied after the spiking stage, using a similar sampling method from June to September in each successive sampling time in both 2004 and 2005, by 30 days intervals.

Additionally, in July 2008, 60 shoots were sampled from transplanted potted shoots in the greenhouse, to study the differentiation statuses of SAM without the influence of low winter temperatures. Potted shoots were transplanted in June 2007. At each sampling time, a 1 cm leaf sheath of the upper and lower SAM in sampled shoots was removed. Then, the samples were placed into FAA solution (formalin-acetic acid-50% alcohol at a volume ratio of 1:1:18) for processing into paraffin sections.

Paraffin-embedded section method

Fixed SAM samples were removed from the FAA solution and dissected into small pieces approximately 1 cm long. Dehydration was performed with different concentrations of ethanol and tert-butyl alcohol. Paraffin-embedding was performed in a 60℃ oven for 72 h, followed with cooling in natural temperature condition (the melting point of paraffin was 56 to 58℃). The paraffin-embedded SAM was sectioned into continuous slices 10-μm thick by a rotary microtome (Yidi YD-202, China). Five or six slices on the central parts of SAM were selected and plated to glass slides. Dewaxing by xylene, rehydration with ethanol, staining and dehydration were performed consecutively. The staining procedure was as follows: 2% $ZnCl_2$ solution for 1 min, 1/25 000 safranin solution for 5 min, 5% tannic acid and 2% orange G solution for 1 min, 5% tannic acid solution for 5 min, and 1% iron alum solution for 2 min (Mu, 2001). SAM slices were then observed under a biological microscope (Motic BA400, China).

The staging of the SAM determination

Currently, no classification standard exists for staging SAM differentiation in *L. chinensis*. In this study, we utilized the classification method according to wheat because *L. chinensis* spikes are similar morphologically to wheat spikes (Gardner et al., 1985). A SAM at the 'early stage' was hemispherical in shape and the length was less than the width (Figure 1a). A SAM at the 'elongation stage' was comparatively elongated and the length was more than the width (Figure 1b). A SAM at the 'single ridge stage' was highly elongated and the length was much greater than the width and simultaneously a single ridge (a ring bulge) appeared in the lower part of the SAM (Figure 1c). A SAM at the 'double ridge stage' was greatly elongated and the length was much greater than the width. Large quantities of double-ring bulges are formed and the sublayer of a double ring-shaped bulge grows into bract primordium and the upper layer grows into spike primordium (Figure 1d). Subsequent stages, after the double ridge stage, included a 'glume and lemma stage' and a 'terminal spikelet' stage.

Data analysis

All statistical analyses were performed using SPSS 13.0 (SPSS Inc, Chicago, IL, USA). The data are expressed by mean ± S.E. for 3 determinations.

RESULTS

SAM differentiation after regreening before spiking date

In shoot samples on April 21 of each experimental year (2004, 2005), the results of paraffin sectioning showed

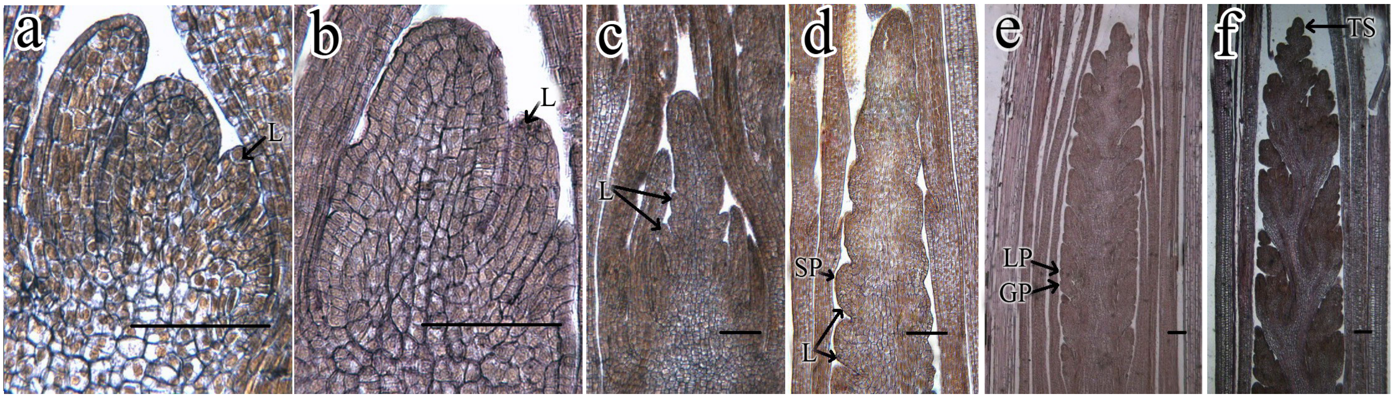

Figure 1. *Leymus chinensis* classification (magnification is listed in parenthesis) a) early stage (400 times), b) elongation stage (400 times), c) single ridge stage (100 times), d) double ridge stage (100 times), e) glume and lemma stage (40 times), f) terminal spikelet stage (40 times). L) leaf primordia, SP: spike primordia, GP: glume primordia, LP: lemma primordia, TS: terminal spikelet, Bar = 0.1 mm.

