SIB-COMPATIBILITY IN S_1 LINES

OF <u>MEDICAGO</u> <u>SATIVA</u> L.

by

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A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of

The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Crop Breeding

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Ames, Iowa

1961

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INTRODUCTION

Full utilization of hybrid vigor in alfalfa has not yet been successfully put into commercial practice. Due to the inherent nature of the crop, and especially the absence of desirable inbred lines, commercial hybrid seed production has entailed much effort and expense, thereby rendering the cost of production of hybrid seed to be high.

Commercial production of hybrid seed of alfalfa can be achieved, however, without the necessity of inbreeding. Alfalfa has a perennial nature of growth and can easily be propagated asexually. Furthermore, production of large numbers of inbred lines is not feasible due to the limits imposed by the loss of self-fertility which accompanies inbreeding. Non-inbred clones possessing high combining ability, as determined on the basis of their polycross progeny performance, can be used as parents in hybrid seed production. In order to assure a consistent and high level of performance of the commercial hybrid seed, vegetative propagation of parental clones adequate to plant the entire acreage desired for the production of hybrid seed, is necessary.

This need for extensive vegetative propagation of parental clones has been a definite obstacle in the production of hybrid seed of alfalfa. The additional costs of producing seed in this manner have not as yet been justified

based on the comparative performance of hybrids and the best yielding synthetic varieties.

The necessity for vegetative propagation could be obviated through the use of inbred lines with restored self-fertility. Results of numerous studies point to the fact that inbreeding in alfalfa is accompanied by a drastic reduction in self-fertility and as a direct consequence, the number of generations of selfing possible is highly limited. With brother-sister mating, which is another form of inbreeding, it is believed that some of the sterility problems encountered in straight selfing may be overcome, or at least postponed.

The primary objectives of the present study were: (a), to determine the nature of sib-compatibility among S_1 plants of alfalfa derived from clones of low, intermediate, and high self-fertility, (b), to compare fertility of S_1 plants from sib-crossing, continued selfing, and backcrossing to the parental clones, and (c), to determine the possibility of using advanced generation S_1 lines for the commercial production of hybrid alfalfa seed.

REVIEW OF LITERATURE

Mode of Pollination of Alfalfa

Alfalfa is a naturally cross-pollinated crop. Pollination studies conducted over a wide range of environmental conditions have confirmed that a high degree of natural cross-pollination occurs in this crop. Burkhart (1937), in Argentina, found an average of 84.5 per cent cross-fertilization; in Canada Knowles (1943) and Bolton (1948) obtained 94.2 per cent and 90 per cent, respectively. In the U. S. Tysdal et al. (1942) reported an average of 89.1 per cent cross-pollination whereas Pedersen (1953) obtained 86.1 per cent.

There is also a considerable amount of self-fertilization in alfalfa but studies have shown that generally there is a marked reduction in seed-setting following self-pollination. Knowles (1943) working with self-fertile selections and random Grimm plants found an average of 1.65 seeds and 0.56 seeds per flower selfed, respectively, whereas after cross-pollination 4.60 seeds and 3.70 seeds per flower, in the same order, were obtained. Similarly Bolton (1948) obtained an average of 1.58 seeds per flower following self-pollination as opposed to the average cross-fertility of 5.54. Tysdal and Kiesselbach (1944) have reported that in open-pollinated plants 12.6 per cent of the population were found to be between zero and 20 per cent self-fertile and among selfed plants 60.2 per cent

had less than 20 per cent self-fertility. The results just presented all point to the fact that cross-pollination in alfalfa is more potent than self-pollination.

There is also a wide variation in the degree of self-fertility among individual plants as reported by Williams (1931). Wilsie (1951) observed a range in self-fertility of single plant selections ranging from 0.12 to 1.84 seeds per flower tripped. Other studies which show remarkably wide range in self-fertility are those of Wilsie (1948), McAllister (1950), and Koffman (1959). Sandal (1946) found a marked difference in self-fertility between low-fertility and high-fertility groups of clonal lines. On the average 0.15 seeds and 1.25 seeds per flower tripped, respectively, were obtained. Cooper et al. (1937) obtained an average of 1.25 seeds per flower tripped for the high seed-setting plants and 0.07 seed per flower for the low seed-setting group.

"Tripping" as described by Bolton (1948) is a term which refers to the process whereby the staminal column is released from the tissues attaching it to the keel and wing petals and strikes against the standard petal. A consideration of this process in relation to the effective pollination of the alfalfa flower has been dealt with repeatedly. Kirk and White (1933) were of the opinion that self-fertilizing plants of alfalfa do not necessarily require tripping of the flowers for fertilization to occur. He observed that anthers of

ordinary alfalfa shed their pollen in the bud stage of develop-
ment and pollen grains are deposited on the stigma in abundance.
The same observation was made by Armstrong and White (1935).
The abnormal positional relationship of the stigma to the an-
ther does not prevent pollination of the untripped flower
(Brink and Cooper, 1936). Piper et al. (1914), Hay (1925) and
Tysdal (1946) observed that about 5 to 6 per cent of the
flowers in some plants can achieve self-fertilization in the
absence of tripping. Carlson (1928) and Engelbert (1932) made
similar observations, but it is generally agreed that self-
pollination with the aid of tripping would result in a much
greater increase in seed-setting of the plants. This is sub-
stantiated by the results obtained by Piper et al. (1914),
Southworth (1928), Carlson (1928, 1930), Clarke and Fryer
(1930) and Brink and Cooper (1936). Armstrong and White
(1935) were of the opinion that in the act of tripping the
stigmatic surface is ruptured and that a stigmatic content is
released which initiates pollen germination. Pollen penetra-
tion is also facilitated. Pollen germination was found to
have occurred in 84 per cent of the tripped flowers and in
less than 1 per cent of the untripped flowers.

Piper et al. (1914) and Knowles (1943) have reported
cases of automatic tripping. Armstrong and White (1935)
maintain that the high seed-setting capacity of certain
selected autogamous plants is due largely to the spontaneous

tripping of their flowers.

There are many factors that may induce automatic tripping. Knowles (1943) has found that temperature is the most important of the weather factors in bringing about mechanical tripping. Piper et al. (1914) stressed the importance of intense sunshine. Tysdal (1946) stated that rain, sun, and wind may cause some tripping although Gray (1925) found wind to be a very ineffective agent. Stewart (1926) believed that alternating spells of cloudy and bright weather with moderate wind and occasional showers are favorable for automatic tripping. But the most important factor is the visitation by insects. Insect agents which cause tripping of flowers are mainly responsible for self- and cross-pollination of alfalfa. The role of leaf cutter bees, bumble bees and honey bees and alkali bees has been emphasized (Dwyer and Allman, 1933; Hadfield and Calder, 1936; Jones and Olson, 1943; Hare and Vansell, 1946; Vansell and Todd, 1946; Pedersen and Todd, 1949).

Self-sterility and Cross-compatibility in Alfalfa

There are a number of factors inherent in the alfalfa plant which may be associated with the sterility problems usually encountered in this crop.

Polyploid nature of alfalfa

Fryer (1930), in a survey of chromosome numbers of 17 species, has shown that the genus Medicago includes species with 14, 16 and 32 chromosomes, but that only tetraploids are present in Medicago sativa. Meiotic studies made by Reeves (1930) and Cooper (1935) revealed that 16 bivalents at Metaphase I are most frequent in tetraploid alfalfa, strongly suggesting a condition of allotetraploidy. On the other hand in a study of diakinesis cells from 12 plants Grun (1949), as reviewed by Atwood and Grun (1951), found an average of 0.62 quadrivalents per cell. Individual plant averages ranged from 0.21 to 0.89 quadrivalents per cell. As many as four quadrivalents per cell were noted, which is suggestive of an autotetraploid origin.

Armstrong (1954) has advanced the theory that tetraploid alfalfa is an alloploid originating from closely related diploid species. The theory is based on the following results obtained. A hexaploid was obtained from crossing a tetraploid and an induced octoploid. Meiotic behavior in the hexaploid showed a low frequency of univalents indicating that the A and B genomes are partially homologous. It was observed also that the quadrivalent frequency in the octoploid was more than three times as high as in the tetraploid, suggesting a lack of complete homology between the two genomes.

Atwood and Grun (1951) argued that the presence of laggard chromosomes frequently observed would indicate that aneuploids could be expected to occur in relatively high frequency, especially true if the species is highly auto-ploid, by virtue of the fact that a metabolic balance could be maintained better in an aneuploid from an autoploid than that from an alloploid. The results of the studies of Led-ingham (1940) also are cited as evidence of autoploidy. Since an intermediate condition between true alloploidy and autoploidy seems to exist in $\underline{M}$. $\underline{sativa}$, the term "segmental alloploidy" as first described by Stebbins (1947) is sug-gested. This condition may account for the non-consistency in the nature of inheritance of characters. All 36 genetic studies involving 25 characters reviewed by Atwood and Grun (1951) indicated a disomic pattern of inheritance. However, in most of these studies F_2 and backcross segregations were used and tetrasomic inheritance could not be confirmed until F_3 and later generations were observed. The first convincing evidence of tetrasomic inheritance was reported by Stanford (1951) involving purple-flower color, confirmed later by the work of Twamley (1955), Dudley and Wilsie (1956), Davis (1956), and Markus and Wilsie (1957).

The inherent polyploid nature of alfalfa which results in meiotic irregularity where plants are rendered both poor pollen and seed parents and which may perpetuate an unstable

chromosome condition in the hybrids, contributes to the self-sterility nature of the plant (Armstrong, 1952, 1954; Farley and Hutchison, 1941).

Somatoplastic sterility

The collapse of ovules during the early stages of post-fertilization development has been observed in alfalfa, particularly after self-pollination. According to Brink and Cooper (1939) ovule collapse is frequent in 48 to 72 hours after pollination. Brink and Cooper (1938, 1939, 1940) observed that about 34.4 per cent of the fertile ovules in the selfed series collapsed in the ovary at 72 to 144 hours after pollination in comparison to the 7.1 per cent found in the crossed series. In Medicago falcata, more than 80 per cent of the self-fertilized ovules and less than 12 per cent of the cross-fertilized ovules were aborted, according to Miller (1960). Cooper et al. (1937) found also that while on the average 3.1 ovules per flower in a high-seed setting group were fertilized, only 1.25 seeds per flower developed to maturity. In a low seed-setting group the average number of fertile ovules found was 2.5 but only 0.07 seed per flower was formed. It was observed also that the embryos of the low seed-producing group developed more slowly.

His tological studies made by Brink and Cooper (1939) showed that an abnormal growth took place in the somatic

tissues adjacent to the embryo sac prior to the collapse of the ovule. Such growth was characterized by the finely vacuolate and densely staining nature of the cytoplasm of the cells immediately adjacent to the endosperm, and the resemblance of such cells to the tapetal cells was gradually lost. This condition was first observed on the funicular side of the ovule in the region of the vascular bundle before the breakdown of the embryo sac became noticeable. The breakdown of the endosperm ensued, starting at the chalazal region and it continued toward the embryo.

Ovule collapse, which is commonly referred to as "somatoplastic sterility", is explained by Brink and Cooper (1939) on a basis associated with food translocation. It is believed that the reserved food being translocated to the inner integument and the endosperm, a dominant tissue of the embryo sac, can be equally shared if parallel growth takes place between the two competing tissues. Failure of the endosperm to keep pace with growth offers the maternal tissues a competitive advantage. Consequently the endosperm starves and the ovule eventually collapses. The visible food reserves immediately available are observed to gradually disappear just after fertilization and this is the most critical stage in ovule development. It is maintained also that the reason for ovule collapse occuring in lesser frequency in crosses is the fact that the endosperm of crosses grows at a relatively

faster rate than that of selfs. Farley and Hutchison (1941) found that in the hybrids of a British Columbia variety the proliferation of the endoflexial portion of the integument normally occurs before the initiation of growth of the endosperm thereby the competition which otherwise would lead to somatoplastic sterility is minimized.

