

BREEDING AND GENETICS

Development of Random-mated Populations Using Bulked Pollen Methodology: Cotton as a Model

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ABSTRACT

Random mating has been used successfully to break linkages in cross-pollinated crops. In self-pollinated crops, however, the requirements for cross-pollination can markedly influence the feasibility of random mating. The objective of this study was to evaluate Miravalle's bulked-pollen method for its ability to simulate random mating in cotton (*Gossypium hirsutum* L.). Pollen from a selection from Auburn G1-213 glandless (gl_2gl_2 , gl_3gl_3) and 'TM-1' glanded (GL_2GL_2 , GL_3GL_3) cottons were mixed at five different proportions of blooms (1:13, 1:26, 1:39, 1:52, and 1:65) from the two genotypes to serve as a model for Miravalle's bulked-pollen method in upland cotton. Observed progeny genotypic ratios were nearly as expected except for the 1:26 treatment, which had an excess of glandless progeny. Crosses containing 'Stoneville 825' nectariless (ne_1ne_1 , ne_2ne_2) developed with this methodology were also assayed for the nectariless trait, and the trait segregated as expected. Combining the glanded data resulted in the expected segregation. Cotton pollen mixed well and the final boll set was excellent. The results of this study indicate that the bulked-pollen methodology can be used to develop a random-mated population.

Improving yield and quality are important objectives for crop breeders. In cotton (*Gossypium spp.*), however, yield increases are often associated

with reduced fiber quality. For example, lint yield is negatively correlated with fiber qualities of strength and length within upland cotton (Meredith, 1984). The negative association between yield and fiber quality has hampered breeding efforts for the improvement of multiple traits. These linkages between desirable and undesirable loci can slow down genetic progress through traditional breeding programs, such as selfing and selection. It is desirable to break the linkage blocks in cotton that associate undesirable traits with high yield and quality.

Random-mating procedures have provided an important means to effectively break the negative associations between yield and quality in several self-pollinated crops. Humphrey et al. (1969) developed an eight-line synthetic population by random mating in tobacco (*Nicotiana tabacum* L.). Genetic male sterility, as well as cytoplasmic male sterility, was used in sorghum (*Sorghum bicolor* L. Moench) to develop three random-mating populations that had desirable recombinant traits (Nordquist et al., 1973). Genetic male sterility was used in the development of random-mating populations in soybean (*Glycine max* L. Merr.) by allowing natural hybridization for seven generations without selection (Burton and Brim, 1981). Frey and Holland (1999) reported conducting nine cycles of recurrent selection in oat using hand pollinations to achieve random mating of selected lines in each cycle. Random mating has also been successfully used in cotton (*Gossypium hirsutum* L.) to break linkages between fiber strength and yield with correlations improving from -0.34 to 0.05 (Miller and Rawlings, 1967). Hybridization of two parents followed by five generations of intercrossing via insects improved yield by 9% and maintained fiber strength. Only two cycles of inter-mating were used to reduce the correlation between lint yield and fiber strength from -0.54 to -0.38 (Meredith and Bridge, 1971).

Random-mating has also been successfully used in cross-pollinated crops. Covarrubias-Prieto (1987) evaluated the effectiveness of random-mating methodology for increasing genetic variability in populations formed from either related or unrelated single crosses in maize (*Zea mays* L.). These stud-

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ies indicate the feasibility of using random-mating procedures in the improvement of two traits which are negatively correlated.

In most cases, random-mating schemes have involved hand pollinations. When few parents are used, hand pollinations may be adequate to simulate random mating; however, hand pollinations become time consuming and expensive with more than three parents. Miravalle (1964) proposed a bulked-pollen method for inter-mating cotton populations and described the method using four different cotton strains, but the randomness of using his method was not investigated. A random-mating population involving a large number of parents can provide a better chance to combine desirable traits for selection, so it is important to determine the sample size or the number of crosses required to maintain representation in a random-mating population.

In this study, the influence of sample size and pollen combination on randomness and accuracy of Miravalle's bulked-pollen method was investigated using cotton as a model. This research provides information on the use of a bulked-pollen technique to develop a random-mating population for both self-pollinated and cross-pollinated crops.

MATERIAL AND METHODS

Glandless and nectariless are two important phenotypic markers in cotton and are controlled by recessive genes. Glandless is controlled by two recessive genes gl_2gl_2 , gl_3gl_3 and can be easily observed in seeds, which can be used to detect the pollen segregation ratio. The nectariless trait is controlled by the duplicated recessive genes ne_1ne_1 ne_2ne_2 and is observable in early stages of plant development (Meyer and Meyer, 1961). An expedient way to determine if a cotton seed is the result of a hybrid is to use pollen from glanded plants to fertilize glandless plants.

