Self-Incompatibility in Two Alfalfa Populations


ABSTRACT

Self-incompatibility provides a useful mechanism for pollination control in alfalfa. Meehan et al. (1955) found hundreds randomly selected seedlings from each of two well-adapted multiple-pest-resistant alfalfa populations (W10 A3 C3 and W10P8 A5 C5) were screened for self-incompatibility. Twenty-eight plants were selected following these evaluations in the greenhouse. Unmaturated hand pollinations of the self-incompatible plants, using plants with the single-gene, completely dominant white-cotyledon character as the pollen source, resulted in the production of 90.0% hybrid seed. Plants were intercropped in the greenhouse using honey bees, Apis mellifera L. Growth chamber forage-yield trials of half-sib progeny indicate that certain combinations of self-incompatible plants exist that will yield significantly better than the best population. Twelve elite self-incompatible plants selected after the initial screening produced (i) < 0.15 seeds/plant when self-pollinated, (ii) < 50% viable pollen, and (iii) > 3 seeds/plant when used as the male or female in crosses with other plants. Pollen-petal interaction studies of the self-incompatible plants showed that the pollen had normal viability and either failed to germinate upon self-pollination or pollen tube growth was abnormal. Pollen tubes that grew near the nectaries either formed bulbous structures and terminated, or formed several beaklets, or continued to grow past all of the styles resulting in the failure of fertilization. However, normal fertilization and seed set were obtained when the self-incompatible plants were used both as male and female in crosses with other plants. The 12 elite self-incompatible plants were shown to have the capacity to produce somatic embryos in vitro, interesting that these plants could be propagated utilizing artificial seed technology. Evidence to date indicates that the use of the self-incompatibility system may provide a mechanism for successfully producing high-yielding hybrid alfalfa cultivars.

ALFALFA BREEDERS have long speculated on the value of hybrid cultivars in maximizing forage production of alfalfa fields. Childers and Barnes (1972) discussed this topic in detail and concluded that hybrid cultivar production could revolutionize the alfalfa industry. Nearly 50 years ago, Tydal et al. (1942) discussed techniques for hybrid production. One technique they proposed involved the use of self-incompatibility, however, they concluded that the self-incompatibility mechanism in the plants they studied was not sufficiently stable, and the technique was discounted. Twenty-five years later, the concept of using cytoplasmic male sterility maintainer and fertility restorer lines in a three-way hybrid cross was introduced by Davis and Greenblatt (1967). This system was utilized to produce the first successful hybrid alfalfa cultivar (Viands et al., 1983). However, due to difficulties in obtaining sufficient seed on the male-sterile lines, attributed to the nonpreference of pollinators to collect insects for the pollen-deficient male sterile lines, hybrid cultivar production using cytoplasmic male sterility was not considered economically practical. The 10 to 15% increase in forage production from the hybrid cultivar did not sufficiently offset the significant increase in cost of seed production for the USDA-ARS, Plant Sciences Inst. Sybryn and N. R. O'Neill, Res. Lab. Beltsville, MD 20702. Contribution of the USDA-ARS. Received 4 Aug. 1989. *Corresponding author.