Partial self-incompatibility

The greater potency of cross-fertilization over self-fertilization has been indicated earlier. Stevenson and Bolton (1947) have shown that cross-fertilization often occurs when foreign pollen is applied one hour or longer after tripping without emasculation. Brink and Cooper (1938) stated that in partially self-incompatible plants the male gametophytes, although not necessarily impotent on the individual from which they arise, are found to be less effective in effecting fertilization than unrelated male gametophytes. Cooper and Brink (1940) found that with self-pollination only about 14.6 per cent of the eggs in the ovules had become fertile, whereas 66.2 per cent fertile eggs were obtained following cross-pollination.

Partial self-incompatibility is, to a great extent, the result of the inability of the growing male gametophyte to reach the eggs in the ovules of the same plant. Cooper and Brink (1938, 1940) observed that after selfing, few pollen

tubes were able to advance beyond the mid-region of the ovary so that most of the fertile ovules were concentrated in the apical half of the ovary. After cross-pollination the tubes usually reached the base of the ovarian cavity and were able therefore to accomplish fertilization in a greater number of ovules. After self-pollination there was a uniform gradient in fertility which occurred from the apex of the ovary downwards, the ovules in the most basal positions rarely showing signs of fertilization. A similar gradient was observed following cross-pollination, but there was a greater proportion of fertilized ovules in each position in the ovary in comparison with that observed after selfing. In diploid alfalfa there was an equal frequency of cross-fertilized ovules in the basal and apical portions of the ovary, according to the observations of Miller (1960). It is believed that in selfing, pollen tubes may seldom reach the basal ovules in the ovary. It was observed that pollen tube growth, seen at 30 hours and at 48 hours after pollination, was more rapid in cross matings than in the selfs. Cooper and Brink (1940) concluded that partial self-incompatibility is due only in part to restricted pollen tube growth. There was evidence that frequently after selfing the pollen tubes also by-passed the micropyles of the ovules containing the unfertilized egg.

Pollen sterility

Pollen sterility also is a factor contributing to low self-fertility. Non-viability of pollen may be brought about by abnormalities in meiosis (Armstrong, 1952, 1954; Farley and Hutchison, 1941) or the physiological disturbances in the maturing pollen (Armstrong, 1952). Bolton and Fryer (1937) noted two classes of sterile pollen; one consisted of clear, empty-appearing grains and the other apparently normal grains which did not germinate. Brink and Cooper (1936) state that occasionally the amount of aborted pollen may be large enough to limit seed formation. Studies conducted by Armstrong and White (1935) and Clarke and Fryer (1930) have shown similar results. Engelbert (1932) has shown a case in which one plant was found to possess sterile anthers and did not produce seed. He further concludes that plants producing a large percentage of sterile pollen are generally poor seed producers.

In a test of pollen viability on an artificial medium Bolton and Fryer (1937) obtained wide variations among different plants, similar to the results found by Sexsmith and Fryer (1943). The pollen viability ranged from 13.3 to 64.0 per cent for the sterile group in contrast to the 50.5 to 87.9 per cent obtained for the fertile plants.

In summary, the incompatibility associated with selfing or crossing is due to the failure of pollen to fertilize the

egg and secondly, the collapse of fertilized ovules resulting

from somatoplastic sterility. Cooper and Brink (1940) be-

lieve that the observed 98 per cent of the difference between

the potential and actual fertility following self-pollination

and the 67 per cent difference in cross matings are accounted

for by the frequency of fertilization and the collapse of fer-

tile ovules within the first 6 days. In one instance Cooper

(1935) observed that in spite of the abundance of growing

pollen tubes, less than half of the ovules in the ovary con-

tained fertilized eggs. There were observed in heavy setting

lines, for example, 10 to 12 ovules present in the ovary, yet

an average of only 3 or 4 seeds per pod developed.

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Genetics of Incompatibility Known in Other
Forage Legume Species

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The genetics of self- and cross-incompatibility has been

studied extensively in other forage legumes, especially the

clovers. The "diploid personate type of multiple oppositional

allelomorphs" mechanism similar to that found in <u>Nicotiana</u> by

East and Manglesdorf (1925), and East and Yarnell (1929) has

been found to operate also in red clover, sweet clover, alsike

clover and white clover. The theory is that if a pollen grain

carries the same allelomorph as one of those in the pistil the

resulting pollen tube fails to grow far enough to allow fer-

tilization. On the other hand, if a pollen grain bears an

allelomorph different from either of those in the pistil,

pollen tube growth is normal and fertilization is brought about. Williams and Williams (1947a, 1947b) have confirmed its action in red clover (Trifolium pratense) and found 37 out of 40 S_x and S_y alleles tested to be different. The same mechanism has been found to operate in alsike clover (Trifolium hybridum) as shown by Brewbaker (1953).

Atwood (1940), found that multiple oppositional alleles are also in operation in white clover (Trifolium repens). The same worker (1942) found that of the 26 alleles tested in the first series, 25 (or 96 per cent) proved to be different and in the second series, 34 (or 83 per cent) of the 41 alleles were different. It is believed that the total number existing in nature is much larger. In another study, Atwood (1944), working with 2 series, found 36 (73 per cent) out of 49 alleles tested were different. In the second series, 39 (80 per cent) out of 49 proved to be different.

In sweet clover (Melilotus officinalis), Brink (1934) first observed that the cause of self-incompatibility seemed to be the reduced rate of germination of the pollen on its own stigma, failure of many of the tubes formed to become established in the style, and the slow growth of those which penetrate the pistil. With cross-pollination the pollen germinated freely in large numbers at the base of the style after 24 hours. Evidence has been furnished by Gettys and Johnson (1944) that the oppositional factor type of sterility operates.

In addition to the $S_x S_y$ multiple allelomorphic series controlling self- and cross-incompatibility, a fertility factor (S_f) has been discovered in the clovers. Atwood (1942, 1945) has shown its occurrence in <u>Trifolium</u> <u>repens</u>. The single factor, S_f, is a member of the multiple allelic series and acts as a dominant or partly dominant allele to the other members of the series in affecting pollen tube growth. As an illustration, in a heterozygous $S_f S_x$ plant used as a female the S_f functions as a partly dominant factor when all pollen normally would have been inhibited to germinate. But the S_f factor in the pollen has no effect in stimulating other pollens to function in the pistil which bears a similar allele. Furthermore, although the pollen bearing the S_f factor penetrates a style bearing the same allele, it does not compete equally with other pollen bearing entirely different alleles from those in the pistil.

Gettys and Johnson (1944) have demonstrated the presence of an S_f gene in sweet clover. Its occurrence in red clover also has been shown by Williams and Williams (1947a). The practical use of the fertility gene in a breeding program for red clover was outlined by Rinke and Johnson (1941). Atwood (1942) suggested the applicability of its use in a white clover breeding program.

It is not known whether the genetics of self- and cross-incompatibility occuring in the clovers applies directly to

alfalfa. The study in alfalfa is made complicated by the polyploid nature of this crop. Miller (1960), using diploid alfalfa (M. falcata) in the study, hypothesized that self-incompatibility is controlled by two sets of genes derived from the duplication of a single locus. The competitive interaction of two different S genes in the pollen was believed to overcome self-incompatibility. Dean (1942) has advanced an hypothesis based on the autotetraploid inheritance of oppositional alleles in M. sativa.

Genetic studies in other species have shown that self-incompatibility genetically controlled by the multiple oppositional alleles in the diploid may be overcome at the polyploid level. Lewis (1943) illustrated this in Oenothera organensis. Where inhibition of pollen tubes is weak in the diploid it disappeared altogether in the tetraploid. The incompatibility reaction in tetraploids is dependent not on the style being a tetraploid but entirely on the pollen being diploid. He further concluded that growth of heterogenic pollen grain with 2 different S genes on styles with one or with both genes is due to a competition of the S genes for a basic antigenic substance. Atwood and Brewbaker (1953) found that in autoploid white clover, heterogenic pollen may not always be functional where there is a dominance relationship involved among the different S allelles. For example, a dominance relationship $S_2 \gg S_3 > S_4 > S_1$ was observed.

Improvement in self-fertility from the diploid to the
tetraploid level has been observed in the following species:
Solanum spp (Cadman, 1942; Johnstone, 1939), Pyrus communis
(Crane and Lewis, 1942), Trifolium repens (Atwood and Brew-
baker, 1953), Trifolium hybridum (Brewbaker, 1953), and
Trifolium nigrescens (Brewbaker, 1955). On the other hand,
no apparent improvement in self-fertility was made in the
autoploids of Brassica spp and Raphanus sativus (Howard, 1942),
Taraxacum Koksaghyz (Bannan, 1946), Secale cereale (Lundqvist,
1947), Ananus sativus (Kerns and Collins, 1947), and Parthenium
argentatum (Gerstel and Riner, 1950). It needs to be proven
whether or not tetraploid alfalfa has an improved self-
fertility over its diploid relatives.

Environmental Factors Affecting
Pollen Tube Growth

Specific factors have been demonstrated to exert in-
fluence on pollen tube growth. According to Bolton and Fryer,
(1937) relatively high temperature conditions accelerate
pollen tube growth, although in their results certain individ-
ual plants reacted somewhat differently at two different tem-
perature treatments. A linear relationship was obtained by
Sexsmith and Fryer (1943) between temperature and pollen tube
growth, the length increasing with an increase of temperature
within the range from 70 to 100° F. At 50° F. no germination
occurred in one half hour although a slight bulge at the germ

pores of the pollen grains was produced. Grandfield (1945) found that the alfalfa flowers developing into pods decreased in number as temperature went above 100° F. The optimum was 80° F.

Pollen tube growth reactions to temperatures in alfalfa may not always be constant (Bolton and Fryer, 1937). In Oenothera organensis, Lewis (1943) observed that the reactions of the pollen genotypes to temperature were different. In the diploid, tube growth of pollen possessing a supposedly incompatible genotype had an optimum of 15° C. but the compatible tube growth had an optimum at 30° C. In the tetraploid, the S_3S_6 and S_3S_4 pollen had an optimum growth at 30° C. The optimum for the S_4S_6 genotype was at 15 to 20° C.

Moisture and relative humidity also have been found to influence pollen tube growth. Martin (1915) stated that the water requirement for pollen germination was dependent upon a certain ratio between the moisture delivered by the stigma and the moisture of the air surrounding the stigma. Grandfield (1945) found that the number of flowers setting pods increased as the relative humidity decreased from 90 to 10 per cent. At 80° F. there was a difference of 24 per cent between the percentage of pods setting at 10 and 90 per cent relative humidity.

Environmental Factors Affecting Seed Setting

It has been indicated previously that factors which affect pollen tube growth such as temperature and moisture, and flower-tripping agents like rain, sunshine, wind and pollinating insects play important roles in influencing seed setting, due to both self- and cross-fertilization. The amount of water supplied to the crop also may have a decided effect on seed setting. Stewart (1926) stated that the more important alfalfa seed producing areas are located in the arid and semi-arid regions of the U. S. He believed that with an abundant supply of moisture the alfalfa plant grows rapidly, giving a high yield of forage but low seed yield. Engelbert (1932) suggested that in Ontario, Canada the limiting climatological factor for seed yield was the amount and distribution of rainfall in summer. Excessive rainfall in July appeared to be the cause of failure in seed production whereas a limited amount of rainfall in the same month offered the best condition for seed production. In Colorado, Blinn (1920) observed an instance in which the abnormally wet weather in spring prevented setting of seeds. Grandfield (1945) reported that high plant reserves tend to increase seed production especially if there is a relatively low supply of soil moisture. Moderate air temperature, low humidity, and soil moisture below the optimum amount tend to produce the type of vegetative growth which stimulates the storage of high organic reserves, resulting in

a physiological condition conducive to seed setting. Tysdal (1946) found that at a thick rate of planting (plants spaced 8 inches each direction) the plants growing in the soil with a relatively low moisture supply produced more seed than those under a high moisture condition. At 32-inch spacing, conditions of high and low soil moisture resulted in practically the same yield.

