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Note on the efficiency of artificial hybridization in soybean

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Summary

During a soybean breeding programme, different crossing techniques were applied to several sets of parents belonging to maturity groups 00 to I. The hypocotyl and flower colour trait was used as a genetic marker in order to distinguish successful cross-pollinations from selfings. All parental material was grown in the field, female parents were cultivated in pots to allow easier handling of plants during pollination. Around 64 % of pods containing hybrid seeds were obtained when female flowers were emasculated and pollinated using a microscope, whereas only 18 % hybridization was achieved when pollinations were done with the naked eye and without emasculation. Percentage of hybridity decreased from 18 to 6.5 from the first to the second crossing period, which is explained by cleistogamous flowering due to low temperatures during the second crossing period.

Key-words: soybean, *Glycine max,* crossing method.

Zur Effizienz unterschiedlicher Kreuzungstechniken bei Sojabohne

Zusammenfassung

Im Rahmen eines Kreuzungsprogramms bei Sojabohne wurden verschiedene Kreuzungstechniken an Elternkombinationen der Reifegruppen 00 bis I angewandt. Die Hypokotyl- sowie die damit gekoppelte Blütenfarbe wurden als genetischer Marker verwendet, um zwischen erfolgreichen Kreuzungen und Selbstbestäubungen unterscheiden zu können. Elternpflanzen wurden ausschließlich im Freiland herangezogen, wobei die weiblichen Kreuzungseltern der leichteren Manipulierbarkeit wegen in Töpfen kultiviert wurden. Ein Anteil von 64 % an Hybridsamen — bezogen auf den gesamten Samenansatz — konnte dann erreicht werden, wenn Kastration und Bestäubung unter einem Mikroskop durchgeführt wurden. Eine Bestäubung mit freiem Auge und ohne Kastration erbrachte nur 18 % erfolgreiche Kreuzungen. Dieser Anteil von 18 % Hybridisierungen aus der ersten Kreuzungsperiode sank auf 6,5 % in einer zweiten Periode, was durch niedrigere Temperaturen und das damit verbundene kleistogame Blühverhalten der Sojabohne zu erklären ist.

Schlüsselworte: Sojabohne, *Glycine max,* Kreuzungstechnik.

1. Introduction

Artificial hybridization between parental genotypes is the first step to initiate segregating populations for breeding varieties. In soybean the small size of the perfect flower, the environmental conditions which influence the suitability of flowers for hybridization and the time-consuming crossing procedure have resulted in the development of several different crossing techniques (Diaz-Carrasco et al. 1984, Fehr 1987, Paschal 1976). Most of the soybean breeders use emasculation of female flowers before pollination, although the removal of anthers is not necessary under favourable growing conditions (Walker et al. 1979). In northern soybean growing areas however, small and cleistogamous flowers are formed during periods of low temperature, which are considerably lowering seed set and promote selfings when cross-pollination is attempted (Gridnev and Kochegura 1988, Kadlec 1988, Schuster 1985, Schori et al. 1988). Natural outcrossing rates of soybean in the field are below 1 % in general, although outcrossing percentages up to 4.5 % have been reported from certain environments (Gumisiriza and Rubaihayo 1978). Therefore, special techniques based on genetic male-sterility have been introduced to enhance natural outcrossing (Boerma and Moradshahi 1975) in order to enable intercrossing designs and to manage recurrent selection procedures without extensive hand pollination (Werner and Wilcox 1990).

In the recent study we compared two different hybridization procedures (emasculation, nonemasculation) and their effects on the numbers of successful crosses vs. undesirable selfings in different combinations of early-maturing soybean genotypes. The influence of environmental conditions on the rate of successful crosses, which is of special relevance to soybean breeding programmes in cool environments, is also reported.

2. Materials and methods

Parent sets "Dom/Apache", "Dom/B 152", "Dom/Birka", "Dom/Silvia", "Dunajka/Apache", "Dunajka/Dawson", "Dunajka/Silvia", "Evans/Birka", "Evans/VSB 2844" and "Evans/Dawson", which are part of a soybean breeding programme were used in the present study. All female parents were homozygous for the recessive allele determining flower colour *(w1w1)*, had white flowers and no coloration in the hypocotyl at the seedling stage. The male parents were considered homozygous for the dominant flower colour allele *(W1W1)*, had purple flowers and purple pigmentation of hypocotyls. The male parents (pollen donors) were grown in the field in single rows at four different sowing dates in ten days intervals to ensure a long period of pollen availability. Plants of female parents were grown outdoors in pots (15 cm diameter, three plants per pot) in a mixture of 2 soil : 1 perlite at two sowing dates with a 14 days interval.