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hybrid as compared to conventional synthetic culti-
vars (Viands et al., 1988).
Recently, we began to reevaluate the possibilities for
hybrid cultivar production using self-incompatibility
as first described by Tysdal et al. (1942). Self-incom-
patibility is defined in this paper as the inability to
produce seed upon selfing; however, both the male
and female gametes are functional when outcrossed.
Self-incompatibility is of particular interest in light of
the advances in technologies relating to the use of so-
matic embryos derived from tissue culture in the pro-
duction of artificial seeds (Redenbaugh et al., 1986).
Once this technology is improved, artificial seeds may
be used to vegetatively propagate genetically uniform
plants in seed-production fields. Self-incompatible
plants could be multiplied vegetatively and planted as
mixtures of the two lines in the same row. Fertile pol-
len would be produced on both parents but would ger-
minate on and fertilize only the other line. Hybrid
seed could be harvested from all of the plants in the
field rather than from a portion of the field, as required
with hybridization systems using male sterility. Even
without the benefit of artificial seed technology, plant
breeders could use the self-incompatible system to
produce F1 hybrids in the breeder-seed generation in
small plantings using conventional vegetative propa-
gation followed by a generation of conventional seed
increase, with the high probability of carrying some
heterosis to the certified generation. In addition, per-
petuation of self-incompatibility in generations fol-
lowing the F1 would allow the possible production of
double-cross hybrids in sufficient and economical
quantities.
We investigated the feasibility of selecting self-in-
compatible plants from two alfalfa populations as a
mechanism for producing hybrid alfalfa with in-
creased forage production (Egin and Bauchan, 1986).
Specific objectives were to (i) select self-incompatible
plants based on the results from crosses using a cross-
 pollen viability; (ii) evaluate half-sib progenies from
self-incompatible plants for their forage yield potential
in a greenhouse (iii) observe pollen tube-plastid
interactions of self-incompatible plants when selfed
and outcrossed to ascertain if protogynous hybridization
is possible and determine the ability of self-
compatible plants to produce somatic embryos in vitro,
for potential use as a method of asexual prop-
ergation.
MATERIALS AND METHODS
Selection of Self-incompatible Plants
Five hundred randomly selected seedlings each from two
well-adapted multiple pest-resistant alfalfa populations,
W10 AC3 and BMP AC3 (Egin and Otaezaki, 1984),
were grown in the greenhouse at Beltsville, MD, in the spring of
1985. Greenhouse temperatures ranged from 20 to 25°C. Tops
were removed from the first growth. When the second
growth reached flowering, a minimum of 30 florets on each
plant were self-pollinated. Pollen quantity was estimated (1
= no pollen, 2 = small quantity of pollen, and 3 = medium
to high quantity of pollen) for each plant, based on obser-
vations of quantities accumulated on a folded red plastic
pot label used for self-pollination. Approximately 30 d after
selling, pods were harvested and the number of seeds pro-
duced per floret tripped was calculated.
Plants were dissected to determine more than 1 seed
flower tripped in self-pollinations were or male sterile. Self
fertility of 66 putative self-incompatible plants were tested in
the greenhouse twice more, using the same procedure
described above. During the second evaluation period, self-
flowering was conducted during July and August 1985, when tem-
peratures in the greenhouse ranged from 30 to 40°C, the third
evaluation period was during January and February
1986, when temperatures ranged from 15 to 25°C. Pollen
viability was measured using cotton blue stain (Hauser
and Morrison, 1944). Pollen was collected from 10 florets
stamen, mounted on a microscope slide, and the percentage of
viable pollen among 1000 pollen grains was determined.
Only those pollen grains that stained blue and appeared per-
fectly spherical were considered to be viable.
Determination of female fertility of the 66 putative self-
incompatible plants was made by pollinating 30 florets on
each of the subject plants with pollen from unrelated self-
sterile plants selected from 'Arc'. These self-sterile plants were
selected on the basis that they produced >75% viable pollen and
>3 seed flower when selfed. Plants that pro-
duced no seed when unselfed were considered to be female
sterile and were thus discarded. Pollen from the self-incom-
patible plants was used to pollinate 30 florets of a male-
sterile plant, W15 N52 (received from E.T. Bingham, Uni-
versity of Wisconsin, Madison, WI).
In addition, during the third evaluation period, pollen
from a plant carrying the red-root gene (Barons and Hanson,
1967) in the triples or quadruples condition was used to
pollinate 20 florets on each self-incompatible plant, to de-
termine if the presence of foreign pollen on the suspect
plants stimulated the plant to accept its own pollen. A single
such red-root plant was propagated and used as the pollen
source. The red-root clone was selfed, to determine if 100% of
the progeny would have red roots. Homozygous recessive
plants have white roots. The red-root clone was also crossed
to self-sterile and male-sterile plants, to check for differences
in the pollen receptivity compared to the suspect self-com-
patible plants. The progeny were studied in parallel.
self-filled flats (10 by 10 cm) and placed in the greenhouse
at 25°C with 70% relative humidity. Four to six weeks after
planting, roots of the seedings were rated for the presence of red pigmen-
tation. Evaluation of the red pigmentation was enhanced by
digging the roots into a solution of 0.5 M HCl.
Twenty-eight plants of self-incompatibility were selected for further study due to their consistent results regardless of the time of year in which the
crossing occurred.
Evaluation of Half-Sib Progeny
The 28 plants that exhibited self-incompatibility were
planted randomly or a caged bench in the greenhouse and
intercrossed using henry bees as pollinators. Half-sib seed
was harvested from each plant. Progeny from the 28 plants
were evaluated for their yield potential in a growth chamber
study. Progeny were planted in pastured soil-filled flats
(52 by 5 by 7 cm) and placed in a growth chamber main-
tained at 24 to 27°C with 70% relative humidity. Progeny
were arranged in a randomized complete-block design with
seven replicates. Fifteen seeds of each half-sib family were
planted per row per replicate; two rows each of the parent
progenies (W10 AC3 and BMP AC3) were also planted in
each flat. Yield data were obtained from three harvests
at 30-d intervals, starting 30 d after planting. The number
of seedlings and the total weight of the forage from each was
The percentage of hybrid plants formed when the self-incompatible crosses were crossed with the red-root clone was 58.8% (2335 red-root outcrosses of 4066 total seedlings). The red-root clone produced 107 red-root seedlings (92.2%) and nine white-root seedlings (7.8%) when selfed, significantly fewer red-root seedlings than expected based on the crosses made. Two hundred seventy four red-root seedlings were obtained from 277 total seedlings (98.9%) when pollen from the red-root clone was used to pollinate a male-stem plant. Self-fertile plants (selections from 'Arc') produced 110 red-root seedlings of 130 total seedlings (77.7%) when crossed to the red-root clone. Thus, there appeared not to be a stimulation of the self-incompatible plants to accept their own pollen when in the presence of foreign pollen. This also demonstrated the significant reduction of setting (1.4% vs. 22.3%) when self-incompatible plants are used in crosses.