Other factors influence seed setting. Aicher (1917) believed that there was a close correlation between the number of days of sunshine in the summer and the amount of seed set. Blinn (1920) expressed the opinion that the amount of heat and light influenced seed setting. As Dwyer and Allman (1933), Hadfield and Calder (1936), hare and Vansell (1946) and Vansell and Todd (1946) have shown, the success of alfalfa seed production rests mainly on the activities of pollinating insects, especially wild bees and honey bees in the area.

Inbreeding in Alfalfa

Results of a number of studies have shown that the process of inbreeding in alfalfa is accompanied by a drastic reduction in seed yield and also by a decrease in forage yield.

Kirk (1927) reported that, on the average, a pronounced and progressive reduction in vigor of growth was observed for each generation of selfing and he noted that the mean yield of seed in the second generation of selfing was markedly less

than that of open-pollinated strains. Williams (1931) found that the yield of seed from the S_1 plants was only 12.4 per cent of that of the parent plants from which they were derived. A reduction of 46.1 per cent in seed set among S_1 lines was obtained by Sandal (1946). Skory (1947) obtained a decrease of 84 per cent in self-fertility from the first to the second selfed generation. Similarly, Koffman (1959) reported a drop in self-fertility of 80 to 90 per cent in the second and third generations of selfing compared to that of the parental clones. It was observed by Bolton (1948) that while seed yield of S_1 to S_3 lines was exceedingly low, one line in the S_3 was found to produce 60 to 69 pounds of seed per acre.

Wilsie (1958) showed that selfing for one generation resulted in a loss of self-fertility of from 80 to 90 per cent and a loss of vegetative vigor of from 20 to 30 per cent. Loss of vigor and the reduction in seed yield were greater in the S_2 than in the S_1. Results obtained by Tysdal et al. (1942) indicated that forage yield was reduced on an average of 32 per cent in the first selfed generation, with additional decreases in the succeeding selfed generations until a level of 26 to 30 per cent of the original yield was maintained in the seventh and eighth generations. Koffman (1959) found that selfed progenies yielded only 55 to 75 per cent of the forage yield of non-inbred parental clones. Similar results were

obtained by McAllister (1950).

Bolton (1948) showed that progenies of crosses among second generation selfed plants derived from the same parents were intermediate in seed and forage yield between comparable inbred lines and outcross progenies. A partial restoration of self-fertility in sib-matings of S_1 plants was reported by Koffman (1959).

There are other effects associated with inbreeding in alfalfa. Kirk (1927) found a reduction in variability of some important morphologic and physiologic characters. Some chlorophyll deficient seedlings appeared and winter injury damage to some inbreds occurred. Stewart (1934) found a significantly lower variability in selfed progenies for plant height, plant width, stem diameter, leaflet length and width, blossom color, and foliage color.

Breeding Procedures

Progress made in alfalfa breeding has been reviewed by Bolton (1948) and White (1949).

Improvement of seed yield by selection

Kirk (1927) obtained a significant correlation (r = .46) in seed setting between the first and second generations of selfed lines and concluded that the high and low seed producing characteristics are inherited. Selection within self-

fertilized lines was suggested as a means of increasing seed
yield. This idea was abandoned later because of the loss of
vigor resulting from selfing. Tysdal and Kiesselbach (1944)
reported a significant positive correlation (r = 0.3781) be-
tween self-fertility and seed production under poor seed-
setting conditions, but a negative significant correlation
(r = -0.3874) was obtained under good seed-setting conditions.
The positive association under poor seed-setting conditions
was attributed to the fact that selfing was more prevalent and
since selfing results in reduced vigor, the progeny of these
plants would be low in forage productivity. They believed
that selection for self-fertility was not advisable.

Another approach to improving seed yield in alfalfa is
through "maternal line selection" as proposed by Fryer (1939).
This method consisted of 4-year cycles following each other
and continued indefinitely.

In the first year 80 progenies, each containing 50 plants,
were grown from seeds. In the second year each plant was
scored on the basis of the density of pods, and poor plants
and poor progenies were rogued. Plants were scored for fer-
tility again in the third year and only 100 plants, which rep-
resented from 30 to 40 different progenies were selected late
in the fall. In the fourth year further selection was made
and 80 out of the 100 selections made in the previous year,
were allowed to set seed under open-pollination. These were

used to initiate the next cycle of selection. In 3-year tests, the alfalfa stock improved through 10 years of maternal-line selection showed a superiority in yield over three unimproved standard varieties and a selfed line. Pedersen (1953) found that over a 3-year period nine selections based on maternal performance were 38.8 per cent better in seed production than the average of six checks.

Utilization of hybrid vigor for forage yield

Tysdal et al. (1942) showed evidence of considerable hybrid vigor from hand-pollinated crosses between inbred lines, depending on the combining ability of the parents. Single crosses yielded from 60 to 139 per cent of the average of Grimm, Hardistan and Ladak, but the average of 28 hybrids was only 96 per cent of the average yield of the check varieties. Tysdal and Kiesselbach (1944) obtained results from forage yield tests of 31 different F_1 hybrids which showed marked hybrid vigor resulting from crossing. Some double crosses produced by crossing two F_1 hybrids exhibited as much vigor as the single crosses from inbred lines. In the order of yield, the top five hybrids were those built up from 3, 4, 2, 5, and 8 lines respectively. The average yield of the 5 hybrids exceeded that of the highest yielding variety by 23 per cent. Waldron (1920) found 47.5 per cent increase in forage yield in a cross between M. sativa and M. falcata,

as compared to that of the parents.

Tysdal and Kiesselbach (1944) suggested a procedure for the commercial production of F_1 hybrid seed in alfalfa similar to that being followed in corn. Clonal lines used for the production of the hybrid are chosen on the basis of their combining ability. While the best measure of combining ability is average single cross performance, Tysdal and Crandall (1948) concluded that polycross progeny performance was a satisfactory method of testing the combining ability of clones. Wilsie and Skory (1948) obtained a correlation of $r = 0.37$ between general combining ability as measured by the yield of open-pollination progenies and that determined by the average yield of single crosses. Open-pollination progenies and S_1 progenies appeared to be of equal value in measuring the breeding potentialities of the parental clones. Bolton (1948) presented data to show that where large numbers of clones are to be tested, a relatively small number of tester plants may be used for making test crosses to be used in the estimation of combining ability.

Tysdal and Kiesselbach (1944) stressed that selection of parental clones at the start of a breeding program should be directed toward plants that are highly self-sterile. Eventual use of highly sterile materials in the production of F_1 hybrids will insure maximum degree of outcrossing rather than selfing. Furthermore, it was believed that self-sterility is

associated with high cross-fertility. Using hybrid materials
in the study, these authors found a correlation between the
self-sterility of the hybrids and increased seed production
resulting from crossing. Two of the most highly self-sterile
hybrids showed the greatest increase in seed production when
outcrossed. All hybrids showed a greater seed production when
sibbed than when selfed, but self-sterile hybrids showed a
greater increase when outcrossed than did the self-fertile
hybrids. Other workers have obtained contradicting results.
McAllister (1950) found that high self-fertility clones pro-
duced approximately three times as many seeds when selfed or
crossed as did clones of low self-fertility, indicating a
positive relationship between self- and cross-fertility.
Bolton (1948) earlier had obtained a positive correlation
($r = 0.288$) between self- and cross-fertility.

Tysdal and Kiesselbach (1944) presented evidence, from a
relatively small population of selected plants, that self-
sterility was associated with higher forage yield. Selection
of plants high in self-sterility was suggested as a means of
maintaining a high level of productivity. In contrast, Wilsie
(1951) showed that self-fertility appeared to be relatively
independent of combining ability. There was no correlation
between self-fertility and open-pollinated progeny forage
yields and only a slight correlation was obtained between self-
fertility and S_1 progeny yields. The lack of association

between self-sterility and forage yield is further substantiated by the results obtained by Fryer (1939) and Pedersen (1953).

The method of producing double crosses in alfalfa proposed by Tysdal and Kiesselbach (1944) rests on the following main points: (1) selection of parental plants based on superior polycross progeny performance; (2) the use of self-sterility to ensure cross-pollination; (3) vegetative propagation of the parental plants in order to obtain sufficient amounts of single-cross seed; and (4) production of double cross seed.

Armstrong (1952) suggested the following for the commercial production of double-cross seed. Firstly, there should be available two superior lines possessing a sterility mechanism which is due to physiologic disturbances in the maturing pollen but which prove to be good combiners, and another two good combining lines that are self-incompatible but which produce an abundance of pollen. Secondly, the F_1 generations (or single crosses) should be made within the types and not across the types, in order that self-sterility may be retained and crossing between related F_1 plants be reduced to a minimum. The difficulty with this system would be in finding any four clones which would meet the aforementioned requirement.

An alternative plan to that proposed by Tysdal and

Kiesselbach (1944) was outlined by Bolton (1948) for the production of commercial hybrids of alfalfa. It suggested the use of "non-self-tripping, self-fertile" inbred plants in order to avoid the necessity for vegetative propagation. Crossing for the production of both single cross and commercial double cross seed will depend on insect pollination.

The use of synthetic varieties or seed of advanced generations of a double cross, or that originating from a composite of four or more parental clones with good general combining ability, has been highly successful. Tysdal et al. (1944, 1948) showed that clones selected for high combining ability by the polycross method produced a synthetic variety having a significantly higher forage yield than the standard variety or a synthetic produced using clones of poor-combining ability.

MATERIALS AND METHODS

Plant Materials

In the present study the S_1 progenies used were obtained from four clones of alfalfa varying in the degree of self-fertility as determined on the basis of previous observations. The clones may be described as follows:

(1). Clone 585 B-2, which was obtained from the cross C84xV-2 and selected for alfalfa spotted aphid resistance. It was chosen to represent a high self-fertility group. The S_1 plants obtained from this clone were numbered 1 to 42 inclusive (Group I).

(2). Clone 618, a second cycle selection from Synthetic 2187, was selected for resistance to Cercospora medicaginis. It was classified as having a low self-fertility. The S_1 plants were numbered from 101 to 142 (Group II).

(3). Clone 631-101, a selection from Vernal, obtained from x-ray irradiation studies and selected for resistance to Cercospora. It was classified as intermediate in self-fertility but in this study it turned out to be of low self-fertility. The S_1 plants were assigned the numbers from 201 to 242 (Group III).

(4). Clone 5, a low-crown selection from an old golf

course near Des Moines, Iowa. It was chosen to

be representative of a low self-fertility group,

but in this study proved to be intermediate in

self-fertility. The S_1 plants were numbered from

301 to 342 (Group IV).

Procedure

A total of 42 S_1 plants, together with the parental

clone, constituted each of the four groups. The S_1 plants

of each group were chosen at random from a larger S_1 progeny

growing in the breeding nursery. Stem cuttings were taken

from the field in the fall of 1958 and were propagated in

vermiculite in the greenhouse. When the cuttings had rooted,

they were transplanted into 4-inch pots. Three cuttings were

transplanted for each S_1 plant. The growing medium used was

of a sterilized mixture of 2 parts field soil, 1 part peat,

and 1 part fine sand.

Each group or family of plants, was arranged on a single

greenhouse bench in the order of the assigned number. Twelve

plants of the parental clone also were included.

Nutrient solution was applied to the plants at monthly

intervals. As the plants grew the elongating stems were

supported by bamboo stakes held in an upright position. In

order to accelerate flowering, the photoperiod was extended

to 18 hours each day, using 200-watt incandescent lamps.

Determination of self-fertility of each of the S_1 plants and parental clones was made by artificial selfing of at least 300 flowers. Selfing of flowers was accomplished by applying pressure on the keel (which holds the staminal column of the flower) with the tip of a toothpick, and drawing the tip across the exposed stigma. The number of flowers that were selfed, and the date of selfing were indicated. Selfing was performed whenever flowers were available, over a period of time in winter and spring of 1958-59 and 1959-60.

