Introduction of a self-compatible gene of *Lolium temulentum* L. to perennial ryegrass (*Lolium perenne* L.) for the purpose of the production of inbred lines of perennial ryegrass

Toshihiko Yamada

Yamanashi Prefectural Dairy Experiment Station

Nagasaka, Yamanashi 408-0021, Japan

Present address

Hokkaido National Agricultural Experiment Station,

Sapporo, Hokkaido 062-8555, Japan

Key words: inbred line, interspecific hybridization, *Lolium perenne, Lolium temulentum*, perennial ryegrass, self-compatibility

Abstract

Interspecific hybrids between self-compatible species, *Lolium temulentum* L. and self-incompatible species, *L. perenne* L. were obtained using embryo rescue. Two cycles of backcrossing of interspecific hybrids with *L. perenne* were carried out. A 1: 1 segregation ratio of self comatibility and incompatibility was observed in backcross generations. These segregation data confirmed that self-compatibility of *L. temulentum* was controlled by a single gene. It suggested that the self-compatible gene of *L. temulentum* could be introduced to self-incompatible plants of *L. perenne* through interspecific hybridization. It appears that

utilization of a self-compatible gene of *L. temulentum* would be useful for production of inbred lines of *L. perenne*. The possibility of breeding procedures of perennial ryegrass using a self-compatible gene was discussed.

Introduction

The improvement of open-pollinating populations such as cool season grasses and clovers depends essentially upon a change of gene frequencies toward fixation of favorable alleles while maintaining some degree of heterozygosity. Recurrent selection procedures have been useful in breeding programs for these open-pollinating species. One of the greatest problems concerning the utility of such breeding procedures in cool season grasses and clovers is the production and maintenance of inbred lines.

Most outbreeding species of cool season grasses and clovers possess a gametophytic self-incompatibility system. Mutations of the locus for self-incompatibility are well known to give rise to self-fertility in clovers (Atwood, 1942; Rinke & Johnson,1941; Townsend, 1965; Yamada et al., 1989a). Important forage and turf grass, perennial ryegrass (*Lolium perenne* L.) is outbreeding with the system of two locus (S, Z) multi-allelic self-incompatibility (Cornish et al., 1979). Many studies on inbreeding in perennial ryegrass were carried out (Utz & Oettler, 1976; Jones & Jenabzadeh, 1981; Thorogood & Hayward, 1991). Thorogood & Hayward(1991) suggested that one inbred line of perennial ryegrass has been shown to posses a single mutation gene (Sc), independent of the S and Z loci, which is gametophytic in action. Further, self-incompatibility mechanism occasionally breaks down by environmental conditions so that a small number of self seed can be obtained. Wilkins & Thorogood (1992) observed that seed set by selfing of perennial ryegrass plants was markedly increased by heat treatment (34^{o}C).

On the other hand, it is well known that genus *Lolium* L. contains inbreeding species such as *L. temulentum* L.. *L. temuletum* hybridized relatively easily with *L. perenne* (Jenkin, 1935). Inheritance of self-compatibility in *L. temulentum* was examined using backcrossed generations of interspecific hybrids between *L. temulentum* and *L. perenne* with *L. perenne* (Nitzsche, 1983; Thorogood & Hayward, 1992). Self-compatibility in *Lolium temulentum* was controlled in a gametophytic manner by a single gene mutation of either the Z locus or a locus tightly linked to it (Thorogood & Hayward, 1992).

L. perenne is one of the most important forage and turf grasses in the temperate zones of the world. Many varieties for forage and turf have been developed by the breeding procedures of mass selection and synthetics. Production of inbred lines seems to be more promising for the improvement of *L. perenne*. However, attempt of breeding program using inbred lines has been little. In the present study interspecific hybridization between *L. temulentum* and *L. perenne* and two cycles of backcrossing the hybrids with *L. perenne* were carried out for the purpose of the production of inbred lines. The possibility of breeding procedures of perennial ryegrass using a self-compatible gene was discussed.