Field experiments were conducted at the R. R. Foil Plant Science Research Center, Mississippi State, MS. In 2002, a glandless parent, a selection from Auburn G1-213 (gl_2gl_2 , gl_3gl_3) (Shepherd, 1982), was used as a female parent. The male parent was mixed pollen from both the glandless plant and the glanded parent, 'Texas Marker-1' (GL_2GL_2 , GL_2GL_2) (Texas Agricultural Experiment Station; College Station, TX) (Kohel et al., 1970). The bulked pollen ratios were 1:13, 1:26, 1:39, 1:52, and 1:65 (glandless/glanded). Five different ratios were used to consider various parent population sizes.

The bulked-pollen mixtures were created based on the technique described by Miravalle (1964). Unopened buds from glandless and glanded parents were covered with cloth bags the day before anthesis. In addition, unopened buds from the glandless parent were emasculated and the stigma covered with a straw in order to be used as females the following day. The covered buds were collected at 8:00 a.m., separated, and allowed to open under the sun. At 10:00 a.m. pollen was collected by inverting the flower in a 250 mL beaker and gently tapping it. The number of flowers used per beaker was based on the previously described ratios. After tapping the necessary number of flowers, the beaker was covered with aluminum foil and a small hole was punched in it. An empty plastic wash bottle was used to mix the pollen by blowing air into the beaker through the punched hole. After mixing the pollen, it was transferred to a small vial using a glass funnel. Pollen was applied to the previously emasculated glandless flowers by gently introducing the stigma into the vial of pollen and rolling the pollen into the stigma. A straw was used to cover the pollinated stigma and the flower was labeled with the date and pollen ratio.

Bolls from each cross were harvested, counted, and separated by ratio. Each boll was ginned by hand to remove lint from the seed. To evaluate the resultant crosses, each seed was cut to visually check for the presence of gossypol glands. A chi-square goodness-of-fit test was applied to the observed data to verify the resultant ratios.

This bulked-pollen methodology was used to develop a random-mating population from an 11-parent half diallel. F_1 crosses were made by hand emasculating and pollinating in 2002. At least 30 plants for each of the 55 F_1 populations were grown and random mated in the winter nursery at Tecomán, Colima, Mexico, during the 2002-2003 season. Equal numbers of flowers were collected from each of the 55 F_1 populations (pollen was collected and mixed) and emasculated flowers from each F_1 cross were pollinated. The procedure was repeated each day until at least 75 flowers had been pollinated from each cross. One of the parents, 'Stoneville 825' (Stoneville Pedigreed Seed) is nectariless, (ne_1ne_1 , ne_2ne_2). Seed from each of the 55 Cycle-0 (C_0) populations were grown in summer 2003. Because 10 of the 55 C_0 populations involved Stoneville 825 (nectariless), plants in the C_0 populations were scored for the presence of the nectariless trait. Based on the double recessive nature of the nectariless trait (Holder et al., 1968), one out of 88 plants evaluated was expected to be nectariless ($1/16 \times 10/55=1/88$).

RESULTS

Boll retention ranged from 76 to 89 % (Table 1), which is typical for average growing conditions without insect pressure. Average boll set (82%) was less than that reported by Miravalle (1964), but he used gibberellic acid to improve boll set after emasculation (Brown and Lee, 1976). The number of seeds per boll averaged 28, which was two less than reported by Miravalle (1964). Cole (2003) reported that the number of seed set per boll after self-pollination of commercial cotton cultivars usually ranged from 30 to 37 seeds per boll.

Table 1. Number of crosses, percentage of boll retention, and mean seed boll⁻¹ obtained from bulked pollen mixtures of five different ratios of glanded (GL) and glandless (gl) pollen

Ratio (gl:GL)	Number of crosses	Boll set (%)	Mean seed boll ⁻¹
1:13	37	76	28
1:26	64	86	29
1:39	37	81	28
1:52	59	76	27
1:65	63	89	29
Combined	260	82	28

Since both bulked glanded (dominant) and glandless (recessive) plants were used to pollinate the glandless parent, the collected bolls contained both glanded and glandless seeds. The number of seeds for the two types of plants would be expected to reflect the ratio of the two pollen sources used to make each bulk pollen sample. The results fit the expected ratios except for the treatment with a 1:26 ratio of pollen (Table 2). More glandless seeds were produced than expected in the 1:26 ratio. When large

number of seed were classified as in the total seed set, the data fit the expected ratio (5775:5779 and 186:181) (probability > F = 0.11; Table 2).