In order to study sib-mating compatibility relationships, cyclic crosses (e.g. 1 x 2, 2 x 3, 3 x 4 •••• 42 x 1) among the 42 S_1 plants of each group were made by hand pollination. There were 42 cross combinations for each group and including reciprocal crosses, a total of 84 different crosses were actually involved. In addition, check crosses with 2 unrelated clones also were made.

For each cross, ten flowers were used. Two racemes, borne on the same plant, were selected and only 5 flowers from a raceme were used, the rest of the flowers being clipped off.

In making a cross the standard petal of each flower used as female was first cut off close to the base, after which the flowers were tripped. An adequate quantity of pollen was collected from the exposed staminal column of each plant used

as a parent. Emasculation of flowers was achieved by suction, using a finely drawn glass tube connected to an electrically-driven vacuum pump. Reciprocal crosses were made, pollen being applied immediately after emasculation. The racemes bearing the hand pollinated flowers were appropriately tagged.

In each group four series of cyclic crosses were made, usually at intervals of from 7 to 10 days. Each series of crosses served as a replicate and in order to keep environmental variability as low as possible all crosses in a series were completed within a period of one or two days. To provide a measure of the differences in seed setting between days during which a series was completed, check crosses were made each day. After making each series of sib crosses, the pots were re-arranged on the bench in order to eliminate the possible positional effects of the pots on seed-setting. Repotting of plants within the entire group was done whenever necessary in order to complete the desired number of replicated crosses.

Each S_1 plant in each group also was backcrossed to the parental clone, the latter being used as the pollinator. Four replicated series of backcrosses were made. A replication was completed in one day.

The dates during which all the crosses were made are indicated as follows:

Group		Rep I	Rep II	Rep III	Rep IV
A.	**Cyclic crosses**				
Group I	(1-42)	3/7/59	3/17-18/59	4/9-10/59	4/23-24/59
Group II	(101-142)	3/24-25/59	3/18-19/60	-	-
Group III	(201-242)	4/15-16/59	6/22-23/60	-	-
Group IV	(301-342)	3/12/59	4/2-3/59	4/8-9/60	4/12-13/60
B.	**Backcrosses**				
Group I	(1-42)	1/9/60	1/16/60	4/4/60	4/6/60
Group II	(101-142)	4/11/60	-	-	-
Group III	(201-242)	3/23/60	3/26/60	4/2/60	4/5/60
Group IV	(301-342)	1/23/60	1/30/60	4/15/60	4/16/60

Pods were harvested when fully mature, usually about four weeks after selfing or crossing. The total number of pods formed was recorded, and when the pods were threshed the total number of seeds set was determined by counting only those that were well-filled.

For comparative purposes, the fertility data were expressed on the basis of the number of seeds set per flower pollinated.

EXPERIMENTAL RESULTS

Results of Selfing

The results of selfing of the S_1 progenies and S_0 parental clones used in this study are shown in Tables 1, 2, 3 and 4, in the Appendix. The self-fertility data for the parental clones obtained in the present study did not coincide exactly with data obtained previously.

Table 1. Self-fertility of four S_0 clones of alfalfa as determined in two separate experiments

S_0 parental clone	1956-1957		1958-1960	
	Seeds per flower selfed	Self-fertility classification	Seeds per flower selfed	Self-fertility classification
585 B-2 Cl	2.20	High	2.582	High
618 Cl	0.20	Low	0.498	Low
631-101 Cl	1.50	Intermediate	0.538	Low
5 Cl	0.24	Low	0.910	Intermediate

Considering the average self-fertility of the S_1 progenies and corresponding non-inbred parents, the four groups of alfalfa plants used in the present study may be reclassified as follows: Group I (1-42) as a high self-fertility group; Group II (101-142) and Group III (201-242) as low

self-fertility groups; Group IV (301-342) as intermediate in
self-fertility.

Generally, there was a marked reduction in self-fertility
among the S_1 plants as compared with the self-fertility of the
S_0 parental clone (Table 2b). The self-fertility of the S_1
plants in Groups I, II, III and IV, expressed as an average of
the group, was 0.482, 0.051, 0.106 and 0.246 seed per flower
tripped, respectively. The self-fertility of the parental
clone of each group, in the same order, was 2.582, 0.498,
0.538 and 0.910 seed per flower selfed.

There was a remarkable range in self-fertility among the
S_1 plants within a group. This is especially true among those
in the high and intermediate self-fertility groups (Table 2a).
It is of interest to note that one S_1 plant in Group III and
two S_1 plants in Group IV had a self-fertility higher than
that of their non-inbred parents.

The most drastic reduction in self-fertility occuring in
the low fertility groups seems to indicate that the parental
clones for these groups had already a preponderance of the
genes associated with the expression of self-incompatibility,
and that the intensity of self sterility is increased after
one generation of inbreeding, owing to the fact that the in-
dividuals were approaching homozygosity.

In the high self-fertility group, 18 S_1 plants out of
42 studied were observed to be autogamous or capable of

Table 2a. Frequency distribution of S_1 plants on the basis of self-fertility as measured by the number of seeds per flower selfed

Classes (Seed per flower selfed)	Frequency distribution of S_1 plants			
	Group I (1-42)	Group II (101-142)	Group III (201-242)	Group IV (301-342)
0.000 - 0.050		28	17	2
0.051 - 0.100	2	6	8	8
0.101 - 0.150	5	2	7	8
0.151 - 0.200		2	3	8
0.201 - 0.250	5	1	2	3
0.251 - 0.300	4		2	4
0.301 - 0.350	6	1		1
0.351 - 0.400	3			2
0.401 - 0.450	1		1	
0.451 - 0.500	2			
0.501 - 0.550	2			1
0.551 - 0.600	2			1
0.601 - 0.650				
0.651 - 0.700			1	1
0.701 - 0.750	1			1
0.751 - 0.800	2			
0.800 - 0.850				
0.851 - 0.900				
0.901 - 0.950	2			1
0.951 - 1.000	1			
1.001 - 1.050				1
1.051 - 1.100				
1.101 - 1.150				
1.151 - 1.200				
1.201 - 1.250	2			
1.251 - 1.300				
1.301 - 1.350				
1.351 - 1.400	1			
1.401 - 1.450				
1.451 - 1.500				
1.501 - 1.550				
1.551 - 1.600	1			
Total	42	40	41	42

Table 2b. Average self-fertility of S_0 and S_1 plants

Average self-fertility	Group I (1-42)	Group II (101-142)	Group III (201-242)	Group IV (301-342)
Parental S_0 clone	2.582	0.498	0.538	0.910
S_1 plants	0.482	0.051	0.106	0.246
Average S_1 self-fertility expressed as a percentage of the self-fertility of the parental S_0 clone	18.67	10.24	19.70	27.03

setting seeds without the aid of artificial tripping. The parental clone of this group possessed the same autogamous characteristic. Practically all the plants in this group produced a large number of flowers per plant and an abundance of pollen. In Group II, the group lowest in self-fertility, eleven S_1 plants out of a total of 42 included were observed to have produced a very small quantity of pollen. Generally, the S_1 plants produced limited numbers of flowers per plant. In Group III, a low self-fertility group, four plants did not produce adequate pollen. Flower production was not a problem. In Group IV, five S_1 plants produced very little pollen but the others were good pollen producers. Flowers were borne in large numbers except for two plants.

Results of Cyclic Sib-mating

The data on seed setting following sib-mating in the high fertility group are presented in Table 5 in the Appendix. Cross-fertility among sib-matings ranged from 0.125 to 2.550 seeds per flower crossed. The analysis of variance is shown in Table 3a. Significant differences in seed set were obtained among sib-crosses as shown by the significance of the mean square for crosses at the 1 per cent level. Evidently, genetic factors are involved which determine the compatibil-ity relationship of any two sib lines. It is believed that the S_1 individuals were segregating and were therefore different in genetic make-up for factors determining self- or cross-incompatibilities. Using the L.S.D. value, comparisons were made in seed set of two sib-crosses involving a common female parent but differing in the pollen source. Out of

Table 3a. Analysis of variance for seed setting obtained from sib-crosses among S_1 plants in Group I

Source of variation	Degree of freedom	Mean square	Computed F- value
Crosses	85	134.35	6.71**
Replications	3	42.82	2.14
Error	255	20.03	

**Exceeds the 1 per cent level

Table 3b. A partitioning of the sum of squares for seed
           yield among sib-crosses

Source of variation	Degree of freedom	Mean square	Computed F- value
Sib-crosses	83		
Cross combinations	41	152.29	3.26**
Reciprocals	1	2.86	
Combinations x reciprocals	41	46.75	2.55**
Error	249	18.32	

**Exceeds the 1 per cent level

42 possible sets of comparisons, two showed a statistically
significant difference at 5 per cent level, whereas six pairs
showed significant difference at the 1 per cent level. Non-
significance obtained in the remaining sets of comparisons
indicates a similarity in the compatibility factors contained
by the fertilizing pollen of the two sources involved. Sim-
ilarly, comparisons in seed set were made between two sib-
crosses involving two different female plants hand-pollinated
with pollen coming from the same plant. Ten pairs of com-
parisons showed significant differences at the 5 per cent level
whereas four sets had differences significant at the 1 per
cent level. The results obtained point to the fact that cross
compatibility is dependent on the genotype of both the

maternal and paternal lines which complement each other.
Statistical differences between reciprocal crosses were ob-
tained in eight cases, out of 42 sets of reciprocal crosses,
five of which had a significance at 1 per cent level. A sig-
nificant difference was also obtained between reciprocals of
the check crosses. It would seem that in some combinations,
the order of pairing in making the cross is very important.

It is of interest to note that eight compatible sib-
crosses even produced as much seed as that of a check cross
which involves two unrelated S_0 clones. While many crosses
were highly compatible, other crosses, on the other hand,
were not as compatible and produced very limited amounts of
seed.

The mean square for replication is non-significant. The
four replicated series of crosses were all made in the spring
of 1959.

The sum of squares representing the sib-crosses is fur-
ther partitioned into orthogonal comparisons (Table 3b). The
between-reciprocals component represents two groups, one of
which includes the crosses in which all the 42 S_1 plants were
used as female parents while the other contains all crosses
involving the same 42 S_1 plants but used as pollen sources.
The sib-crosses in one group are therefore the reciprocals of
the entries in the other group. The new error term used is
also a re-calculated value after eliminating the entries for

the check crosses.

The mean square for cross combinations is significant at the 1 per cent level. A combination represents the average seed set of its reciprocal crosses. Certain combinations were certainly more compatible than others. The mean square for combinations x reciprocals interaction is also significant at the 1 per cent level, indicating that, generally, the same cross combination did not perform consistently in reciprocal crosses.

The average cross-fertility for the sib-crosses is 0.970 seed per flower crossed. The average self-fertility of the 42 S_1 plants and parental clone is 0.482 and 2.582 seeds per flower, respectively. Although a comparison of seed setting between sibbing and selfing may not be statistically valid, by virtue of the fact that the averages were obtained from two separate experiments, yet there is reason to believe that the process of sib-mating in this particular group would result in a greater quantity of seed setting in comparison to straight selfing. In the first generation of selfing the majority of the plants would have obtained some degree of duplication for the genetic factors associated with the self-incompatibility mechanism and selfing of these plants would result in a reduction in seed setting, as has been actually demonstrated. The S_1 population, however, is still highly heterogeneous so that sib-mating among plants, due to

complementary gene action, would overcome such sterility problems accruing from straight selfing.

The results of the sib-mating study in Group IV are presented in Table 8 in the Appendix. The analysis of variance is shown in Table 4a. The degrees of freedom are diminished by 5, a figure corresponding to the number of missing data. It will be noted that the mean square for crosses is significant at the 1 per cent level. Cross-fertility ranges from 0.075 to 3.175 seeds per flower crossed. It is indicated that while some sib-crosses are highly compatible, other combinations are not. Eight sib-crosses gave as much seed set as one of the reciprocals of the check crosses. Comparisons are made between two sib-crosses in which a common female parent and two different male parents are involved. Comparisons are made also between crosses involving the same pollen source but differing in the seed parents. Six and fourteen sets of comparisons, respectively, showing statistically significant differences were obtained. A comparison between reciprocal crosses showed that five pairs of reciprocals showed significant differences in seed setting.

