Chloroplast DNA evidence for non-random selection of females in an outcrossed population of soybeans \([\text{Glycine max (L.)}]^*\)

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Summary. Restriction fragment length polymorphisms (RFLPs) were used to assess chloroplast DNA (cpDNA) variation in a population of soybeans subjected to continuous cycles of forced outcrossing. This population was derived by crossing 39 female lines with four male-sterile (Ms2ms2) maintainer lines and advancing each generation by selecting only outcrossed seed borne on male-sterile (ms2ms2) plants. Analysis of the original 39 female lines revealed three groups based on cpDNA RFLPs. These three groups had been previously documented in soybeans, and the distribution of these groups among the female parents of this population was similar to that observed in germ plasm surveys of soybean. Thirty-four of the female parents had group I cpDNA, 3 had group II, and 2 had group III. Plants collected from this population after seven cycles of outcrossing were scored for four morphological traits (flower color, pubescence color, seed color, and pubescence type) known to be controlled by alleles at single nuclear loci. The frequencies of the phenotypes observed in this study indicated that the population underwent random mating with respect to flower and pubescence color, but deviated from random mating at the other two loci. Analysis of 158 of these same plants collected from the population after seven cycles of outcrossing revealed no individuals with group II or group III cpDNAs. The fixation of the group I cpDNA marker in this outcrossing population was judged to result primarily from selection against individuals in the population with the rare cpDNAs.

Key words: Glycine max – Chloroplast DNA – RFLP – Population – Male-sterile

Introduction

Population improvement in self-pollinated crop species such as soybeans (\text{Glycine max}) and barley (\text{Hordeum vulgare}) can be facilitated with the use of male sterility (Brim and Stuber 1973; St. Martin 1981; Suneson and Ramage 1963). The segregation of genetic male sterility in a population of ordinarily autogamous individuals provides a means for producing allogamous individuals because seeds produced on male-sterile (MS) segregants are the result of cross-fertilization events. New genetic combinations can therefore be generated without the labor-intensive task of performing hand emasculations and pollinations.

The value of male-sterile-facilitated population improvement methods may be limited by various short-term and long-term effects of the mating system on variation in the population (Jain 1969). In barley populations segregating for male sterility, natural selection changed the frequency of alleles at loci specific for isozymes and seed storage proteins (Alexander et al. 1990). Soybean populations segregating for male sterility have undergone successful recurrent selection for increased seed yield (Nelson 1987; Burton et al. 1990), changes in seed composition (Burton and Brim 1981), and other quantitative traits. Little is understood, however, about the effect of selection for genetic male sterility per se on the frequencies of specific alleles controlling other traits in soybeans. Selection for male sterility could affect allele frequencies at other loci as a result of linkage, epistasis, and/or pleiotropy. Natural selection on the phenotypic
variation found among male-sterile plants will also influence the frequency of alleles at other loci in these populations. These effects could dramatically shift allele frequencies at specific loci and dictate the utility of male-sterile facilitated recurrent selection. It is therefore important to assess the impact of the mating system on genetic variation in these populations.

Maintaining cytoplasmic variation can be a desired objective in population improvement programs (Weissinger and Albertson 1984). Because the inheritance of the mitochondria and plastids is maternal in most plant species, a continuous cyclic selection of female parents could affect cytoplasmic diversity in these populations. Cytoplasmic genes have been demonstrated for herbicide resistance, susceptibility to disease, and other agronomically or physiologically important traits (Oxoby and Hughes 1989; Harvey et al. 1972; Robertson and Frey 1984; De Broux et al. 1990).

Extranuclear DNA markers are useful in assessing the effect of repetitive selection for cytoplasmic genotypes in a male-sterile population. Recent studies have demonstrated the existence of variation in the chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) among G. max lines (Close et al. 1989; Grabau et al. 1989) that can be used as markers defining specific cytoplasmic genotypes. Hatfield et al. (1985) demonstrated that the chloroplast DNA is inherited maternally in soybeans. Therefore, the number and frequency of cpDNA genotypes in populations with cpDNA variation can be used to assess changes in cytoplasmic diversity that occur within the population over time.

