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GENETIC CONTROL OF PARTHENOGENESIS IN KENTUCKY BLUEGRASS: RESULTS FROM A SEXUAL x APOMICTIC CROSS

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Abstract

Apomixis, as it exists in *Poa pratensis* L., permits to combine genotype fixation with propagation by seed. The process of apomictic seed production involves the formation of embryo sacs without meiotic reduction (apospory) and embryos without egg cell fertilization (parthenogenesis). Further information on the genetic control of apomixis was obtained by analyzing aposporous parthenogenesis and the segregation of molecular markers in a progeny resulting from a “sexual” (S) x “apomictic”(A) cross. Data from the 35 F_1 plants examined so far have shown that parthenogenesis segregated 1:1, confirming control by a dominant gene, simplex in the parthenogenetic parent. Analysis of variance and regression were used to find single markers from the A and S parents affecting the trait in the 15 parthenogenetic progenies. A minimum of 4 genes from the sexual parent and 1 from the apomictic parent influencing the expression of parthenogenesis appeared to segregate in this cross.

Keywords: Apomixis, Genetic control, Molecular markers, Parthenogenesis, *Poa pratensis* L.

Introduction

Kentucky bluegrass (*Poa pratensis* L.) is a facultative apomict with an extremely versatile mode of reproduction (Mazzucato *et al.*, 1995; Barcaccia *et al.*, 1997). Apomictic reproduction involves the parthenogenetic development of unreduced eggs from aposporic embryo sacs and leads to progenies of maternal origin. Sexual reproduction through either outcrossing or selfing is also possible in this species.

On the genetic control of the mode of reproduction in *P. pratensis*, independent cyto-embryological and molecular progeny tests have indicated that a single dominant allele controls parthenogenesis (Matzk, 1991; Barcaccia *et al*., 1998). Indeed, the two components of apomixis, apospory and parthenogenesis, appear to be closely associated in *P. pratensis*: sexual, non parthenogenetic plants completely lack aposporic activity in ovules and apomictic plants are highly aposporic and parthenogenetic (Barcaccia *et al*., 1997). The question remains as to whether apospory and parthenogenesis are determined by the same or by different genetic factors (Savidan, 2000).

Marker-assisted selection (MAS) would be very useful for selecting plants expressing apomixis at high level without the need of progeny testing in *P. pratensis* breeding programs. In this work, a progeny segregating for parthenogenesis was characterized with PCR-based molecular markers, and the molecular data from the parthenogenetic plants were subjected to analysis of variance and multiple regression in an attempt to determine the minimum number chromosome regions controlling the expression level of parthenogenesis.

Materials and Methods

A completely sexual clone ($S1/B_I$ $2 \cong 5x \cong 35$) was crossed with a highly apomictic genotype ($RS7/H \cong 4x \cong 28$). These plants were selected within different F_1 progenies resulting from “sexual” x “sexual” and “apomictic” x “apomictic” crosses and characterized,

respectively, as B_I hybrid and (poly)haploid (Barcaccia *et al*., 1997). Total genomic DNA was isolated from 70 F_1 plants using the CTAB procedure (Doyle and Doyle, 1990). All DNA samples were investigated by AFLP (Vos *et al.*, 1995) and SAMPL (Morgante and Vogel, 1994) markers. So far the presence and degree of parthenogenesis has been assessed on 35 of the 70 F_1 plants using the auxin test (Matzk, 1991) according to Mazzucato *et al*. (1996). Segregation of parthenogenesis (presence *vs.* absence) was tested by χ^2. Individual markers significantly affecting the percentage of parthenogenetic embryo sacs (arcsin transformed) were initially selected by single factor ANOVA. The significant markers from the sexual (S) and apomictic (A) parents were separately subjected to regression analysis for selecting independent markers using the SAS Stepwise procedure (Statistical Analysis System, SAS Institute, Inc., Cary, NC, USA). ANOVA (SAS GLM procedure) was then performed on the markers selected by stepwise regression to test their effect on seed set. The contribution of these markers to the variation of the degree of parthenogenesis was estimated by R^2, calculated as the ratio of marker to total sums of squares. Least squares means were calculated for the two classes (presence *vs.* absence) of each significant marker.