In a first crossing period (from July 7 to July 19), hybridizations were carried out by four persons with similar crossing experience. The two different procedures of artificial hybridization were applied to flower buds of female parents approx. one to two days before regular opening of the flowers:

A. A binocular microscope with 15 × and 30 × magnifications was used to prepare flowers of female parents. Sepals, petals and the ring of anthers were removed by thin forceps. Whenever an anther could be detected precociously releasing pollen to the stigma, the respective flower was discarded. Flowers of male parents containing mature anthers were collected at full bloom and whole flowers were used to pollinate a female stigma after removal of the corolla. Pollination was done immediately after emasculation. Only one to two flower buds per raceme were used for hybridization.

B. All manipulations were carried out with the naked eye and female flowers were not emasculated before pollination.

In a second crossing period (from July 25 to August 9), hybridizations were carried out by the same persons without using a microscope and without emasculation of flowers (=procedure B). During the second period of crossing significantly lower temperatures (minimum night temperatures below 15 degrees centigrade) were recorded in comparison with the first crossing period (minimum night temperatures between 22 and 24 degrees centigrade).

Two to three weeks after pollination all additional flowers and flower buds except the crosses were removed. At maturity, pods from crosses were identified by their lack of sepals and dry seeds were harvested. Finally, seeds were germinated in pots in the greenhouse and hybrid plants were determined by hypocotyl pigmentation and flower colour. Absence of hypocotyl pigmentation in the seedling stage and subsequent expression of the white flower character were used to discriminate undesired selfings. The number of successful crosses among the total number of pods harvested was recorded with reference to the procedure of hybridization and to the crossing period.

3. Results

At maturity, 74 pods containing one to three seeds each (grand mean = 1.86 seeds/pod) were harvested from a total number of 368 flower buds pollinated during the first period of crossing. This represents an average seed set of 20.1 % ranging from 14 to 30 % among different parental combinations. A comparison of the efficiency of the two hybridization procedures is given in table 1. The percentages of crosses (successful hybridizations) were 64 in procedure A (emasculation and pollination with binocular microscope) and 18 in procedure B (pollination without emasculation). In one particular pod a hybrid seed as well as a selfed seed were found. Following procedure A, approx. 35 % of the flower buds selected for pollination had to be discarded because of cleistogamous flowering and precocious pollen release, which could be detected by microscope. According to a chi-squared statistic (2 × 2 contingency table), differences between the two procedures in terms of selfings vs. crosses were significant at the 0.1 % level.

Table 1

Efficiency of two soybean hybridization procedures

Procedure	Total no. of pods harvested	No. of crosses	% successful crosses
A. Emasculation and pollination with binocular microscope	36	23	64
B. Pollination without prior emasculation with naked eye	38	7	18

(Chi-square = 15.85 for difference between no. of crosses, df = 1, $P < 0.001$)

The effect of different crossing periods on the efficiency of hybridization using procedure B is shown in table 2. The decrease in the rate of successful hybridizations between the first and the second crossing period is statistically significant at the 10 % level (2 × 2 contingency table).

Table 2

Effect of different crossing periods representing warmer and cooler temperatures at flowering stage on the efficiency of hybridization using procedure B (pollination without prior emasculation with naked eye)

Crossing period	Total no. of pods harvested	No. of crosses	% successful crosses
1. July 7 to July 19 (warm temperatures)	38	7	18
2. July 25 to August 9 (cool temperatures)	46	3	7

(Chi-square = 2.81 for difference between no. of crosses, df = 1, $P < 0.1$)

4. Discussion

Referring to our results it is suggested that both emasculation of flowers before pollination and use of a binocular microscope to enable careful manipulations and discrimination of self-pollinated flowers are a prerequisite to gain acceptable rates of soybean hybrids. In addition, the use of appropriate genetic markers to monitor the success of hybridization is obligatory, when self-pollination is predominating. This is particularly important if unfavourable European growing conditions and early maturing genotypes belonging to maturity groups 00 to I are considered. In contrast to these results, WALKER et al. (1979) did not find significant differences between emasculation and nonemasculation in terms of undesired selfings when hybridizing under favourable growing conditions and obtaining substantially higher percentages of hybrids. Considering the time necessary for castration, they recommended a nonemasculation procedure for hybridization.

In our study, the lower rate of successful hybridizations in the later crossing period is attributed to low day and night temperatures recorded during this period. A low temperature regime promotes the formation of cleistogamous flowers (SCHUSTER 1985) which are unsuitable for artificial hybridization. This indicates the high degree of environmental sensibility of the soybean flowering process which justifies the growing of parental material in growth chambers (GRIDNEV and KOCHEGURA 1988) or greenhouses, although this is associated with special problems and might not be feasible for all genotypes (FEHR 1980).

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