Materials and methods

Four strains of *Lolium temulentum*, PI 176624, 197439, 249725 and Ba 3081 used in the present study. PI 176624, 197439 and 249725 were obtained from the Hokuriku National Agricultural Experiment Station, Japan, and original germplasm was introduced from the United States Department of Agriculture with the UJNR (United States – Japan Conference on Natural Resources Development) project on exchange of genetic resources. Ba 3081 was introduced from the Institute of Grassland and Environmental Research, UK. Diploid turf type varieties of *Lolium perenne,* "All*Star", "Prelude" and "Saturn" were used. The interspecific hybrid plants were produced by the crossing hand– emasculated *L. temulentum* plants with *L. perenne* plants. Embryo rescue technique using B5 (Gamborg et al., 1968) agar medium containing 3 % sucrose was applied to produce interspecific hybrids.

The resulting interspecific hybrids were backcrossed with *L. perenne* twice with diploid turf type varieties of *L. perenne*, "Advent ", "Manhattan II" and "Prelude". Because interspecific hybrids were male sterile, it used as the female parents without emasculation. Further hand – emasculated self-compatible plants in BC1 were crossed with *L. perenne,* "Advent". Fertility and self-compatibility in backcrossed generations (BC_1, BC_2) were checked by the investigation of seed set. The spikes of each plant were bagged at the time of ear emergence in 5 x 25 cm glassine paper bags where they remained until mature. Plants setting no self seed, 1-5 self seeds per spike and more than 5 self seed per spike were classified as self-incompatible, partially self-compatible and fully self-compatible, respectively.

Results

Reciprocal crosses between *L. temulentum* and *L. perenne* were possible. Because of big size of florets in *L. temulentum*, it was used as the female parent. Development of hybrid embryos was observed, but many of them were aborted on and after 14th days after pollination, due to endosperm failure. Four strains of *L. temulentum* were crossed with *L. perenne* and no significant difference on development of embryos was observed. The embryo culture technique involved the culture of fertilized embryos 12 – 14th days after pollination. In total, one hundred and seventy-one embryos were cultured, of which thirty-four germinated (Table 1). Seventeen hybrid plants were transplanted into soil. All of them grew into the flowering stage. Hybrid plants showed intermediate between *L. temulentum* and *L. perenne* (Table 2). No significant difference on the number of hybrid plants obtained was found among different cross combinations.

All of hybrid plants were male sterile and backcross progeny (BC_1) was produced by crossing these hybrids as the female parents with a particular variety and open-pollination of three varieties of *L. perenne*. Some plants of backcross progeny were male sterile. Fully self-compatible plants setting more than 5 seeds per spike, partially self-compatible plants setting 1 to 5 seeds per spike and self-incompatible plants were observed in backcross generation (Table 3). Fully self-compatible and partially self-compatible plants were expressed as the self-compatible plants. Segregation of self-comatibility and self-incompatibility was agreed with 1: 1 ratio in the first backcross generation (Table 5).

Some of hand- emasculated self-compatible plants in BC_1 were backcrossed with *L. perenne* (Advent). In the second backcross generation, no male sterile plant was observed. Segregation of self-compatibility and self-incompatibility was also 1: 1 in the second backcross generation (Table 4,5). These segregation data confirmed that self-compatible gene of *L. temulentum* was controlled by a single gene for self-incompatibility and introduction of a self-compatible gene to *L. perenne* was possible.

Discussion

Genus *Lolium* L. can be divided into two groups: outbreeding species such as *L. perenne* L. and *L. multiforum* Lam. and inbreeding species such as *L. temulentum* L. and *L. remotum* Schrank. There are a clear tendency for highest seed set and germination in crosses between inbreeding species and between outbreeding species, respectively (Jauhar, 1993). When crosses were made between an inbreeding species and an outbreeding species, embryo rescues were required. The present study also showed that *L. temulentum* hybridized relatively easily with *L. perenne* as either the male and the female parent although embryo-rescue techniques were required to obtain adequate number of hybrid plants. Within the genus *Lolium* the inbreeding species and outbreeding species constitute two distinct groups, between which at least partial barriers to hybridization exist (Jauhar, 1993). Borrill (1976) surmised that inbreeding species were derived from the outbreeding group.

The present data on segregation of self-compatibility and self-incompatibility coincided with the results of Thorogood & Hayward (1992). It was confirmed that self-compatible gene of *L. temulentum* was controlled by a single gene. These findings suggested that self-compatible gene of *L. temulentum* could be introduced to self-incompatible plants of *L. perenne* through interspecific hybridization.