**Evaluation of Half-Sib Progenies**

Yields of half-sib families from two plants (168 and 189) from the parent population BMP8 AC3 were significantly (P < 0.05) greater than the yield of the parent population (Table 2) indicating that these two plants combined well with the other self-incompatible plants. None of the half-sib lines tracing to W10 AC3

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**Table 1. Average number of seeds per plant**

<table>
<thead>
<tr>
<th>Plant</th>
<th>Self pollinated</th>
<th>Related foreign pollen</th>
<th>Crossed to male sterile</th>
<th>Pollen viability %</th>
<th>seed set</th>
<th>%</th>
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<td>456</td>
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<td>3.6</td>
<td>68</td>
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<td>801</td>
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<td>3.0</td>
<td>3.3</td>
<td>57</td>
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<td>2.2</td>
<td>1.0</td>
<td>40</td>
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</tr>
<tr>
<td>BMP8 AC3</td>
<td>1.14</td>
<td>3.5</td>
<td>2.3</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W10 AC3</td>
<td>1.15</td>
<td>2.2</td>
<td>3.3</td>
<td>45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Plants advanced to 203 were selected from BMP8 AC3 and those numbered 338 to 580 were selected from W10 AC3. Determined by counting the number of pollen grains stained with cotton Blue 30000 pollen grains.

One of the 12 'diogen' self-incompatibilities.

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differed significantly (P > 0.05) from the parent pop-
ulation (Table 2). Twelve elite self-incompatible plants were subse-
quently selected based on fertility and pollen viability. These lines produced (i) <0.1% seed floret1 when self
pollinated, (ii) >30% viable pollen, and (iii) >3 seed
floret1 when foreign pollen was applied to the self-
incompatible or when the pollen from the self-incom-
patible was used as the pollen parent (Table 1).