The mean square for replication also is statistically significant at the 1 per cent level. Crosses made in the first two replications were made in the spring of 1959 whereas the last two replications were completed in the spring of 1960. The plants grown in 1960 were derived from cuttings of

Table 4a. Analysis of variance for seed setting obtained from sib-crosses among S_1 plants in Group IV

Source of variation	Degree of freedom	Mean square	Computed F- value
Crosses	85	328.00	7.15**
Replications	3	2,846.81	62.05**
Error	250	45.88	

**Exceeds the 1 per cent level

Table 4b. A partitioning of the sum of squares for seed yield among sib-crosses

Source of variation	Degree of freedom	Mean square	Computed F- value
Sib-crosses	83		
Cross combinations	41	405.29	7.66**
Reciprocals	1	12.96	
Combinations x reciprocals	41	52.87	1.36
Error	244	38.86	

**Exceeds the 1 per cent level

plants grown in 1959. Twice as much seed setting was obtained in 1960 as compared to that obtained in 1959. It is indicated that the amount of seed setting following sib-mating is subject also to fluctuations owing to differences in environmental

conditions occuring at the time crosses are made. The conditions in 1960 were more favorable to seed setting.

The sum of squares for crosses was broken down into three components (Table 4b). The mean square for combinations is statistically significant at 1 per cent level. The combinations x reciprocals component has a non-significant mean square. The results of the statistical analysis show that certain combinations are more compatible than others. The relative degree of seed setting among cross combinations was consistent in reciprocal crosses.

The average amount of seed set among the sib-crosses was 1.061 seeds per flower crossed. The average self-fertility of the 42 S_1 progenies and the S_0 parent was 0.246 and 0.910 seeds per flower, respectively. Apparently, loss of fertility is much slower following sib-mating than when selfing is performed.

Cyclic sib-crosses also were made for the S_1 plants belonging to Groups II and III which are both classified as low in self-fertility. The results are presented in Tables 6 and 7 in the Appendix. Only two replicated series of sib-crosses were made in the two groups since the amount of seed setting was found to be exceedingly low, from the practical point of view. The results obtained indicate that no additional advantage could be attained in sib-mating among self lines which are inherently very poor in self-fertility. The

problem encountered in making sib-crosses in Group II was the limited amount of pollen available from each S_1 plant. In making the actual crosses, however, pollen was collected in adequate amount, enough to cross-pollinate each female flower. Failure in seed setting was therefore the result of the incompatibility relationships between sibs rather than the lack of adequate cross-pollination.

The S_1 plants in Group III, although including a few plants which produced pollen in limited quantity, were, on the whole, better pollen producers than the plants in Group II. Seed setting in the sib-crosses also was exceedingly low.

It is believed that the populations of Groups II and III were homogeneous to an extent and were composed of S_1 plants the majority of which were identical in the factors associated with self- or cross-incompatibility. Results of sib-mating therefore would be little different from those obtained by selfing.

Results of Backcrossing

Table 9 in the Appendix shows the result of backcrossing of the S_1 plants in Group I (1-42) to the parental clone from which they were derived. Seed setting ranges from 0.950 to 4.675 seeds per flower, with an average of 3.012 seeds. The analysis of variance is shown in Table 5. The mean square for crosses is statistically significant at the 1 per cent

level indicating that the S_1 progenies were different in the factors controlling fertility. The mean square for replications also is highly significant, showing that seed setting is partly dependent on environmental factors.

The analysis of variance for seed setting obtained in the backcrossing of the S_1 progenies in Group III (201-242) is presented in Table 6. Significant differences in seed setting are obtained between backcrosses as shown by the statistically significant mean square. The range in seed set is from 0.70 to 3.625 seeds per flower, with an average of 2.131 seeds (Table 11, Appendix).

The analysis of variance for Group IV (301-342) is shown in Table 7. Statistical differences in seed set among backcrosses are obtained. Differences among replications are also

Table 5. Analysis of variance for seed setting following backcrossing of S_1 plants to parent clone in Group I

Source of variation	Degree of freedom	Mean square	Computed F- value
Crosses	39	276.14	11.31**
Replications	3	126.45	5.18**
Error	115	24.41	

**Exceeds the 1 per cent level

Table 6. Analysis of variance for seed setting following backcrossing of S_1 plants to parent clone in Group III

Source of variation	Degree of freedom	Mean square	Computed F- value
Crosses	39	264.19	7.50**
Replications	3	33.06	0.94
Error	116	35.22	

**Exceeds the 1 per cent level

Table 7. Analysis of variance for seed setting following backcrossing of S_1 plants to parent clone in Group IV

Source of variation	Degree of freedom	Mean square	Computed F- value
Crosses	39	550.51	13.09**
Replications	3	350.25	8.33**
Error	117	42.06	

**Exceeds the 1 per cent level

statistically significant. The range in cross-fertility is from 0.950 to as high as 5.900 seeds per flower, the average being 2.793 seeds.

In Group II (101-142) only one replication of backcrosses is available. The average cross-fertility of the group is

0.813 seed per flower.

It is of interest to note that in all cases, the average seed set of a group following backcrossing exceeds that of selfing of the corresponding S_0 parental clone (Table 8). There is indication that improvement in fertility among the S_1 plants may be achieved by crossing them back to the parental clone from which they were derived.

Correlation Studies

Correlation studies made for the S_1 plants in Group I are shown in Table 9. As can be noted, the self-fertility of the S_1 plants is correlated with the fertility of the same plants following backcrossing. It shows that both procedures were equally good in measuring genetic differences relating

Table 8. Seed set from selfing S_0 parental clones and back-crossing S_1 plants to parental clones

| | Number of seeds per flower crossed or selfed | | | |
	Group I	Group II	Group III	Group IV
Self-fertility of parental clone	2.582	0.498	0.538	0.910
Average cross-fertility of the S_1 plants back-crossed to parental clone	3.012	0.813	2.131	2.793

to fertility among the S_1 plants. Self-fertility and back-cross fertility both are associated with the average cross-fertility of the clones as male and female parents. There is, however, a lack of association when each of the S_1 clones was considered only as a male parent in the process of sib-mating.

Table 9. Correlations of seed setting following selfing, backcrossing, and sib-mating of 42 S_1 plants in Group I (1 to 42) .

	Self-fertility	Fertility following backcrossing	Fertility following sib-mating (S_1 plants involved as female parents)
Fertility following backcrossing	0.3702*		
Fertility following sib-mating (S_1 plants involved as female and male parents)	0.3540*	0.3810*	
Fertility following sib-mating (S_1 plants involved as female parents only)	0.3285*	0.5183*	
Fertility following sib-mating (S_1 plants involved as male parents only)	0.2843	0.1524	0.5063**

*Exceeds the 5 per cent level

**Exceeds the 1 per cent level

The correlation studies made for Group IV S_1 plants are presented in Table 10. The correlation value obtained between selfing and backcrossing is statistically non-significant, although it is just a little lower than the tabular value at the 5 per cent level. But there is a good association between self- and average sib-cross-fertility (as

Table 10. Correlations of seed setting following selfing, backcrossing, and sib-mating of 42 S_1 plants in Group IV (301-342)

	Self-fertility	Fertility following backcrossing	Fertility following sib-mating (S_1 plants involved as female parents)
Fertility following backcrossing	0.3042		
Fertility following sib-mating (S_1 plants involved as female and male parents)	0.4159**	0.6257**	
Fertility following sib-mating (S_1 plants involved as female parents only)	0.4861**	0.5671**	
Fertility following sib-mating (S_1 plants involved as male parents only)	0.2966	0.2506	0.7780**

**Exceeds the 1 per cent level

maternal and paternal parents) as shown by the significance
of the computed r at 1 per cent level. A similar relation-
ship in fertility between that of backcrossing and sib-mating
is obtained. Statistically non-significant correlation values
were obtained when each of the S_1 clones was considered only
as a pollen parent in the process of sib-mating.

In Group III, a significant correlation was obtained in
seed setting between selfing and backcrossing, the r value
being 0.5297.

The correlation values obtained seem to indicate that S_1
plants showing high self-fertility may generally be found to
possess a high degree of backcross- or sib-cross-fertility
preferably when used as female parents. This positive re-
lationship between self- and cross-fertility is clearly
demonstrated in Tables 11 and 12.

Table 11. Average self-, backcross-, and sib-cross-fertility
(number of seeds per flower) of three sub-groups
of 42 S_1 lines of Group I classified on the basis
of self-fertility

Sub-group	Average self-fertility	Average backcross-fertility	Average sib-cross-fertility
Low self-fertility	0.175	2.828	0.767
Intermediate self-fertility	0.360	2.713	0.906
High self-fertility	0.910	3.511	1.238

Table 12. Average self-, backcross-, and sib-cross-fertility (number of seeds per flower) of three sub-groups of 42 S_1 lines of Group IV classified on the basis of self-fertility

Sub-group	Average self-fertility	Average backcross-fertility	Average sib-cross-fertility
Low self-fertility	0.076	2.046	0.811
Intermediate self-fertility	0.173	2.980	1.084
High self-fertility	0.487	3.338	1.286

DISCUSSION

The low seed set obtained by selfing the S_1 plants used in this study showed that a marked reduction in self-fertility had taken place through one generation of self-fertilization. Self-sterility was most pronounced in the two families derived from low self-fertility clones. Although a drastic loss in self-fertility had occurred in the S_1 progenies of the high and intermediate groups, it is believed that a majority of the S_1 plants could be selfed successfully at least one more generation.

Differences in seed setting among plants in inbred progenies, produced by selfing, backcrossing, and sib-mating indicated segregation of hereditary factors controlling fertility. Selection in the first and subsequent generations of selfing for individuals that are high in self-fertility, if this were desirable, might prove to be effective. Kirk (1927) recognized earlier the effectivity of selection for improved seed yield in the self generations of alfalfa. Bolton (1948) obtained one third generation line yielding over 60 pounds per acre.

Sib-mating is an inbreeding method which might be used as an alternative to straight selfing. As shown by Wright (1921) the approach toward homozygosis is somewhat slower through sib-mating than through selfing, and consequently the drastic drop in self-fertility characteristic of selfing might

be partially prevented or at least delayed. In the two low self-fertility groups in this study, however, sib-mating proved to be impractical because of a high degree of sib-incompatibility. On the other hand, sib-mating among S_1 plants in the high and intermediate self-fertility groups resulted in a significant gain in fertility. A study made by Koffman (1959) has shown similar results.

Restoration of fertility following sib-mating rests on the following assumptions. As a result of selfing, plants tend to become more homozygous for the genetic factors associated with the expression of self-incompatibility, and may, therefore, be self-incompatible. However, the S_1 population undoubtedly is still heterozygous for many factors so that many of these self-sterile plants, with the operation of complementary and modifying factors, would still be capable of producing seeds when cross-fertilized by compatible sibs from the same population.

There are two observable components of self-incompatibility operating in alfalfa. One is associated with the inability of the pollen tube to reach the ovule and effect fertilization, as Cooper and Brink (1940), Brink and Cooper (1938), and Miller (1960) have observed. The other factor is that involving post-fertilization ovule collapse, as has been reported by Brink and Cooper (1938, 1939), Cooper and Brink (1940), and Miller (1960). The genetics involved in the two cases are not

understood at the present time. But it seems, as the present data suggest, that there are actually "plus" genes directly controlling fertility. This may be evidenced from the correlation studies made, which show that self-fertility of the S_1 plants is positively associated with average backcross- and sib-fertility. A positive relationship between self- and cross-fertility has previously been shown by McAllister (1950) and Bolton (1948). Such significant correlations would unlikely be brought about by the operation of the S incompatibility gene systems. Whether or not such "plus" genes are related directly to the expression of embryo abortion is not known. It is believed that factors related to the production of functional gametes may have accounted for the positive association between self- and cross-fertility.

