Medicago truncatula F2 Segregation Data For Leaf Marking, Pod Coiling, & Albinos Involving Cultivars Caliph and Paraggio Crossed With Jemalong

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This study was initiated to find out if there is any inbreeding depression in cultivars of Medicago truncatula. The goal was to measure F2 heterosis for biomass in crosses involving 'Caliph' and 'Paraggio' crossed with 'Jemalong'. This is sometimes done in self-pollinated crops where it is difficult to produce F1 seed. Any positive difference between the F2 and the parents would reflect inbreeding depression in the parents. Rabbit damage ruined the study, but, we were able to collect the segregation data reported herein. We were not expecting segregation for so many traits or to see occasional multifoliolate leaves, but we have seen all these things before in crosses involving M. sativa, coerulea, and falcata, Hence, our goal now is a unified understanding of genomic relationships in Medicago broadly.

Materials and Methods

Seed of Jemalong was obtained from Tom Osborn, University of Wisconsin, and Caliph and Paraggio were obtained from Robin Groose, University of Wyoming. Crosses were made by hand in the greenhouse in April 2001. The greenhouse at this time of year averages 18 C at night and 23 C on cloudy days. During sunny days temperatures reach 28 to 30 C. Greenhouse lights are a mixture of incandescent and mercury vapor which we leave on 24 hours a day. Jemalong was used as the pollen parent because it produced the most abundant pollen, which was collected by tripping flowers on the tip of a pocketknife blade. Newly opened flowers of Caliph and Paraggio were tripped into the Jemalong pollen without emasculation. We were prepared to search for hybrid families on the basis of the segregation for the Jemalong leaf marking in the F2; however, every F2 family segregated. Crossing success probably was due to a relatively cool greenhouse at that time of year. In any case, crossing and backcrossing in 2003 will be continued into the warmer months, and the crossing success monitored with the leaf markers.

Pods from tagged crosses were harvested as soon as they start to turn brown. F1 plants were placed in perforated plastic bags 18 X 22 cm when pods began to ripen in order to catch and retain the pods. Similarly F2 plants in the field were laid on the same type of bag on the ground to retain the pods. Pods generally are threshed after softening on moistened germination paper for about six to eight hours. We scarify seed by rubbing gently with fine sandpaper. Plants are grown in the greenhouse in a 3:1:1 mixture of soil, sand and peatmoss.

F2 seed was used in three different growouts. First, a germination test of 30 unscarified seeds of each F2 family and the parents which was terminated after five weeks. Second, in May 2002, ten F2 scarified seeds of each family were

sown in the greenhouse for transplanting to the field for F3 seed production, pod coiling data, and general observations on fertility, lethality, and leaf markings. Lastly, the data in Table 1 on leaf marking and albinos were collected from a field planting in mid-August 2002 of 60 – 90 scarified F2 seeds of each family and the parents.

Results and Discussion

Interesting differences between the parents and the F2 material were first noted in the germination of unscarified seed. All three parents had hard seed in the germination test, whereas the F2 seed produced at the same time in the same greenhouse was not all hard. All F2s had about 10 of 30 seeds germinated except P X J F2-4 that had 20 of 30 germinated. At six days the hard seeds were scarified with fine sandpaper and returned to the germination paper. Germination and seedling development were monitored for another four weeks and provided observations of weak albinos, chlorophyll deficients, and other lethals that never would have emerged in the field. The parents produced 30 of 30 viable seedlings.

The albino column in Table 1 with the number of plants at emergence in parentheses needs to stand alone because a few seeds germinated and formed plants after the albinos were scored, especially in C X J F2-2. Nonetheless, there was seedling lethality in most F2 families as evidenced by fewer plants classified for leaf marking than recorded emerged in the albino column. At least 60 scarified seeds were planted in all F2 families except P X J F2-8 and 9; thus, there was considerable seedling lethality which merits more study.

The F2 segregations for leaf marking in Table 1 are interesting. Final genetic conclusions will be based on recombinant inbred lines (RILs) in a couple of years. Regarding leaf markings, Lake (1985) reported that a brown blotch centered on the lower mid vein of the adaxial leaflet surface was recessive to a brown-edged, irregular water mark centered on the upper mid vein of the adaxial leaflet surface. Penmetsa and Cook (2000) indicated that the prominent Jemalong leaf mark is recessive to ecotype A20 that lacks the mark, and further indicate that a single gene is involved. In 2001, Thoquet et al. illustrated the Jemalong mark and the occasional random streak of anthocyanin on the adaxial leaflet surface of an Algerian ecotype used in their genetic mapping work, and discuss involvement of a single gene.