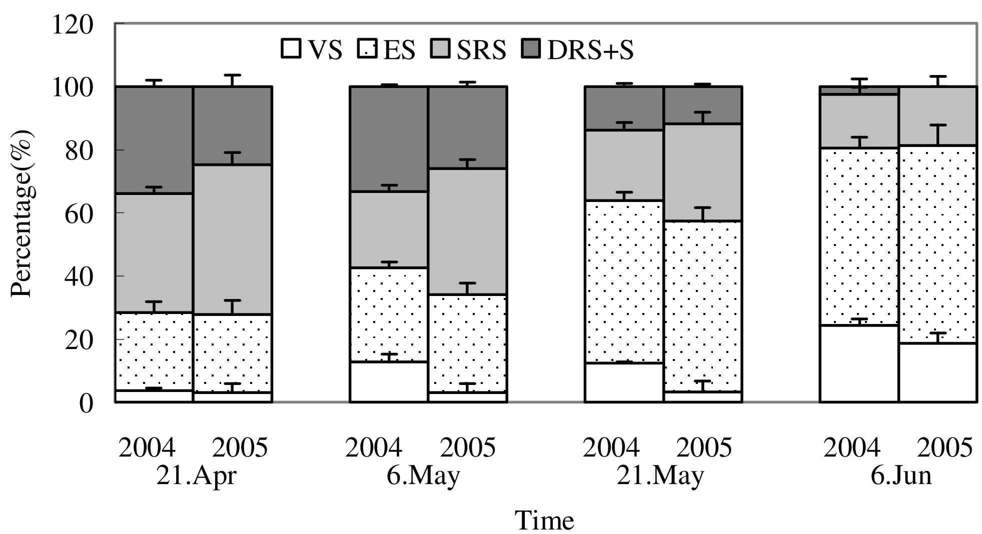

Figure 2. The proportions of each differentiation stage after recruits stage but before spiking stage in 2004 and 2005. VS: Early stage, ES: elongation stage; SRS: single ridge stage; DRS+S: double ridge stage and subsequent stages.

that the proportions of *L. chinensis* developed into Early stage and Elongation stage were 3 to 4% and 24 to 25%, respectively. By spiking date (June 6th) the proportions of these two stages gradually increased and reached 18 to 24% (Early) and 56 to 62% (Elongation). Conversely, the proportions of Single ridge stage and Double ridge stage, for April 21, was 38 to 46% and 25 to 34%, respectively. The proportions of these two stages both declined by spiking date (June 6th). The Double ridge stage, in particular, measured zero in the later stage (Figure 2).

SAM differentiation of vegetative shoots after the spiking date

During June to September sampling time, the paraffin sections from vegetative shoots showed the following proportions: Early stage, 12 to 33%; Elongation, 54 to 74%; and Single ridge, 8 to 17% (Figure 3). These results indicated that these distinct differentiation statuses coexisted at each successive sampling time, and the proportions of each status changed minimally after spiking, despite of the dominance of the Elongation stage. Interestingly, the Double ridge stage and subsequent differentiation stages were not found in the SAM of vegetative shoots during June to September.