As the present correlation data indicate the degree of fertility is somewhat dependent on the maternal parent. Significant correlations were obtained in the S_1 between self-fertility and sib-fertility when the plants were utilized as maternal parents in making the crosses. This may explain why in some cases, reciprocal sib-crosses varied considerably in seed setting. Whitehead and Davis (1954) ascribed the variation in seed setting between reciprocal crosses to the differences in female fertility between the parental plants. It was postulated that alfalfa plants may differ in the number of highly functional ovules, a factor which would determine

female fertility. In Lotus sp., Bubar (1958) found that uniformity in the development of ovules within each ovary determines the self-fertility of a plant. The self-fertile plants were found to have a greater uniformity in the development of the ovules within each ovary than the self-sterile individuals.

From the 42 S_1 plants constituting a group, a total of 861 cross combinations are possible. The 42 cross combinations obtainable in the present experiment may be assumed to be a representative sample of the said theoretical number. Assuming that the 42 self plants represent a true population originating from a selfed non-inbred clone, it follows that the results in seed setting obtained in sib-mating would be similar to those obtainable when sib-mating occurs at random in the field. One point that should not be overlooked, however, is that seed setting under greenhouse conditions may not be essentially similar to that under natural field conditions. Seed setting would be influenced by the environmental conditions especially that of temperature and moisture prevailing in the area, and by the activity of pollinating insects.

Sib-mating as has been noted, is a form of inbreeding much slower than selfing in attaining homozygosity. This is especially true in alfalfa since it is a tetraploid. Bartlett and Haldane (1934) have estimated that in a tetraploid selfing must be done for 3.80 generations as compared to one generation

for a diploid, in order to halve heterozygosis. With brother-sister matings, the number of generations of selfing is 8.72 and 3.26, respectively. Furthermore, alfalfa is characterized by a highly heterozygous nature maintained by the prevalent outbreeding system. Many generations of inbreeding, which may not be possible, would be required to produce approximately homozygous or true-breeding inbred lines. Stewart (1934), however, has observed remarkable uniformity in many observable characters after one generation of selfing.

Parent-progeny crosses made in this study have shown interesting results. There was a consistent and relatively high seed set in each of the groups studied resulting from back-crossing of each S_1 individual to the parent from which it was derived, in comparison with that of sib-mating. Average cross-fertility in these S_1 backcrosses even exceeded the self-fertility of the respective non-inbred clones and approached the level of cross-fertility attained in outcrossing parent clones to an unrelated tester plant. No genetic explanation can be offered at this point. It should be remembered, however, that in making crosses only five flowers to a raceme were used as opposed to the greater number of flowers per raceme utilized in the process of selfing and the greater fertility indicated may be due, in part at least, to physiologic rather than genetic reasons. Furthermore, there were indications that the period during which all backcrosses were

made was favorable for seed setting.

Using the method of path coefficients (Wright, 1934), the coefficient of relationship between the S_1 individual and the non-inbred parental clone during the first backcrossing and that between full sibs may be computed and the two values should be equal. Assuming that a linear relationship exists between fertility and the degree of inbreeding, as previously shown by Fyfe (1957), seed setting in the two systems of mating should be the same. Yet seed setting resulting from back-crossing was higher than that following sib-mating. It should be noted though that in the case of backcrossing the pollen came from a non-inbred. There is reason to believe that the loss of fertility accompanying inbreeding in alfalfa is not only due to the action of the genetically controlled self-incompatibility mechanism but also to a consequent marked re-duction in male potency or general level of vigor.

The possibility of using the backcross progenies espe-cially among those involving self plants whose self-fertility is exceedingly low, as a starting point in an inbreeding pro-gram, deserves further study.

In the light of the preliminary results obtained in the present study, a procedure with respect to inbreeding of alfalfa is presented. A combination of selfing and sib-mating may be adopted in the inbreeding program. The objective would be to isolate reasonably homozygous lines which at the same

time possess some degree of fertility when selfed or sib-mated.
Highly self-fertile non-inbred clones are initially selected
from a population. These clones are selfed and S_1 plants are
grown. The S_1 plants are artificially selfed, assuming that
a procedure for extensive selfing is feasible, and seeds ob-
tained from those with relatively high self-fertility are used
for growing the next generation. Selfing and selection for
high seed setting probably could be continued for a few sub-
sequent generations depending on the self-fertility behavior
of the self progenies. After this point, sib-mating among
members of a line is followed. Sib-mating may be started
early in the first self-generation if further selfing leads
into sterility problems.

The possibility of restoring fertility by making inter-
generation crosses among self plants may be further explored.
This idea is based on the results obtained in the present
backcross study of the low self-fertile groups. Self
progenies in an advanced generation having low cross-fertility
may be crossed to some related high self-fertile plants ob-
tainable in the immediate preceding generation. If this is
economically possible, self-fertility may be restored without
sacrificing much for the loss of homozygosity in the other
characters. Further study is needed along this line.

At the end of the inbreeding program isolated lines must
have a restored fertility when either selfed or sib-mated.

These lines are then tested for combining ability, and those which combine well to give a high level of heterosis could be further evaluated as experimental F_1 hybrids.

The procedures just discussed are applicable only where plants do not exhibit an extreme degree of sterility during the initial stages of inbreeding. In the two families studied, which were derived from clones of low self-fertility, one generation of selfing resulted in a drastic loss of fertility, and sib-mating used as a means of restoring fertility proved to be ineffective. If such is the case, then it would seem that a program of inbreeding would exclude those clones which are low in self-fertility. As previously shown by Wilsie (1951) and McAllister (1950) these may constitute as much as 30 to 40 per cent of a non-inbred population.

SUMMARY AND CONCLUSIONS

A study was undertaken to consider the possibility of using advanced generations of inbred lines of alfalfa for commercial hybrid seed production. Four groups of S_1 plants were used, one of high, one of intermediate, and two of low self-fertility. Selfing, sib-mating and backcrossing of S_1 plants were done concurrently and a comparison of seed setting was made among these systems of mating, on the basis of the number of seed per flower selfed or crossed.

1. There was a marked reduction in self-fertility among the S_1 plants in all groups. Expressed as a percentage of the self-fertility of the non-inbred parental clone, the average self-fertility of the high, intermediate, and two low self-fertility groups was 18.67%, 27.03%, 19.70% and 10.24%, respectively. There was a considerable range in self-fertility within families in the first two groups.

2. Sib-mating was achieved by making cyclic crosses among the S_1 plants. There was a considerable gain in fertility following sib-mating over that of selfing in the high and intermediate fertility groups. Seed setting resulting from sibbing was 2.01 and 4.31 as much as that of selfing in the two respective groups. The gain in fertility obtained in the two low-fertility groups was considered insignificant, for practical purposes.

3. Analysis of variance in the sib-crosses made for the

high and intermediate fertility groups showed statistical differences at the 1 per cent level in seed set among crosses and among cross combinations. There was a wide range of variation in seed setting among the sib-crosses and in some cases it reached the level of cross-fertility of two unrelated non-inbred clones. Factors for fertility were segregating in the S_1 population and seed setting was conditioned by the compatibility relationship of any two self lines. Also, in some instances reciprocal crosses differed significantly in fertility.

4. Results of backcrossing showed statistical differences at the 1 per cent level in cross-fertility among the S_1 plants. There was a consistent increase in seed setting resulting from backcrossing over that of sib-mating in all the groups. Seed setting was even as high, if not higher, than that of the corresponding selfed non-inbred parental clone.

5. The greater fertility obtained following backcrossing over that of sib-mating, despite the fact that the two systems represented the same degree of inbreeding, indicated that loss of fertility accompanying selfing had been the result not only of the operation of the genetic self-incompatibility mechanism but also of the consequent marked reduction in male potency or general level of vigor.

6. Statistically significant correlation values were

obtained either at the 5 or 1 per cent level, except for one, between self-fertility and cross-fertility following sib-mating or backcrossing. The positive association was be-lieved to have been brought about by the inherent differences among the S_1 plants in the production of highly functional gametes, especially in the ovules. The factors involved are thought to be independent of those associated with the mul-tiple oppositional allele series.

7. Consideration is given to the possibility of under-taking an inbreeding program for alfalfa based on techniques which provide a slower rate of inbreeding than that of con-tinued selfing.

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ACKNOWLEDGMENT

APPENDIX

Table 1. Self-fertility of S_1 plants in Group I

Plant number	Number of flowers tripped	Number of pods that developed	Number of seeds that set	Seeds per flower tripped
1	348	146	151	0.434
2	378	24	25	0.066
3	340	140	112	0.329
4	452	257	451	0.998
5	388	173	218	0.562
6	518	331	826	1.594
7	679	205	158	0.233
8	564	118	123	0.218
9	354	211	439	1.240
10	722	112	99	0.137
11	520	203	246	0.473
12	522	205	286	0.548
13	408	191	310	0.759
14	611	131	169	0.276
15	470	53	58	0.123
16	475	141	146	0.307
17	423	109	146	0.345
18	427	166	225	0.527
19	376	210	283	0.753
20	360	204	327	0.908
21	868	77	86	0.099
22	524	111	133	0.254
23	392	124	156	0.398
24	538	310	657	1.221
25	591	170	188	0.318
26	506	237	475	0.939
27	577	133	142	0.246
28	515	163	203	0.394
29	527	226	393	0.745
30	491	123	137	0.279
31	361	104	110	0.305
32	416	159	199	0.478
33	459	52	65	0.142
34	537	194	211	0.393
35	614	55	71	0.116
36	161	84	93	0.578
37	371	96	118	0.318
38	527	374	726	1.378
39	356	43	42	0.118
40	553	124	152	0.275
41	423	93	92	0.217
42	408	81	86	0.210
585 B-2 C1	997	842	2,574	2.582
Average of the S_1 plants				0.482

Table 2. Self-fertility of S_1 plants in Group II

Plant number	Number of flowers tripped	Number of pods that developed	Number of seeds that set	Seeds per flower tripped
101	830	93	87	0.105
102	189	4	2	0.011
103	425	69	72	0.169
104	52	0	0	0.000
105	302	9	10	0.033
106	454	12	9	0.020
107	102	1	2	0.020
108	252	7	5	0.020
109	285	2	2	0.007
110	416	83	142	0.341
111	450	22	22	0.049
112	408	0	0	0.000
113	134	4	4	0.030
114	-	-	-	-
115	-	-	-	-
116	239	4	4	0.017
117	353	11	12	0.034
118	58	0	0	0.000
119	789	154	165	0.209
120	243	0	0	0.000
121	346	3	3	0.009
122	246	21	17	0.069
123	124	0	0	0.000
124	431	35	36	0.084
125	177	6	5	0.028
126	232	13	16	0.069
127	281	29	48	0.171
128	59	0	0	0.000
129	43	1	0	0.023
130	272	0	0	0.000
131	487	17	12	0.025
132	15	1	1	0.067
133	357	14	18	0.050
134	272	30	20	0.074
135	33	1	1	0.030
136	324	15	12	0.037
137	452	17	15	0.033
138	337	16	15	0.044
139	324	43	38	0.117
140	435	18	24	0.055
141	20	0	0	0.000
142	172	0	0	0.000
618 C1	880	295	438	0.498
Average of the S_1 plants				0.051