Pollens Tube Growth Observations

Observations of pollen tubes of the 12 elite self-
incompatible plants indicated that the mechanisms
controlling self-incompatibility were associated with
three types of prezygotic barriers: (i) pollen grains
that did not germinate and penetrate the stigma, (ii)
pollen tubes that penetrated the stigma did not grow
through the style and enter the locale, and (iii) pollen
tubes that grew near the ovaries either formed a bulbous
structure and terminated, formed several branches, or
continued to grow past all of the ovaries (Table 3).
Frequently the pollen tubes formed multiple loops
adjacent to an ovule, but did not enter the ovule. Pre-
zygotic hybridization barriers were not complete,
however. Also, these barriers did not account for all
of the self-sterility in the self-incompatible plants, as
the seeds per floret pollinated was usually less
than the observed percent pollen tubes that reached
the ovaries (Table 3). Studies of the growth of foreign
pollen in the pistils of the elite self-incompatible plants
and the growth of pollen from these plants in the pis-
tils of a male-sterile line showed a normal progression
of pollen germination, pollen tube penetration of the
stigma, growth through the style, and the termination
of growth at the ovary. Some of the pollen tubes en-
countered the same prezygotic hybridization barriers
as those described when selling occurred, however, the

Table 2. Mean fresh weight of the half-sib progeny from self-in-
compatible ($S$) alfalfa plants selected from populations W10 AC3
and BNPB AC3.

<table>
<thead>
<tr>
<th>S1 plant</th>
<th>Fresh wt.</th>
</tr>
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<tr>
<td>95</td>
<td>48.8</td>
</tr>
<tr>
<td>189</td>
<td>47.7</td>
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<tr>
<td>197</td>
<td>46.3</td>
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<td>194</td>
<td>42.6</td>
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<tr>
<td>745</td>
<td>42.4</td>
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<td>179</td>
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<td>39.8</td>
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<td>508</td>
<td>39.5</td>
</tr>
<tr>
<td>82</td>
<td>39.4</td>
</tr>
<tr>
<td>203</td>
<td>39.1</td>
</tr>
<tr>
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<td>589</td>
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<tr>
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<td>36.8</td>
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<tr>
<td>23</td>
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<td>894</td>
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<td>968</td>
<td>34.8</td>
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<tr>
<td>W10 AC3</td>
<td>36.1</td>
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<tr>
<td>BNPB AC3</td>
<td>35.2</td>
</tr>
<tr>
<td>USD (0.9%)</td>
<td>10.5</td>
</tr>
</tbody>
</table>

1 Self-incompatible plants numbered 10 to 203 were selected from BNPB
and those numbered 536 to 980 were selected from W10 AC3.

Table 3. Observations of pollen tube growth in elite self-incompatible
alfalfa plants upon selling and outcrossing.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Ovules ovary 1</th>
<th>Pollen germinated</th>
<th>Tubes in style</th>
<th>Tubes in locale</th>
<th>Ovules reached</th>
<th>Seed floret1</th>
<th>Ovules developed seed 2</th>
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</thead>
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<tr>
<td></td>
<td>no.</td>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td>no.</td>
<td>%</td>
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<td>Self-pollinated</td>
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</tr>
<tr>
<td>10</td>
<td>10.4</td>
<td>11.2</td>
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<td>4.5</td>
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<td>0.17</td>
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<td>1.59</td>
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<td>0.68</td>
</tr>
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<td>2.5</td>
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<td>1.07</td>
<td>1.08</td>
<td>1.13</td>
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<tr>
<td>203</td>
<td>9.9</td>
<td>5.8</td>
<td>3.2</td>
<td>2.8</td>
<td>4.60</td>
<td>1.06</td>
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<td>5.1</td>
<td>0.73</td>
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1 Plants numbered 10 to 203 were selected from BNPB AC3 and those numbered 536 to 980 were selected from W10 AC3.
Production of Somatic Embryos

Somatic embryos were produced from the callus cultures from all 12 elite self-incompatible plants. The cultures required from 30 to 60 d in the regeneration medium for somatic embryos to form. Plants 45, 52, 203, and 589 developed embryos in the shortest time (30 d), and plants 10 and 710 required >60 d before embryos were detected.

DISCUSSION

Screening of two well-adapted multiple pest-resistant alfalfa populations yielded 3.0% male-sterile, 1.5% female-sterile, and 3.1% self-incompatible plants. Male sterility in alfalfa has been described as either male-sterile and self-incompatible plants. The cultures required from 30 to 60 d in the regeneration medium for somatic embryos to form. Plants 45, 52, 203, and 589 developed embryos in the shortest time (30 d), and plants 10 and 710 required >60 d before embryos were detected.

REFERENCES


Savidge et al. (1983) and Bau-