SAM differentiation of grass growing in the greenhouse over the winter

From June 2007 to July 2008, all grasses grown during

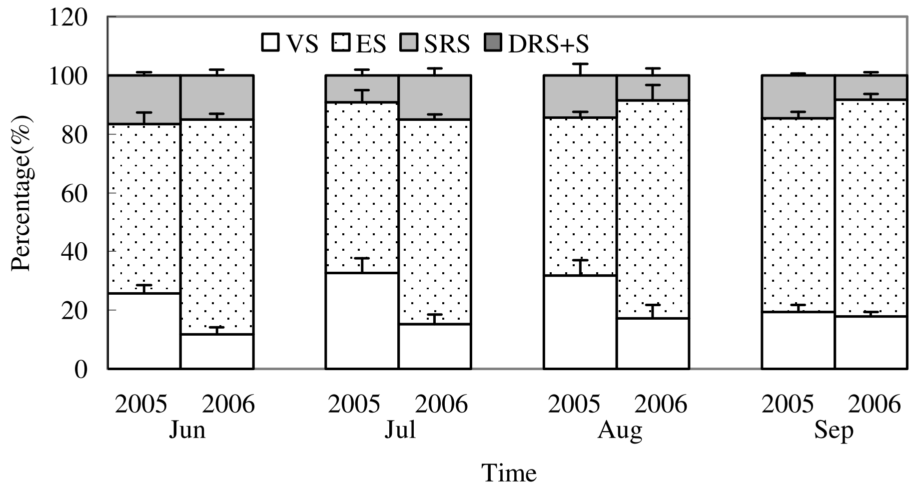

Figure 3. The proportions of each differentiation stage after recruits stage but before spiking stage in vegetative shoots in 2004 and 2005. VS: Early stage; ES: elongation stage; SRS: single ridge stage; DRS+S: double ridge stage and subsequent stages.

the winter in the greenhouse grew continuously, developed new leaves, and increased in height. However, it did not produce spikes. For grass shoots sampled in July 2008, the results showed that the proportions of Early stage, Elongation stage, Single ridge stage, Double ridge stage and subsequent stages were 21.2, 72.7, 6.1, 0 and 0%, respectively.

DISCUSSION

The aboveground parts of *L. chinensis* regreen in mid-April and cease growing in early October, which completes the growing season. A proportion of grass shoots exhibited the double ridge stage and subsequent stages of SAM differentiation in April and May, before the spiking date, but these stages were not detected during periods after spiking. Therefore, we assume that if the SAM differentiates into the double ridge stage and subsequent stages before the spiking date, the grass can eventually spike and develop reproductive shoots. However, for grass in the early stage, elongation stage or single ridge stage after the spiking date, the SAM will, at most, develop into the single ridge stage, and then arrest and become vegetative. The double ridge stage is a critical period to determine whether *L. chinensis* spikes. In the studies of winter wheat and perennial ryegrass, it has been demonstrated that the double ridge stage is a critical period of reproduction (Porter et al., 1987; Toyota et al., 2001; Onishi et al., 2003; Zhang et al., 2007). As a perennial rhizomatous grass, *L. chinensis* may have a developmental process similar to winter wheat and perennial ryegrass.

Why did only a portion of *L. chinensis* shoots exhibiting SAM differentiation subsequently develop into the double ridge stage in the spring? A portion of the grass regreening in the current spring originates from newborn grass in the previous autumn, whereas another portion comes from the underground germination-derived grass in the current spring (Zhang et al., 2009). Compared to the daughter shoots that developed from underground buds in late autumn, the early growth daughter shoots have a longer growing time from in early autumn, which leads to the large variation seen in differentiation status of SAM.

This suggests that the SAM of daughter shoots successful in differentiation into the Double ridge stage may be decided by the length of growing time before the winter. This agrees with past studies, which demonstrated that the spike number was constrained by the daughter shoot number that formed in the autumn of the previous year (Yang et al., 2000; Wang et al., 2010).

However, temperature is a very important factor in SAM development of wheat. A low temperature environment, in particular, is a necessary condition for young spike differentiation, because vegetative growth cannot be transferred into reproductive growth in the absence of low temperatures. Additionally, studies have documented that the most sensitive time for response to low temperature is from the Single-ridge stage to the Double-ridge stage (Haloran and Pennel, 1982; Trione and Metzger, 1970; Porter et al., 1987). Grasses grown in the greenhouse experience a photoperiod similar to an outdoor environment, but do not experience low winter temperatures. Therefore, under greenhouse conditions, *L. chinensis* developed new leaves continuously, increased in height, but never produced spikes with the SAM differentiating only into the early stage, elongation stage and single ridge stage.These results indicate that low winter temperature is a determining factor that restricts the reproduction process of *L. chinensis.* Similar to the developmental process of winter wheat, the SAM of

L. chinensis daughter shoots, developed in the previous early autumn, can differentiate into the single ridge stage and continue complete vernalization and develop to spike. Therefore, the ceiling temperature for effective vernalization needs further study.

Conclusion

We concluded as follows: (1) During and after regreening but before spiking, the SAM differentiation of spiked shoots was simultaneously observed in the early stage, elongation stage, single ridge stage, double ridge stage and subsequent stages. However, in un-spiked shoots, the double ridge stage and subsequent stages were not observed. (2) Similar with the un-spiked shoots, the daughter shoots cannot spike, and the SAM differentiation was observed only in the early stage, elongation stage and single ridge stage, which was produced in autumn and without low winter temperature. (3) Our results indicated that double ridge stage is a critical period to determine whether *L. chinensis* can spike, and low winter temperature also played an important role in spiking of this species.