Results and Discussion

The auxin test proved to be an efficient method for estimating the genetic capacity for parthenogenesis in *P. pratensis.* Of the 35 F_1 plants examined so far, 15 were parthenogenetic and 20 sexual, a ratio not significantly different from 1:1 (χ^2 = 0.71, 1df). This confirms genetic control by a single dominant gene in the simplex state in RS7 (Matzk, 1991; Barcaccia *et al*., 1998). In the 15 parthenogenetic plants, the degree of parthenogenesis ranged from 1.0 to 91.6% (Table 1). This may reflect the involvement of modifying genes, as earlier hypothesized by Matzk (1991).

Based on one way ANOVA, 12 markers out of 144 from the sexual parent, and 4 out of 147 markers from the apomictic parent significantly affected the degree of parthenogenesis. Four and one of these markers were selected as independent by stepwise regression among S and A markers, respectively (Table 2). Preliminary data from marker linkage mapping confirm independence (not shown). Overall, the S and the A marker explained 43.7% and 48% of the variation, respectively.

Our results indicate that a minimum of four genes influencing the expression of parthenogenesis from the sexual parent and one from the apomictic parent segregate in this cross. Examination of parthenogenesis in a higher number of progenies is necessary to confirm these results. If several independent genes are involved in the control of the degree of parthenogenesis, these will have to be taken into account in MAS for apomictic types in *P. pratensis*, so that the gene conferring the parthenogenetic capacity and those influencing its degree can be tracked together.

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Table 1 - Results of the auxin test and degree of parthenogenesis in the parthenogenetic F_1 plants tested.

Plant No.	Seeds examined	Sterile seeds %	Seeds with endosperm %	Seeds without endosperm			Degree of parthenogenesis[(2)]
				With embryo[(1)]	Without embryo	%	
2	610	12.5	20.2	4	407	67.4	1.0
6	196	15.3	29.1	3	106	55.6	2.8
7	113	8.0	18.6	76	7	73.5	91.6
9	394	60.9	27.9	3	41	11.2	6.8
14	134	31.3	36.6	18	25	32.1	41.9
15	335	8.7	40.9	128	41	50.4	75.7
17	161	16.1	21.7	1	99	62.1	1.0
26	403	22.3	21.8	191	34	55.8	84.9
29	466	35.0	16.7	37	188	48.3	16.4
31	115	23.5	34.8	1	47	41.7	2.1
32	93	21.5	15.1	4	55	63.4	6.8
33	215	2.3	22.8	23	138	74.9	14.3
34	56	32.1	1.8	1	36	66.1	2.7
36	261	18.0	1.9	3	206	80.1	1.4
37	110	11.8	22.7	2	70	65.5	2.8

(1) Formed by parthenogenetic egg cells following the auxin test

(2) Ratio of the number of seeds with embryo, without endosperm, and the total number of seeds without endosperm

Table 2 - Results of the analyses of variance for S and A markers and LS means of the marker classes.

Marker[1]	DF	Type III SS	F	Pr > F	R^2 (%)[2]	Degree of parthenogenesis LS Means[3]	
						Marker present	Marker absent
CCA/AAT/12-S	1	0.537	40.16	0.0001	22.4	0.11	0.48
CCA/AAT/2-S	1	0.153	11.45	0.007	6.4	0.17	0.39
AS1/AAC/6-S	1	0.232	17.32	0.002	9.7	0.16	0.41
AS1/ACT/16-S	1	0.125	9.33	0.012	5.2	0.37	0.18
Error	10	0.134	-	-	-	-	-
CAA/AAC/11-A	1	1.153	12.09	0.004	48.2	0.48	0.04
Error	13	1.240	-	-	-	-	-

[1] -S: markers from the sexual (maternal) parent; -A: marker from the apomictic (paternal) parent

[2] Coefficients of determination were calculated as the ratios of marker to total sums of squares

[3] Back-transformed mean values