In the 11 parent experiment, 12 plants with the homozygous nectariless types (*ne₁ne₁, ne₂ne₂*) were observed and 7.5 were expected. After one cycle of random mating, 649 nectaried (*Ne₁Ne₂*) plants were observed and 653.5 were expected. The observed number fit the expected ratio of 1 out of 16 ($\chi^2 = 2.71$; probability > F = 0.10).

Miravalle (1964) did not report any segregation ratios to validate the methodology. The results of this study support the use of bulked-pollen method proposed by Miravalle's (1964) for the development of a random-mating population.

DISCUSSION

Random mating has been successfully used to break the negative associations between important traits in both self- and cross-pollinated crops. The theoretical concerns that should be addressed so that the proposed inter-mated breeding program can be effectively used are as follows: (1) the minimum sample size that can provide the randomness of a certain allele in a random-mating population, (2) a method to assure the randomization of the pollen from several sources, (3) the number of parents to be used in the development of a random-mating population, and (4) the number of generations of inter-mating needed to break linkages. Data from this study shows that a large population (over 2000) is needed to assure random assortment of gametes. This is relatively easy to achieve in breeding programs when hundreds of pollinations are made. A minimum of 75 pollinations per line were used in this study, and with 28 seed per boll around 2000 seed were created per genetic cross. The results from observing the recessive phenotypic

Table 2. Observed and expected numbers of glanded (GL) and glandless (gl) seeds from bulked pollen mixtures of glanded and glandless pollen

Ratio (gl:GL)	GL		gl		χ^2	Prob. > F
	Observed	Expected	Observed	Expected		
1:13	714	721.50	63	55.50	1.09	0.31
1:26	1502	1523.40	80	58.60	8.12	0.004
1:39	821	811.20	11	20.80	4.74	0.03
1:52	1195	1185.20	13	22.80	4.29	0.04
1:65	1543	1538.33	19	23.67	0.93	0.27
Combined	5775	5779.63	186	181.37	0.12	0.11

markers in upland cotton in this study (glandless and nectariless) addressed the second concern and revealed that it is possible to effectively randomize pollen. Since a limited number of genotypes were used, this study did not address the importance of genotypes and the environment. Some genotypes may carry genetic factors interfering with randomness in fertilization. The genotypes in the large random-mating population represent germplasm from the Acala, Texas High Plains, Delta, and Eastern cotton gene pools. Although the 11 parents were not an extensive sample, it fairly represents these gene pools. One can be reasonably certain that the techniques used in this study should simulate random mating in an upland population. The environment is another issue. In the second study, crosses were made both in Mississippi and Mexico at different times of the year, and significant variance from the expected should have been revealed but were not.

The number of parents used in the development of a random-mated population is not as critical for recombination purposes other than insuring genetic variability. Hanson (1959b) suggested using at least four parents in a random-mating population to increase genetic potential over the traditional use of two parents. Reducing the expected portion of the progenitor chromosome is more effective through additional generations of inter-mating rather than additional parents, and this occurs primarily in the first four to five generations (Hanson, 1959a). He suggested using three or four generations of inter-mating in self-pollinated species. Breaking linkages is dependent on the length of chromosomes, the number of linked genes between the two traits of interest, how tightly the genes are linked, and the number of generations of inter-mating.

The ultimate goal of a random-mating scheme in any self-pollinated species is the development of inbred lines. In the process of inbreeding, recombination occurs to the extent that this process should be considered when deriving a plan for random-mating populations. Hanson (1959a) showed that continued reduction in size of linkage block does occur during selfing. In a system where there is a combination of selfing and inter-mating, recombination is reduced although not significantly. Miller and Rawlings (1967) reported that a combination of 50% selfing and 50% random mating only reduced the effective recombination by 17% compared with complete random mating. In the current random-mating population

using 11 parents, it appears that four generations of inter-mating followed by selfing should suffice to bring about increased recombination.

Miravalle (1964) developed the bulked-pollen methodology to extend the use of parental lines in development of a random-mating population, but the randomness of his method could not be evaluated in his experiment. In this study, cotton was used as a model with two recessive phenotypic markers to evaluate his methods through different pollen ratios.

In summary, results of this study substantiate the use of pollen mixtures and bulk pollination to achieve random-mating in cotton, as proposed by Miravalle.

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