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ScienceDirect
Field Crops Research 101 (2007) 259–268
Field Crops Research
www.elsevier.com/locate/fcr

Insect-mediated cross-pollination in soybean [*Glycine max* (L.) Merrill] I. Agronomic performance☆

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Received 29 May 2006; received in revised form 22 November 2006; accepted 1 December 2006

Abstract

In soybean, manual cross-pollination to produce large quantities of F_1 hybrid seed for yield trials is difficult and time-consuming. Conversely, insect-mediated cross-pollination has been shown to produce large quantities of hybrid seed in soybean and could facilitate the identification of heterotic patterns. The objective of our study was: (1) evaluate F_1 hybrid soybean plants from single crosses for yield and agronomic traits over several environments and (2) compare hybrid performance of the single crosses to lines developed from three-way crosses and backcrosses. In 2003, F_1 seed of single-crosses and their parent lines were evaluated in replicated experiments at three locations. Also in 2003, three-way crosses, and BC_1F_1 seed were produced. In 2004, three-way crosses, BC_1F_1 crosses, and their parent lines were evaluated at one location. High-parent heterosis (HPH) in single-crosses for grain yield ranged from −41.11% to +11.19%; for protein content from −4.34% to +3.53%, and for oil content from −13.22% to −0.84%. In three-way crosses, HPH for yield ranged from −25.21% to −4.50%, for protein from −2.72% to +1.92%, and for oil from −5.87% to −1.20%. For BC_1F_1 crosses, HPH for yield ranged from −15.65% to +41.97%, for protein from −2.57% to +1.69%, and for oil from −2.47% to +2.22%. Although positive heterosis levels were observed across all populations tested to determine the economic feasibility it is imperative that more tests of more cross-combinations be evaluated in replicated environments. Extensive research in different environments must be conducted to determine what parental combinations will produce the highest heterosis levels, and to develop criteria for selecting the parents with the best combining ability. This will be important to maximize agronomic performance that can economically justify the use of hybrids in soybean production.

Keywords: Grain yield; Heterosis; Male sterility; Soybean

1. Introduction

The phenomenon of heterosis or hybrid vigor (Falconer and Mackay, 1996) has been studied extensively in allogamous and other plant species with mixed reproductive systems, i.e., maize [*Zea mays* (L.)], sorghum [(*Sorghum bicolor* (L.) Moench], pearl millet [(*Pennisetum glaucum* (L.) R. Br.], rapeseed [*Brassica napus* (L.)], onion [*Allium cepa* (L.)], sunflower [*Helianthus annuus* (L.)] cotton [*Gossypium hirsutum* (L.)], and tomato (*Lycopersicon esculentum* Mill.). Heterosis is commercially exploited in seed production, where F_1 hybrids have shown to considerably increase seed yield. Additionally, the use of hybrids has other benefits such as the possibility of developing genotypes of earlier maturity, uniformity of the harvested products, and the stacking of several other useful traits including disease and herbicide resistance (Palmer et al., 2003). Important related issues added to these benefits are the intellectual property-right system in hybrid commercialization

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doi:10.1016/j.fcr.2006.12.003

and protection of parental inbreds used in hybrid production. These latter issues allow private seed companies to legally protect their products and parentage of hybrids (Wehner, 1999).

In spite of these advantages, the use of hybrids has some limitations. For instance, in some of the self-pollinated crops, hybrids for commercial plantings are considered impractical because of the strict mechanisms of self-pollination that greatly reduces cross-pollination (Palmer et al., 2001). This is the case for rice [*Oriza sativa* (L.)], wheat [*Triticum* spp. (L.)], tobacco [*Nicotiana tabacum*. (L.)], and soybean [*Glycine max* (L.) Merr.]. However, in recent years success has been achieved in the commercial use of hybrid rice (Virmani, 1997, 1999), and in the development of hybrid wheat (Jordaan et al., 1999), and pigeon pea [*Cajanus cajan* (L.) Millsp.], (Plant Breeding News IV, ed. 107, Nov, 1999). In soybean, use of hybrid seed for commercial production is not yet a reality. In spite of difficulties in manually producing hybrid seed in soybean, interest in its use for commercial production has remained high and studies have been conducted to determine heterosis values for traits of economic importance. Average mid-parent yield heterosis determined for 2, 8, 27, 18, and 18 hybrid combinations were 28, 13, 8, 2, and 8%, respectively (Brim and Cockerham, 1961; Hillsman and Carter, 1981; Nelson and Bernard, 1984) suggesting that it is possible to find parent combinations that could give a significant economic increase to seed production. These results, however, cannot be extrapolated to commercial production fields since estimates were collected with spaced F_1 plants. To evaluate hybrid combinations on a large scale and to obtain meaningful results about heterosis in soybean, large amounts of hybrid seed need to be produced.