Table 3. Self-fertility of S_1 plants in Group III

Plant number	Number of flowers tripped	Number of pods that developed	Number of seeds that set	Seeds per flower tripped
201	643	23	30	0.047
202	472	22	11	0.023
203	792	152	168	0.212
204	727	23	20	0.028
205	517	34	31	0.060
206	770	68	81	0.105
207	556	2	2	0.004
208	608	11	6	0.009
209	258	0	0	0.000
210	684	8	8	0.012
211	390	96	114	0.292
212	574	66	83	0.144
213	730	104	138	0.189
214	764	81	87	0.114
215	180	0	0	0.000
216	650	0	0	0.000
217	323	20	25	0.077
218	763	229	308	0.403
219	634	105	156	0.246
220	527	34	35	0.066
221	-	-	-	-
222	596	5	4	0.007
223	609	78	76	0.125
224	267	8	5	0.019
225	577	43	44	0.076
226	600	107	111	0.185
227	544	40	34	0.062
228	587	50	40	0.068
229	707	3	4	0.005
230	511	42	12	0.023
231	664	76	84	0.126
232	599	0	0	0.000
233	712	34	29	0.041
234	583	29	23	0.039
235	841	46	49	0.058
236	650	111	126	0.194
237	524	93	76	0.145
238	674	27	18	0.027
239	403	42	36	0.089
240	758	323	500	0.660
241	429	101	115	0.268
242	451	32	47	0.104
631-101 C1	483	162	260	0.538
Average of the S_1 plants				0.106

Table 4. Self-fertility of S_1 plants in Group IV

Plant number	Number of flowers tripped	Number of pods that developed	Number of seeds that set	Seeds per flower tripped
301	397	78	79	0.199
302	663	62	78	0.118
303	548	157	102	0.186
304	306	185	315	1.029
305	742	109	121	0.163
306	394	27	20	0.051
307	341	53	55	0.161
308	84	16	19	0.226
309	416	65	74	0.178
310	379	46	49	0.129
311	755	185	233	0.309
312	530	49	49	0.092
313	693	62	65	0.094
314	519	16	12	0.023
315	387	168	273	0.705
316	509	257	475	0.933
317	386	128	147	0.381
318	269	62	75	0.279
319	667	80	85	0.127
320	765	196	222	0.290
321	375	94	77	0.205
322	282	19	15	0.053
323	717	346	488	0.681
324	515	10	9	0.017
325	598	77	65	0.109
326	620	68	84	0.135
327	615	139	173	0.281
328	495	80	76	0.153
329	493	32	29	0.059
330	707	45	44	0.062
331	476	57	65	0.136
332	424	62	68	0.160
333	350	56	43	0.123
334	561	40	33	0.059
335	738	143	214	0.290
336	727	204	166	0.228
337	451	171	240	0.532
338	394	65	76	0.192
339	385	123	138	0.358
340	227	14	18	0.079
341	468	52	66	0.141
342	561	210	328	0.585
5 C1	499	227	454	0.910
Average of the S_1 plants				0.246

Table 5. Seed set data (per 10 flowers) for cyclic
sib-crosses among S_1 plants of Group I

Sib-crosses	Replication				Average number of seed per flower
	I	II	III	IV	
1 x 42	1	3	0	1	0.125
1 x 2	3	2	2	3	0.250
2 x 1	11	8	2	6	0.675
2 x 3	6	1	2	2	0.275
3 x 2	2	6	3	4	0.375
3 x 4	1	12	19	8	1.000
4 x 3	4	8	8	7	0.675
4 x 5	8	19	34	19	2.000
5 x 4	28	20	15	26	2.225
5 x 6	11	21	12	8	1.300
6 x 5	17	14	26	22	1.975
6 x 7	3	7	10	7	0.675
7 x 6	4	6	6	8	0.600
7 x 8	3	4	4	5	0.400
8 x 7	2	5	6	6	0.475
8 x 9	5	6	6	8	0.625
9 x 8	5	14	7	11	0.925
9 x 10	7	15	7	10	0.975
10 x 9	23	7	12	7	1.225
10 x 11	9	16	6	8	0.975
11 x 10	10	6	6	14	0.900
11 x 12	6	14	21	15	1.400
12 x 11	5	11	1	11	0.700
12 x 13	5	2	4	6	0.425
13 x 12	13	17	9	14	1.325
13 x 14	9	17	20	13	1.475
14 x 13	10	19	14	7	1.250
14 x 15	2	2	5	1	0.250
15 x 14	9	7	8	3	0.675
15 x 16	17	22	12	10	1.525
16 x 15	11	16	8	10	1.125
16 x 17	12	3	11	10	0.900
17 x 16	10	8	9	9	0.900
17 x 18	19	8	12	16	1.375
18 x 17	23	24	26	15	2.200
18 x 19	11	15	19	13	1.450
19 x 18	9	2	12	2	0.625
19 x 20	11	13	10	13	1.175
20 x 19	12	11	16	17	1.400
20 x 21	4	11	18	10	1.075
21 x 20	8	9	9	10	0.900

Table 5 (Continued).

| Sib-crosses | Replication | | | | Average number of seed per flower |
	I	II	III	IV	
21 x 22	5	5	11	14	0.875
22 x 21	8	6	3	10	0.675
22 x 23	17	6	7	10	1.000
23 x 22	5	6	15	4	0.750
23 x 24	9	8	10	16	1.075
24 x 23	6	3	3	6	0.450
24 x 25	8	3	10	8	0.725
25 x 24	6	13	12	11	1.050
25 x 26	12	13	11	14	1.250
26 x 25	6	5	4	3	0.450
26 x 27	13	14	12	15	1.350
27 x 26	18	11	9	9	1.175
27 x 28	11	7	15	5	0.950
28 x 27	8	4	7	6	0.625
28 x 29	16	11	15	25	1.675
29 x 28	25	14	34	29	2.550
29 x 30	20	6	8	9	1.075
30 x 29	18	7	7	24	1.400
30 x 31	19	14	16	23	1.800
31 x 30	2	1	6	5	0.350
31 x 32	2	0	8	3	0.325
32 x 31	9	3	10	1	0.575
32 x 33	4	5	12	11	0.800
33 x 32	1	0	5	2	0.200
33 x 34	3	4	6	1	0.350
34 x 33	7	6	9	0	0.550
34 x 35	9	4	10	4	0.675
35 x 34	9	4	4	7	0.600
35 x 36	10	18	7	9	1.100
36 x 35	17	23	12	7	1.475
36 x 37	24	16	10	13	1.575
37 x 36	14	20	17	8	1.475
37 x 38	9	12	14	8	1.075
38 x 37	19	12	12	10	1.325
38 x 39	13	12	15	11	1.275
39 x 38	7	7	12	9	0.875
39 x 40	5	3	17	8	0.825
40 x 39	5	13	4	4	0.650
40 x 41	8	0	18	11	0.925
41 x 40	13	6	13	7	0.975
41 x 42	4	5	10	0	0.475

Table 5 (Continued).

Sib-crosses	Replication				Average number of seed per flower
	I	II	III	IV	
42 x 41	10	7	10	9	0.900
42 x 1	4	6	6	1	0.425
592 A-3 x 652-25	30	19	16	21	2.150
652-25 x 592 A-3	22	33	52	37	3.600
Replication average	9.988	9.605	11.174	9.802	
Average of the sib-crosses					0.970
L.S.D. (5%) =					0.620
L.S.D. (1%) =					0.815

Table 6. Seed set data (per 10 flowers) for cyclic sib-
crosses among S_1 plants of Group II

| Sib-crosses | Replication | | Average number of seed per flower |
	I	II	
101 x 142	-	0	.000
101 x 102	3	4	.350
102 x 101	0	4	.200
102 x 103	3	2	.250
103 x 102	0	6	.300
103 x 104	0	4	.200
104 x 103	0	0	.000
104 x 105	0	0	.000
105 x 104	-	0	.000
105 x 106	0	0	.000
106 x 105	1	4	.250
106 x 107	0	4	.200
107 x 106	0	0	.000
107 x 108	0	0	.000
108 x 107	0	0	.000
108 x 109	2	1	.150
109 x 108	0	0	.000
109 x 110	1	5	.300
110 x 109	3	11	.700
110 x 111	0	3	.150
111 x 110	3	6	.450
111 x 112	-	0	.000
112 x 111	0	1	.050
112 x 113	0	0	.000
113 x 112	-	0	.000
113 x 114	0	-	.000
114 x 113	0	-	.000
114 x 115	0	-	.000
115 x 114	0	-	.000
115 x 116	0	0	.000
116 x 115	0	1	.050
116 x 117	0	0	.000
117 x 116	3	2	.250
117 x 118	-	3	.300
118 x 117	0	2	.100
118 x 119	2	1	.150
119 x 118	-	12	1.200
119 x 120	-	2	.200
120 x 119	0	0	.000
120 x 121	0	0	.000
121 x 120	-	0	.000
121 x 122	0	3	.150
122 x 121	1	6	.350
122 x 123	-	0	.000

Table 6 (Continued).

Sib-crosses	Replication		Average number of seed per flower
	I	II	
123 x 122	0	0	.000
123 x 124	0	0	.000
124 x 123	-	0	.000
124 x 125	0	4	.200
125 x 124	1	1	.100
125 x 126	2	3	.250
126 x 125	0	2	.100
126 x 127	4	3	.350
127 x 126	-	4	.400
127 x 128	-	1	.100
128 x 127	-	0	.000
128 x 129	-	0	.000
129 x 128	-	0	.000
129 x 130	-	0	.000
130 x 129	-	0	.000
130 x 131	0	0	.000
131 x 130	-	0	.000
131 x 132	0	0	.000
132 x 131	1	0	.050
132 x 133	0	2	.100
133 x 132	0	0	.000
133 x 134	4	3	.350
134 x 133	1	1	.100
134 x 135	2	4	.300
135 x 134	1	4	.250
135 x 136	1	-	.100
136 x 135	0	-	.000
136 x 137	0	0	.000
137 x 136	0	0	.000
137 x 138	0	1	.050
138 x 137	2	6	.400
138 x 139	1	5	.300
139 x 138	0	2	.100
139 x 140	5	1	.300
140 x 139	1	11	.600
140 x 141	-	0	.000
141 x 140	0	0	.000
141 x 142	-	0	.000
142 x 141	-	0	.000
142 x 101	1	4	.250
5 Cl x 618 Cl	51	70	6.050
618 Cl x 5 Cl	23	29	2.600
Average of sib-crosses			0.140

Table 7. Seed set data (per 10 flowers) for cyclic sib-
crosses among S_1 plants of Group III

Sib-crosses	Replication		Average number of seed per flower
	I	II	
201 x 242	0	2	.100
201 x 202	0	0	.000
202 x 201	0	0	.000
202 x 203	2	0	.100
203 x 202	2	0	.100
203 x 204	0	3	.150
204 x 203	23	6	1.450
204 x 205	0	7	.350
205 x 204	0	1	.050
205 x 206	6	8	.700
206 x 205	0	1	.050
206 x 207	-	-	-
207 x 206	4	-	.400
207 x 208	2	1	.150
208 x 207	0	0	.000
208 x 209	0	1	.050
209 x 208	2	0	.100
209 x 210	3	1	.200
210 x 209	0	0	.000
210 x 211	8	0	.400
211 x 210	0	5	.250
211 x 212	2	3	.250
212 x 211	11	4	.750
212 x 213	8	3	.550
213 x 212	0	3	.150
213 x 214	7	20	1.350
214 x 213	6	12	.900
214 x 215	-	-	-
215 x 214	3	-	.300
215 x 216	5	-	.500
216 x 215	-	-	-
216 x 217	0	0	.000
217 x 216	0	0	.000
217 x 218	2	0	.100
218 x 217	0	2	.100
218 x 219	6	4	.500
219 x 218	0	3	.150
219 x 220	0	9	.450
220 x 219	2	3	.250
220 x 221	0	-	.000
221 x 220	-	-	-
221 x 222	-	-	-

Table 7 (Continued).