A population of soybeans segregating for a nuclear male-sterility gene was used in the study presented here. It had been subjected to forced outcrossing within the population for seven cycles by advancing only the progeny of male-sterile plants. The first objective of this research was to determine the number and frequency of distinct cpDNA variants among the original female parents of this population. The second objective was to determine the number and frequency of cpDNA genotypes in a random sample of individuals collected from the population after seven cycles of forced outcrossing.

Materials and methods

Population derivation

The derivation of the soybean population used in this study, SG1, has been described by Specht et al. (1985). The initial synthesis of SG1 was accomplished by making all possible two-way crosses between 39 female parental lines and four male parental lines. The pollen donors were male-fertile Ms2Ms2, plants selected from near-isogenic, male-sterile maintainer lines of the adapted cvs 'Beeson' (Maturity Group II), 'Wells' (II), 'Williams' (III), and the genetic type T259H (III). The SG1 parents are listed in Table 1. Except for the Clark and Harosoy lines, Mandarin 13177, and PI 360.844 ('Raiden'), the female parents form the ancestral basis of contemporary soybean cultivars and cytoplasm (Specht and Williams 1984). The 156 parental matings were accomplished by manual pollinations made in summer field nurseries during the period of 1978–1981. The F1 plants (genotypically 1 Ms2Ms2:1 Ms2Ms2) were selfed and individually threshed to obtain F2 seed.

Outcrossing cycles

For the first outcrossing cycle of SG1 in 1982, a composite of equivalent amounts of F2 seed from each of the 156 matings (74,880 total seeds) were planted in an isolated nursery using the following procedure. The seed was divided into 240 planting packets, each containing two randomly selected F2 seeds per cross (312 seeds). Half of these packets were planted in the 36.6 x 61.0 m nursery, which consisted of 120 two-row plots. The remaining 120 packets were planted 3 weeks later in the same nursery in a direction perpendicular to the first planting. The staggered planting dates created more opportunity for pollen transfer between plants of differing maturities and reduced the temporal limitations to effective random mating. The cross-hatched row pattern established male-sterile maintenance by insect vectors. Insect-mediated pollen transfer from male-fertile (MF) plants generated the seeds produced on male-sterile (MS) plants. In subsequent cycles, a random selection of outcrossed seeds from the previous cycle were planted in an isolated nursery at a single planting date.

Harvest

At flowering, plants were randomly selected in the nursery, and a minimum of 200 MS plants were identified on the basis of the absence of pollen development. These plants were tagged and served as a reference for other MS plants at harvest. Because of the wide range of maturities among plants in this population, harvest was performed in late October after a hard freeze. Tagged MS plants exhibited a delayed senescence and reduced pod set compared to MF plants. Thus, additional MS plants could be identified and were randomly collected based on these criteria. No conscious selection was made on the basis of plant size, color, or architecture. Since disease also could have contributed to reduced pod set, plants with disease symptoms were discarded. MS plants were gathered, threshed, and this outcrossed seed was bulked to generate the seed used to produce the next cycle of the population. Approximately 2,000 MS plants were harvested during the first three cycles of this population. After the third cycle approximately 400 MS plants were harvested using the procedures described above. MF plants from each cycle were also gathered at random and threshed for storage as bulked seed in a cold room.

Plant material and DNA extraction

The seed source for the SG1 parental genotypes used in this study was the soybean germ plasm collection (R. Nelson, USDA-ARS, University of Illinois, Urbana, IL 61801 and E. Hartwig, USDA-ARS, Delta Branch Experiment Station, Stoneville, MS 38776). This collection was the same source for seeds used in the initial synthesis of SG1. No cytoplasmic heterogeneity was observed within a line, and cytoplasmic genotypes characterized in this study were consistent with those determined for parental lines in other studies (Close et al. 1989). Seeds of parental lines were grown in the greenhouse and growth chamber. Seeds harvested from MF plants of the initial cycle (cycle-0 selfed progeny) and seventh cycle (cycle-7 selfed progeny) of SG1 were grown in the field in 1990. Individual plants were tagged and young leaves were removed from parental