In soybean, naturally occurring mutations that impair male function have been found, identified, and their inheritance studied (Graybosch and Palmer, 1988). Use of these male-sterile lines to produce hybrid seed would require that the male-sterile female-fertile system be biologically stable, and that an efficient mechanism of pollen transfer from male to female parent be found (Palmer et al., 2001). However, in soybean, male sterility has been used primarily to generate random mating populations, or to facilitate hybridization in pure-line development programs (Specht and Graef, 1992; St. Martin and Ehounou, 1989; Carter et al., 1983). Despite the fact that soybean is a self-pollinated species, soybean flowers possess most of the floral characteristics of entomophilus plants (Juliano, 1976; Erickson and Garment, 1979; Arroyo, 1981; Erickson, 1983; Delaplane and Mayer, 2000; Horner et al., 2003), suggesting that insect vectors could be a practical means by which pollen can be transferred. Previous studies have shown that insect-mediated cross-pollination may be used to produce large quantities of hybrid seed, which along with selection practiced for insect attractiveness in male-sterile lines has made possible the production of hybrid seed on a larger scale than before. Thus, it will be possible to evaluate replicated row plot tests. Hybrid F_1 soybean seed production using insect-mediated cross-pollination presents several advantages over manual cross-pollination: (1) larger amounts of seed are produced on the male-sterile plants; (2) it is less time and labor consuming; (3) it makes possible producing hybrid seed from a larger number of cross-combinations (which in turn would facilitate the identification of heterotic patterns); (4) conventional yield trials can be conducted with larger amounts of F_1 seed (the testing of spaced F_1 plants, single-row plots, short-length plots, etc., is avoided) (Lewers and Palmer, 1997; Lewers et al., 1996, 1998; Ortiz-Perez et al., 2004).

The objectives of our study were: (1) evaluate F_1 hybrid soybean plants from single-crosses for yield and agronomic traits over several environments, and (2) compare hybrid performance of the single-crosses to lines developed from three-way crosses and backcrosses using male-sterile female-fertile and male-fertile parents and bees from the families Megachilidae, Halictidae, Anthophoridae, and Andrenidae as insect pollen mediators.

2. Materials and methods

2.1. Plant materials

The female parents were soybean lines with nuclear male-sterile mutations. The selected female parents were male-sterile, female-fertile lines segregating for *ms2* in two different genetic backgrounds: (L75-0587) (Bernard et al., 1991), and *ms2* (A00-39 and A00-41) (Cervantes-Martinez et al., 2005); *ms3* (T284H) (Chaudhari and Davis, 1977); *ms6* (T295H) (Skorupska and Palmer, 1989); *ms8* (T358H) (Palmer, 2000); *ms9* (T359H) (Palmer, 2000). The female lines segregating for *ms3*, *ms6*, *ms8*, and *ms9* are non-allelic to each other, and phenotypic expression of male sterility was different for each line. These mutant female lines also showed differences for seed-set, thus, they were selected for high and low seed-set from a group of male-sterile lines previously evaluated in a three-year experiment at Ames, IA (Ortiz-Perez et al., 2006). The male parents were eight male-fertile, female-fertile lines chosen for attractiveness to pollinator insects and/or agronomic characteristics.

2.2. Single-cross hybrid seed production

Single-cross hybrid seeds were produced by using a randomized complete block design (RCBD) with five replications and eight entries. Each entry was the combination of one female parent and one male parent. Plots were six rows wide, with the first and the sixth planted with males and the four center rows were planted with the females. Each row was 4.8 m long, spaced 76 cm between rows and 1.2 m among plots, seeding rate was 14 seeds per meter. The eight single-combinations were cross-pollinated using alfalfa leaf cutter bees (*Megachile rotundata* F.) as pollinator in the summer of 2002 at Plainview, Texas. At stage R1 (Fehr et al., 1971), beginning flowering, alfalfa leaf cutter bee pupae were placed in a container on the base of a bee board, consisting of a wooden board with 10.6-cm by 10.6-cm wood cylinders, 1.2 m long, with closely spaced holes 0.47 cm in diameter, and 8.89 cm deep. Approximately 10,000 pupa were placed per bee board, and only one bee board was placed in the center of the