ACKNOWLEDGEMENTS

The research was funded by National Natural Science Foundation of China (31172259), and the Fundamental Research Funds for the central universities (10SSXT145).

REFERENCES

Chen M (1983). Study on production capability improvement in artificial grasslands. Grassland Ecol. Syst. Res., 3: 193-209 (Chin).

Gardner JS, Hess WM, Trione EJ (1985). Development of the young wheat spike: a SEM study of Chinese spring wheat. Am. J. Bat., 72: 548-559.

Haloran GM, Pennel AL (1982). Duration and rate of development phases in wheat in the environment. Ann. Bot., pp. 49-115.

Kuo PC (1987). Flora Reipublicae Popularis Sinicae. Science Press, Beijing, p. 19 (Chin).

Li H, Yang YF, Lu XS (2004). Quantitative analysis of reproductive tiller characteristics of *Leymus chinensis* populations on the Songnen Plain of China. Acta Pratacult. Sci., 13: 50-56 (Chin).

Li XF, Liu J, Liu GS (2001). Correlation and Path Analysis on Quantitative Characters of *Leymus chinensis*. Acta Agrestia Sin., 11: 42-47 (Chin).

Ma HL, Wan T, Wang FG (1984). Characteristics of seed setting and causes of low seed yields in aneurolepidium chinense, 2: 14-20 (Chin).

Mu CS (2001). The developmental morphology on spike characters of Rice variety. Tokyo University Doctors Paper, pp. 44-45.

Mu CS, Zhang BT, Cui S (2004). The leaf age progression of vegetative shoot s and it s relationship with aboveground biomass of *Leymus chinensis* in different habitats. Acta Pratacult. Sci., 13: 75-79 (Chin).

Onishi K, Sano Y, Nakashima H (2003). Developmental Fates of Axillary Buds as a Major Determinant for the Pattern of Life History in *Lolium*. Plant Prod. Sci., 6: 179-184.

Porter JR, Kirby EJM, Day W, Adam JS, Appleyard M, Ayling S, Baker CK, Beale P, Belford RK, Biscoe PV, Chapman A, Fuller MP, Hampson J, Hay RKM, Hough MN, Matthews S, Thompson WJ, Weir AH, Willington VBA, Wood DW (1987). An analysis of morphological development in Avalon winter wheat crops with different sowing dates and at ten sites in England and Scotland. J. Agric. Sci. Camb., 109: 107-121.

Toyota M, Tsutsui I, Kusutani A, Asanuma K (2001). Initiation and Development of Spikelets and Florets in Wheat as Influenced by Shading and Nitrogen Supply at the Spikelet Phase. Plant Prod. Sci., 4: 283-290.

Trione EJ, Metzger RJ (1970). Wheat and barley vernalization in a precise temperature gradient. Crop Sci., 4: 380-398.

Wang JF, Xie JF, Zhang YT, Gao S, Zhang, JT, Mu CS (2010). Methods to Improve Seed Yield of *Leymus chinensis* based on Nitrogen Application and Precipitation Analysis. Agron. J., 102: 277-281.

Wang RZ, Ripley EA, Zu YG, Nie SQ (2001). Demography of sexual and biomass allocation of grassland and dune *Leymus chinensis*: An analog for response to environment change. J. Arid Environ., 49: 289-299.

Yan H, Zhao W Yin SJ, Shi DC (2006). Different physiological responses of Aneurolepidium chinense to NaCl and Na_2CO_3. Acta Pratacult. Sin., 15: 49-55 (Chin).

Yang YF, Li JD (2001). The ecological plasticity of the quantitative characters for ear heads of *Leymus chinensis* population in natural meadow in northeast China. Acta Ecol. Sin., 21: 752-758.

Yi J, Li QF, Gu AL (2004). Advances on Biology Characteristics the Rhizomatous Grasses. J. Arid L and Res. Environ., 15: 1-16 (Chin).

Zhang JT, Mu CS, Wang DL, Wang JF, Chen GX (2009). Shoot population recruitment from a bud bank over two seasons of undisturbed growth of *Leymus chinensis*. Bot. Sci., 87: 1242-1249.

Zhang L, Takahashi T, Fujimoto K, Yamaguchi S, Matsuzawa T (2007). Factors in the Reduction in Grain Number in Winter Wheat by Early-Sowing in Yamaguchi. Plant Prod. Sci., 10: 189-198.

Zhou C, Yang YF (2006). Allometry regulation of leaf module for two ecotypes of *Leymus chinensis* in Songnen Plain China. Acta Pratacult. Sin., 15: 76-81 (Chin).