In the first backcross generation 10 % of plants were male sterile, however, in the second backcross generation no male sterile plant was observed. These findings might be accounted for by irregularity in chromosome pairing, and chromosome pairing might be increased with backcross generations. Percentage of partially self-compatible plants was decreased in BC_2 generation. Thorogood & Hayward (1992) also investigated the increase of seed set in BC_2 self-fertile plants. Such low seed set of self-compatible plants in the first backcross generation might be also influenced by irregularity in chromosome pairing. It appears that percentage of partially self-compatible plants will be decreased with the progress of the generations of backcrossing.

Production of inbred lines is more promising for the improvement of outbreeding species. *L. perenne* lines possessing self-compatible gene of *L. temulentum* will be produced by several cycles of backcrossing with *L. perenne*. Breeding materials are crossed with self-compatible lines of perennial ryegrass. Yamada et al. (1989a,b) proposed recurrent selection programs for white clover using self-compatible plants. Inbreeding may be used to remove deleterious genes from breeding materials in the process of recurrent selection. Turf type varieties of perennial ryegrass were used for materials in the present study. Dwarf plant type which accumulated many available genes such as disease resistance and stress tolerance genes is most required in a turf grass variety. Therefore, breeding procedures using inbred lines may be more promising in case of breeding program for turf. Further practical evaluation in the field is essential for establishment of breeding procedures using self-compatible gene.

Acknowledgements

The author is indebted to Dr. K. Tase, the Hokuriku National Agricultural Experiment Station, Joetsu, Japan (present address: Yamanashi Prefectural Dairy Experiment Station) and Dr. D. Thorogood, the Institute of Grassland and Environmental Research, Aberystwyth, UK for providing strains of *Lolium temulentum,* and to Mrs. Y. Obi for technical assistance. The author would also like to thank Dr. H. Yamaguchi, Hokkaido National Agricultural Experiment Station, for critical reading of the manuscript.

References

Atwood, S.S., 1942. Genetics of self-compatibility in *Trifolium repens*. J. Am. Soc. Agron. 34: 353-364.

Borrill, M., 1976. Temperate grasses. *Lolium, Festuca, Dactylis, Phleum, Bromus* (Gramineae). In: N.W. Simmonds (ed.) , Evolution of crop plants. pp. 137-142. Longman, London.

Cornish, M.A., M.D. Hayward & M.J. Lawrence, 1979. Self-incompatibility in ryegrass. I. Genetic control in diploid *Lolium perenne* L. Heredity 43: 95-106.

Gamborg, O.L., R.A. Miller & K. Ojima, 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50: 151-158.

Jauhar, P.P., 1993. Cytogenetics of the *Festuca – Lolium* Complex. Relevance to breeding. In: R. Frankel, M. Grossman, H.F. Linskens, P. Maliga & R. Riley (Eds.), Monographs on theoretical and applied genetics, vol. 18. 243 pp. Springer-Verlag, Berlin.

Jenkin, T.J., 1935. Interspecific and intergeneric hybrids in herbage grasses. II. *Lolium perenne* x *L. temulentum*. J. Genet. 31: 379-412.

Jones, R.N. & P. Jenabzadeh, 1981. Variation in self-fertility, flowering time and inflorescence production in inbred *Lolium perenne* L. J. Agric. Sci. 96: 521-537.

Nitzsche, W., 1983. Inheritance of the mode of fertilisation in *Lolium* spp. Z. Pflanzenzuchtg. 90: 243-248.

Rinke, E.H. & I.J. Johnson, 1941. Self-fertility in red clover in Minnesota. J. Am. Soc. Agron. 33: 512-521.

Thorogood, D. & M.D. Hayward, 1991. The genetic control of self-compatibility in an inbred line of *Lolium perenne* L. Heredity 67: 175-181.

Thorogood, D. & M.D. Hayward, 1992. Self-compatibility in *Lolium temulentum* L: its genetic control and transfer into *L. perenne* L. and *L. multiflorum* Lam. Heredity 68: 71-78.

