Ecological Genetic Study on Flowering Phenology in Red Clover

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The 21st International Grassland Congress / 8th 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

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Key words : red clover , SSR marker , flowering traits , local adaptation

Introduction Red clover (*Trifolium pratense* L .) is an important forage legume and adapted to a wide range of environments . Much genetic variability within this species has been characterized by genetic analyses using isozymes as well as DNA markers . In red clover , genetic linkage maps of some segregated populations were constructed with a few thousands DNA makers including SSR , EST , and RFLP . The purpose of our study is to clarify the mechanism of local adaptation of red clover populations . In this study , we focused flowering habit because red clover was thought to divide two ecological groups with different maturity in previous studies . We used SSR markers associated flowering time to assess genetic differentiation for flowering traits .

Materials and methods Three red clover cultivars ; Natsuyu , Corvus and Nordi were used in this study . Natsuyu and Corvus are early maturing cultivars bred at Japan and Switzerland , respectively , while Nordi is late maturing one bred at Norway . The experiment was carried out with 2 × 2 factorial design for each cultivar , with day length (14 hs or 18 hs) and vernalization (first mature leaf stage or second true leaf stage) as the factor . Each treatment was represented by 20 or 25 plants . The total of 90 plants of each cultivar were analyzed by eight SSR markers which was chosen from the public DNA databases (http ://www .clovergarden .jp/) and developed by K lliker *et al* . (2006) . All markers located at the vicinity of QTLs for flowering traits in individual linkage group (Harrmann *et al* . 2006) . Four SSR primer sets were used to detect polymorphism .

Results After 29 weeks from seedling , all plants in Natsuyu and Corvus were flowered , but almost plants of Nordi grown under 14 hs day length didn't flower . In all cultivars , flowering date was earlier 18h than 14h and vernalization at second true leaf than at first mature leaf (Table 1) . It indicated that day length is main factor of flowering in red clover ; while vernalization accelerates it , but not absolute factor . It confirmed five to eight alleles in each primer set (Table 2) . A difference among cultivars was observed for allelic frequency in each primer set . RCS0131 and RCS4430 loci showed different pattern of allelic frequency between early maturing and late ones .

Table 1 *Effect of day length and vernalization of flowering date in each cultivar .*

Leafage at vernalization	First mature leaf		Second true leaf		ANOVA		
	14h	18h	14h	18h	Day length(A)	Vernalization(B)	A × B
Natsuyu	53 .3	46 .8	43 .9	37 .0	* *	* *	ns
Corvus	53 .5	47 .6	38 .6	33 .2	* *	* *	ns
Nordi	—	76 .06	—	64 .28	—	ns	—

Conclusions Red clover had a wide range of variety of sensitively to day length and requirement for vernalization between and within cultivars . It was confirmed that different allelic frequency was observed among cultivars . SSR loci having different pattern of allelic frequency between early maturing and late ones could be useful in monitering population dynamics in red clover population to clarify the relationship between local adaptation and flowering phenology .

Table 2 *Allelic frequency was used in each cultivar .*

Locus	Cultivar	Allelic frequency							
		Ⅰ	Ⅱ	Ⅲ	Ⅳ	Ⅴ	Ⅵ	Ⅶ	Ⅷ
TPSSR16	Natsuyu	0 .29	0 .28	0 .13	0 .21	0 .01	0 .07	—	—
	Corvus	0 .25	0 .08	0 .17	0 .28	0 .14	0 .08	0 .01	—
	Nordi	0 .30	0 .30	0 .10	0 .20	—	0 .10	—	—
TPSSR23	Natsuyu	0 .25	0 .49	0 .20	0 .03	0 .04	—	—	—
	Corvus	0 .43	0 .21	0 .14	0 .07	—	0 .04	0 .09	0 .02
	Nordi	0 .08	0 .40	0 .38	0 .08	0 .01	0 .01	0 .02	—
RCS0131	Natsuyu	0 .26	0 .28	0 .17	—	0 .13	0 .16	—	—
	Corvus	0 .23	0 .23	0 .16	0 .06	0 .08	0 .13	0 .09	0 .02
	Nordi	0 .17	0 .17	0 .33	0 .03	0 .10	—	0 .10	0 .10
RCS4430	Natsuyu	0 .55	0 .19	0 .14	0 .06	0 .06	—	—	—
	Corvus	0 .57	0 .21	0 .11	0 .11	—	—	—	—
	Nordi	0 .71	0 .14	0 .14	—	—	—	—	—

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