Construction of a Transformation-Competent Artificial Chromosome (TAC) Library of *Leymus multicaulis*

Y. L. Zhou
Zhongkai University of Agriculture and Technology, China

Y. Y. Xu
Hunan Institute of Engineering, China

M. L. Zhao
Beijing Academy of Agriculture and Forestry Sciences, China

Zh. Zh. Cao
Gansu Agriculture University, China

P. Chen
Zhongkai University of Agriculture and Technology, China

The XXI International Grassland Congress / VIII International Rangeland Congress took place in Hohhot, China from June 29 through July 5, 2008.

Proceedings edited by Organizing Committee of 2008 IGC/IRC Conference

Published by Guangdong People's Publishing House

Construction of a transformation-competent artificial chromosome (TAC) library of *Leymus multicaulis*

Y .L . Zhou[1,4] ,Y .Y . Xu[2] , M .L . Zhao[3] , Zh .Zh . Cao[4] , P . Chen[1]
[1] College of Agriculture and Gardenning , Zhongkai University of Agriculture and Technology , Guangzhou Guangdong 510225 , PR China ,E-mail zhouyl@yahoo .cn
[2] Hu nan Institute of Engineering , Xiangtan Hu nan 411104 , PR China
[3] Beijing Agriculture Biotechnology Research Center , Beijing Academy of Agriculture and Forestry Sciences , Beijing 100089 , PR China
[4] Gansu Agriculture University , Lanzhou Gansu 730070 , PR China .

Key words : *Leymus multicaulis* ,transformation-competent artificial chromosome (tac) , genomic library , high molecular weight dna , insert size

Introduction *Leymus multicaulis* (2n = 4x = 28 = 14Ⅱ , XmNs) is an important forage of *Leymus Hochst* of Triticaea of Gramineae plant (Wang Shijin and LI Jianhua ,1993) , which possesses resistance characteristics to dry , salt , barley yellow dwarf virus , budworm , barren conditions and pollution . For utilizing the resistance characteristics of *Leymus multicaulis* , we constructed a transformation-competent artificial chromosome (TAC) genomic library of *Leymus multicaulis* in the pYLTAC747H vector . Such genomic libraries should be useful for gene cloning in *Leymus multicaulis* and other crops .

Materials and methods High molecular weight (HMW) DNA was prepared from tender leaves of *Leymus multicaulis* etiolated seedlings using the nuclei-based method as described (Y .L . Zhou et al . ,2007) . TAC vector DNA preparation ,and purity , partial digestion , size selection of *Leymus multicaulis* HMW DNA , and the ligation and transformation of vector DNA with HMW DNA used the method as described (Y .G . Liu et al . , 2002) .

Results We successfully isolated very pure HMW nuclear DNA which was over 2Mb in size (Figure 1) . A TAC genomic library was constructed from nuclear DNA of *Leymus multicaulis* . The library consisted of 2.4×10^5 clones which were collected as bulked pools each containing 500 clones and stored in 5×96-well plates . The library has insert sizes of genomic DNA of approximately 5Kb～200Kb and average insert size of 50Kb (Figure 2) , representing approximately 2～4 haploid genome equivalents .

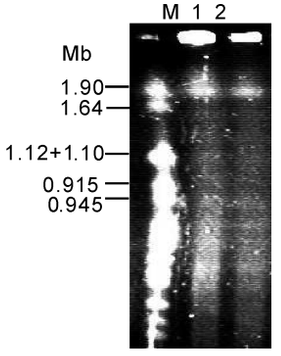

Figure 1 *Size determination of Leymus multicaulis HMW DNA by plused-field gel electrophoresisM : Yeast Chromosome PFG Marker (NEB) , 1 .5ug ; 1 : 1 plug ; 2 :1/2 plug .*

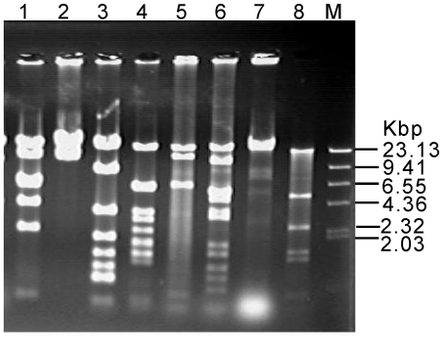

Figure 2 *Recombinant TAC clones digested with restriction enzyme HindIIILine1-8 : clones with TAC747H vector ; M : λDNA-HindIII molecular marker .*

Conclusions Most isolated *Leymus multicaulis* DNA was greater than 2 Mb , and was suitable for constructing a TAC library . Vector pYLTAC747H , which is 18 ,900 bp , was used to construct the TAC library . Constructing a large-insert genomic DNA library is essential for gene map-based cloning . Such libraries will be used to clone many genes . With its high stability and good genomic coverage , the TAC library provides an efficient platform for gene cloning and functional complementation of target genes in *Leymus multicaulis* .

References

Sh .J . Wang J .H . Li , 1993 . A preliminary introduction and evaluation of forage germplasm resources in tribe triticeae . *Acta Prataculture Sinica* 2(1) , 60-69 .
Y .L . Zhou , Y .Y Xu , M .L Zhao , Zh Zh . Cao , Zh .P . Wang , 2007 .An Efficient Protocol for Preparation of High Molecular Weight DNA of Nuclei from *Leymus Multicaulis* .*Pratacultural Science* 24(10) , 52-56 .
Y .G . Liu , HM Liu , L .T . Chen , W .H . Qiu , Q .Y . Zhang , H . Wu , C .Y . Yang , J . Su , Z .H . Wang , D .S . Tian , M .T . Mei ,2002 . Development of new transformation-competent artificial chromosome vectors and rice genomic libraries for efficient gene cloning . *Gene* 282 (1-2) , 247-255 .