Townsend, C.E., 1965. Self-compatibility studies with diploid alsike clover, *Trifolium hybridum* L. I. Frequency of self-compatible plants in diverse populations and inheritance of a self-compatibility factor (S_f). Crop Sci. 5: 358-360.

Utz, H.F. & G. Oettler, 1978. Performance of inbred lines and their top crosses in perennial ryegrass (*Lolium perenne* L.). Z. Pflanzenzuchtg. 80: 223-229.

Wilkins, P.W. & D. Thorogood, 1992. Breakdown of self-incompatibility in perennial ryegrass at high temperature and its uses in breeding. Euphytica 64: 65-69.

Yamada, T., H. Fukuoka, & T. Wakamatsu, 1989a. Recurrent selection programs for white clover (*Trifolium repens* L.) using self-compatible plants. I. Selection of self-compatible plants and inheritance of a self-compatibility factor. Euphytica 44: 167-172.

Yamada, T., S. Higuchi, & H. Fukuoka, 1989b. Recurrent selection of white clover (*Trifolium repens* L.) using self-compatibility factor. Proc. XVI Int. Grassl. Congr. : 299-300.

Table 1. Results of interspecific hybridization between *Lolium temulentum* and *L. perenne* by embryo rescue technique.

Combination	Number of cultured embryos	No. of seeds germinated	No. of plants obtained
L. temulentum X Prelude	98	21	11
L. temulentum X All*Star	48	10	5
L. temulentum X Saturn	25	3	1
Total	171	34	17

Four strains of *L. temulentum* (PI 176624, 197439, 249725 , Ba3081) were used as female parents.

Table 2 Some morphological traits of *Lolium temulentum, L. perenne* and interspecific hybrids

	Leaf length (cm)	Leaf width (cm)	Spike length (cm)
L. temulentum *	25.8	7.7	23.3
L. perenne **	8.7	4.2	12.5
Interspecific hybrids***	13.6	6.4	19.1

*Average of four strains (PI 176624, 197439, 249725 , Ba3081)

**Average of three varieties (Prelude, All*Star and Saturn)

***Average of seventeen plants

Table 3. Fertility and segregation of self-compatible and self-incompatible plants in BC_1 generation

	Male fertility		Self- [2]		
BC_1 line	fertile	sterile	fully compatible	partially compatible	incompatible
No.4 x Advent	3	0	2	0	1
No.6 x Advent	3	0	3	0	0
No. 10 x Advent	8	0	4	0	4
No. 10 x Manhattan II	8	0	4	0	4
No. 10 x Prelude	6	0	1	1	4
No. 1 open[1]	10	0	4	2	4
No. 3 open	4	4	1	1	2
No. 4 open	9	1	4	1	4
No. 5 open	9	1	2	2	5
No. 6 open	9	1	3	4	2
No. 8 open	6	2	2	1	3
No. 9 open	9	1	2	2	5
No. 14 open	5	0	1	2	2
No. 16 open	8	0	1	2	5
Total	97	10	34	18	45

1) Open crossed with diploid varieties (Advent, Manhattan II, Prelude).

2) Classification was described in text.

Table 4. Fertility and segregation of self-compatible and self-incompatible plants in BC_2 generation

	Male fertility		Self- [1)]		
BC_2 line	fertile	sterile	fully compatible	partially compatible	incompatible
(No. 10 x Advent)-1 x Advent	8	0	5	0	3
(No. 10 x Advent)-2 x Advent	12	0	7	0	5
(No. 10 x Advent)-3 x Advent	8	0	3	1	4
(No. 10 x Advent)-4 x Advent	13	0	6	0	7
No. 1 open-1 x Advent	18	0	8	0	10
No. 1 open-2 x Advent	15	0	6	2	7
No. 4 open-1 x Advent	8	0	5	0	3
No. 4 open-2 x Advent	9	0	2	2	5
Total	91	0	42	5	44

1) Classification was described in text.

Table 5. Segregation ratio of self-compatible and self-incompatible plants in backcross generations

	Self-compatible*	Self-incompatible	χ^2 (1:1)
BC_1	52	45	0.51^{ns}
BC_2	47	44	0.10^{ns}

* Total of fully self-compatible and partially self-compatible plants.