Sib-crosses	Replication		Average number of seed per flower
	I	II	
222 x 221	1	-	.100
222 x 223	8	-	.800
223 x 222	-	-	-
223 x 224	0	5	.250
224 x 223	0	0	.000
224 x 225	0	0	.000
225 x 224	0	2	.100
225 x 226	0	2	.100
226 x 225	0	2	.100
226 x 227	0	1	.050
227 x 226	0	0	.000
227 x 228	0	1	.050
228 x 227	0	1	.050
228 x 229	0	4	.200
229 x 228	0	0	.000
229 x 230	0	4	.200
230 x 229	0	0	.000
230 x 331	0	0	.000
231 x 230	4	1	.250
231 x 232	-	0	.000
232 x 231	3	2	.250
232 x 233	2	4	.300
233 x 232	-	0	.000
233 x 234	0	0	.000
234 x 233	5	0	.250
234 x 235	9	2	.550
235 x 234	2	1	.150
235 x 236	2	6	.400
236 x 235	0	2	.100
236 x 237	4	8	.600
237 x 236	3	1	.200
237 x 238	0	15	.750
238 x 237	1	3	.200
238 x 239	2	1	.150
239 x 238	-	5	.500
239 x 240	4	6	.500
240 x 239	6	13	.950
240 x 241	12	-	1.200
241 x 240	2	-	.200
241 x 242	7	-	.700
242 x 241	0	-	.000
242 x 201	0	0	.000
Average of the sib-crosses			.262

Table 8. Seed set data (per 10 flowers) for cyclic
sib-crosses among S_1 plants of Group IV

Sib-crosses	Replication				Average number of seed per flower
	I	II	III	IV	
301 x 342	5	9	31	12	1.425
301 x 302	6	8	39	12	1.625
302 x 301	16	7	18	17	1.450
302 x 303	12	0	15	14	1.025
303 x 302	5	7	38	16	1.650
303 x 304	8	19	42	30	2.475
304 x 303	15	11	37	41	2.600
304 x 305	25	6	24	13	1.700
305 x 304	1	4	16	9	0.750
305 x 306	1	0	2	1	0.100
306 x 305	5	1	5	3	0.350
306 x 307	1	5	11	7	0.600
307 x 306	1	6	0	2	0.225
307 x 308	0	0	7	5	0.300
308 x 307	2	6	10	6	0.600
308 x 309	1	2	8	9	0.500
309 x 308	2	2	10	4	0.450
309 x 310	2	8	5	20	0.875
310 x 309	3	4	10	11	0.700
310 x 311	11[a]	3	9	41	1.600
311 x 310	7	13	21	16	1.425
311 x 312	23	11	32	26	2.300
312 x 311	4	0	29	29	1.550
312 x 313	2	0	1	4	0.175
313 x 312	1	2	1	1	0.125
313 x 314	0	6	0	14	0.500
314 x 313	3	1	18	8	0.750
314 x 315	11	6	22	18	1.425
315 x 314	9	7[a]	16	16	1.200
315 x 316	10	15[a]	26	27	1.950
316 x 315	23	5	44	48	3.000
316 x 317	6	2	3	19	0.750
317 x 316	2	4	8	14	0.700
317 x 318	2	5	7	5	0.475
318 x 317	0	10	12	18	1.000
318 x 319	5	3	4	5	0.425
319 x 318	0	7	13	9	0.725

[a]Computed

Table 8 (Continued).

Sib-crosses	Replication				Average number of seed per flower
	I	II	III	IV	
319 x 320	2	1	8	7	0.450
320 x 319	2	6	15	10	0.825
320 x 321	15	7	11	21	1.350
321 x 320	1	4	6	3	0.350
321 x 322	14	2	18	21	1.375
322 x 321	6a	7	16	16	1.125
322 x 323	4	4	5	4	0.425
323 x 322	5	3	8	4	0.500
323 x 324	2	1	15	12	0.750
324 x 323	0	0	17	8	0.625
324 x 325	1	0	11	6	0.450
325 x 324	2	1	4	0	0.175
325 x 326	3	4	14	7	0.700
326 x 325	7	0	6	9	0.550
326 x 327	2	1	5	8	0.400
327 x 326	14	9	12	21	1.400
327 x 328	1	29	30	28	2.200
328 x 327	7	12	23	16	1.450
328 x 329	5	13	20	30	1.700
329 x 328	14	20a	35	30	2.475
329 x 330	2	5	10	4	0.525
330 x 329	0	1	2	2	0.125
330 x 331	2	3	0	1	0.150
331 x 330	0	1	1	1	0.075
331 x 332	1	3	12	10	0.650
332 x 331	1	7	8	6	0.550
332 x 333	10	13	26	34	2.075
333 x 332	5	1	22	31	1.475
333 x 334	6	6	18	32	1.550
334 x 333	3	3	14	22	1.050
334 x 335	4	9	9	9	0.775
335 x 334	6	5	7	8	0.650
335 x 336	5	2	30	34	1.775
336 x 335	8	7	20	15	1.250
336 x 337	15	8	30	30	2.075
337 x 336	15	10	40	30	2.375
337 x 338	16	27	41	35	2.975
338 x 337	19	42	33	33	3.175
338 x 339	1	4	11	1	0.425
339 x 338	0	3	22	11	0.900
339 x 340	13	6	29	22	1.750

Table 8 (Continued).

Sib-crosses	Replication				Average number of seed per flower
	I	II	III	IV	
340 x 339	4	4	9	1	0.450
340 x 341	1	5	5	8	0.475
341 x 340	2	2	7	11	0.550
341 x 342	2	2	4	4	0.300
342 x 341	4	5	12	17	0.950
342 x 301	4	5	21	21	1.275
652-25 Cl x 5 Cl	6	8	54	60	3.200
5 Cl x 652-25 Cl	42	38	67	68	5.375
Replication average	6.210	6.560	16.71	15.95	
Average of the sib-crosses					1.061
L.S.D. (5%) =					0.939
L.S.D. (1%) =					1.234

Table 9. Seed set data (per 10 flowers) of S_1 plants in Group I after backcrossing to the S_o parental clone

S_1 female parent	Replication				Average number of seed per flower
	I	II	III	IV	
Backcrossed to 585 B-2 Cl					
1	21	17[a]	24	17	1.975
2	42	44	44	38	4.200
3	9	20	21	25	1.875
4	40	37	35	45	3.925
5	34	36	32	33	3.375
6	38	32	45	31	3.650
7	10	5	11	12	0.950
8	27	25	25	38	2.875
9	36[a]	32	35	40	3.575
10	25	29	36	31	3.025
11	36	21	36	28	3.025
12	30	17	22	12	2.025
13	43	38	42	43	4.150
14	28	26	31	38	3.075
15	30	27	28	39	3.100
16	31	18	20	28	2.425
17	28	27	27	25	2.675
18	42	36	39	41	3.950
19	30	27	18	23	2.450
20	53	38	40	38	4.225
21	34	34	38	43	3.725
22	31	33	28	33	3.125
23	15	19	20	21	1.875
24	31	23	33	26	2.825
25	6	17	23	18	1.600
26	37	34	38	42	3.775
27	37	23	32	32	3.100
28	23	23	31	38	2.875
29	41	40	36	42	3.975
30	31	43	33	42	3.725
32	46	39	49	53	4.675

[a]Computed

Table 9 (Continued).

S₁ female parent	Replication				Average number of seed per flower
	I	II	III	IV	
33	17	16	18	17	1.700
34	31	30	20	26	2.675
35	30	27	38	27	3.050
37	39	28	20	38	3.125
38	39	32	41	38	3.750
39	29	21	25	28	2.575
40	19	24	35	31	2.725
41	27	21	25	34	2.675
42	25	25	30	17	2.425
Replication average	3.052	2.760	3.060	3.178	
General average					3.012
L.S.D. (5%) =					0.692
L.S.D. (1%) =					0.916

where S_1 female parent.

Table 10. Seed set data of S_1 plants in Group II after backcrossing to the S_0 parental clone

S_1 female parent	Average number of seed per flower
	Backcrossed to 618 Cl
101	0.7
102	0.0
103	1.6
105	0.3
106	1.0
107	0.2
108	1.1
109	0.8
110	1.5
111	1.2
112	0.2
113	1.0
115	1.2
116	0.3
117	2.1
118	0.0
119	2.1
120	0.0
121	1.7
122	1.4
123	0.0
124	1.9
125	0.4
126	0.9
127	0.9
128	0.0
129	0.8
130	0.0
131	0.6
132	0.1
133	0.4
134	1.6
135	1.0
136	0.4
137	0.8
139	1.0
140	0.9
142	0.8
Average	0.813

Table 11. Seed set data (per 10 flowers) of S_1 plants in
Group III after backcrossing to the S_0 parental
clone

S_1 female parent	Replication				Average number of seed per flower
	I	II	III	IV	
Backcrossed to 631-101 Cl					
201	9	17	14	33	1.825
202	11	9	5	21	1.150
203	33	22	11	11	1.925
204	35	29	15	15	2.350
205	43	24	23	30	3.000
206	13	13	9	16	1.275
207	7	6	7	10	.750
208	11	13	20	15	1.475
209	15	9	12	15	1.275
210	13	10	12	20	1.375
211	34	35	33	33	3.375
212	17	39	35	25	2.900
213	7	23	27	28	2.125
214	25	31	38	22	2.900
216	8	8	9	13	0.950
217	18	34	34	26	2.800
218	28	30	33	26	2.925
219	23	25	32	30	2.750
220	32	37	38	36	3.575
222	10	14	29	21	1.850
223	15	20	12	22	1.725
224	4	11	21	14	1.250
225	15	18	13	10	1.400
226	30	28	20	30	2.700
227	15	30	25	22	2.300
228	18	15	17	16	1.650
229	20	21	13	9	1.575
230	5	10	4	9	0.700
231	34	32	32	30	3.200
232	33	28	33	38	3.300
233	14	6	13	13	1.150
234	21	27	23	25[a]	2.400

[a]Computed

Table 11 (Continued).

S_1 female parent	Replication				Average number of seed per flower
	I	II	III	IV	
235	28	24	28	28	2.700
236	30	19	24	33	2.650
237	16	19	16	26	1.925
238	21	29	7	16	1.825
239	20	12	13	22	1.675
240	34	45	33	33	3.625
241	26	22	15	18	2.025
242	23	33	31	29	2.900
Replication average	2.035	2.192	2.072	2.222	
General average					2.131
L.S.D. (5%) =					0.831
L.S.D. (1%) =					1.100

Table 12. Seed set data (per 10 flowers) of S_1 plants in Group IV after backcrossing to the S_0 parental clone

S_1 female parent	Replication				Average number of seed per flower
	I	II	III	IV	
		Backcrossed	to 5 Cl		
301	40	32	30	43	3.625
302	16	10	23	26	1.875
303	24	37	46	48	3.875
304	35	19	24	34	2.800
305	17	10	20	27	1.850
306	16	7	21	22	1.650
307	14	9	18	22	1.575
309	23	25	27	30	2.625
310	17	14	30	24	2.125
311	49	36	47	45	4.425
312	17	8	32	29	2.150
313	11	21	13	21	1.650
314	20	57	42	23	3.550
315	52	44	61	49	5.150
316	25	19	35	27	2.650
317	15	17	32	22	2.150
318	33	33	42	43	3.775
319	24	25	12	21	2.050
320	24	43	42	44	3.825
321	46	55	66	56	5.575
323	29	28	29	26	2.800
324	14	11	10	3	0.950
325	14	4	15	19	1.300
326	35	33	29	34	3.275
327	34	19	24	24	2.525
328	34	39	35	57	4.125
329	22	14	17	16	1.725
330	18	15	16	18	1.675
331	27	15	21	26	2.225
332	28	19	25	30	2.550
333	43	40	48	41	4.300
334	15	16	17	24	1.800
335	36	23	37	30	3.150
336	34	33	27	30	3.100
337	71	47	54	64	5.900
338	24	20	34	21	2.475

Table 12 (Continued).

S$_1$ female parent	Replication				Average number of seed per flower
	I	II	III	IV	
339	19	21	33	27	2.500
340	9	15	25	28	1.925
341	26	22	25	34	2.675
342	17	19	19	17	1.800
Replication average	2.668	2.435	3.008	3.062	
General average					2.793
L.S.D. (5%) =					0.908
L.S.D. (1%) =					1.202