Caliph and Paraggio both have frequent random streaks of anthocyanin on the abaxial surface of the leaflet, and occasional random streaks on the adaxial surface. Jemalong is free of marking on the abaxial surface (Fig.1). The F2 progeny segregated for parental markings as well as the class with marking on both surfaces of the leaflet (Table 1 and Fig. 1). It will be interesting to see what becomes stabilized in the RILs.

The following is how we interpret the F2 data for leaf marking while waiting for RILs. In a genetic model considering the adaxial Jemalong mark as a recessive

trait, the F2 would contain 25% homozygous C-or P-type: 50% heterozygous: 25% homozygous J-type (the classic 1 : 2 : 1). Remembering that the progeny were scored as J-type when any detectable adaxial mark was present on the midvein, which included weak and prominent marks (see Figure 1), the data make sense if the heterozygotes are included in the J-type to make a 1 : 3 segregation or 25% C- or P-type to 75% J-type. Under our conditions, the J-type evidently can be scored in the heterozygous condition. The data tend toward a 25% : 75% segregation especially in the C X J F2 which was 29% : 71%. As noted in Table 1, the Jemalong-type mark was most prominent in this family. In the P X J F2, probably fewer of the heterzygotes were scored and the distribution was 35% : 65%. Leaf marking is affected by genetic background in these crosses, but we will wait for the RILs before trying to draw any conclusions. We also will try to learn from the literature about leaf marking in legumes in general.

Pod coiling was reviewed by Thoquet et al. (2002) who discuss how anticlockwise pod coiling is characteristic of most Medicago species, and that clockwise is dominant over anticlockwise. They report that the gene for direction of coiling maps on chromosome 7 and had no segregation distortion in their cross. The crosses reported below (Table 2) indicated segregation distortion, with the distortion in opposite directions in the respective crosses.

Albinos (Table 1), chlorophyll deficients, and seedling lethals came as a big surprise among the F2 progeny. The simplest explanation at this point is that one or more of the parents is homozygous for a chromosome structural difference. Thoquet et al. (2002) indicated that their distorted markers are concentrated on linkage groups I, II, and III, and suggest structural reasons for the distortion.

The observation of multifoliolate leaves in the seedling stage of some F1 and F2 progeny, especially in the C X J cross was of great interest to us because we have studied multifoliolate leaves in alfalfa. Evidently, there is a delicate balance in expression of leaf morphology that is easily upset in some genetic backgrounds. It is another trait that is unifying the Medicagos in our thinking.

In future crossing work, we will backcross contrasting leaf markings into Jemalong. This will provide visual genetic markers for genetic research, and to identify crosses. Another trait that would be very useful in M. truncatula we believe, is dwarfism. Dwarf Jemalong would make it a more efficient to use in the greenhouse or growth chambers.

References Cited

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TABLE 1. Segregation for leaf markings and albinos among F2 progeny of Caliph (C) X Jemalong (J) and Paraggio (P) X J. See text for description of C-type, J-type, and C/J-type. Progeny were scored as J-type when any detectable adaxial mark was present on the midvein. Material was seeded at Madison WI in early August 2002, and scored for leaf marking in mid- September.

C X J F2

Family Code	C-type _-_ R_	J/C-type _J_ R_	J-type _J_ -	Albinos & no. of plants at emergence	Notes
1	12	20	4	1 (39)	Expression of
2	26	53	8	15 (90)	Jemalong-type
3	10	27	1	0 (38)	mark was more
4	9	24	3	2 (38)	pronounced
5	7	25	1	1 (43)	in C X J

P X J F2

1	12	21	2	1 (37)	Expression of
2	7	13	3	1 (25)	Jemalong-type
3	18	17	2	0 (45)	mark was less
4	10	25	4	1 (45)	pronounced
5	5	17	2	1 (32)	In P X J
6	5	11	0	0 (38)	
7	12	15	1	0 (44)	
8	10	17	1	0 (28)	
9	5	7	1	0 (16)	

PARENTS

C	87	0	0	0 (88)	
J	0	0	80	0 (82)	
P	87	0	0	0 (87)	

TABLE 2. Segregation for pod coiling

Cross	Clockwise	Anti-Clockwise	Total	Recessive
C X J F2	21	4	25	16%
P X J F2	24	10	34	29%
Total	45	14	59	24%

FIGURE 1. TOP: Adaxial and abaxial leaf surfaces of Caliph and Jemalong, respectively. MIDDLE: F2 segregates with one Caliph adaxial leaf surface (far left) and examples of detectable Jemalong-type mark from weak to strong (far right). BOTTOM: One lateral leaflet twisted upward to show Jemalong-type mark on leaves that also have random streaks of anthocyanin on the abaxial surface.