Cultivar x day period interaction effects on soybean artificial cross efficiency

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ABSTRACT

Efficiency of artificially crossing soybean germplasm was investigated using the BR-16, CD 201 and CD 206 cultivars, which are recommended for cropping in Paraná state, Brazil. The BR-16 x CD 206 and CD 201 x CD 206 hybridizations were carried out at seven different day of times: 8.30 am, 10.00 am, 11.30 am, 1.00 pm, 2.30 pm, 4.00 pm and 5.30 pm under controlled conditions in a greenhouse, in the town of Cambé, northern Paraná state. A randomized complete block design with six replications and treatments in split plots was used. The results indicated that the efficiency, evaluated as percentage of successful pollinations, depended exclusively on the female parent genotype. The BR-16 cultivar requires more careful handling during artificial hybridization because its percentage of successful pollination was consistently lower than that of CD 201. The data confirmed the possibility of conducting artificial hybridizations in soybean throughout the day under controlled conditions in a greenhouse, increasing the effective capacity of carrying out a large number of hybrid combinations.

KEY WORDS: *Glycine max*, hybridization, day time, artificial hybridization, successful pollination.

INTRODUCTION

Artificial hybridization is an extremely important stage in the genetic breeding of a plant species (Borém et al., 1999). A broad genetic base is required to meet the objectives of developing cultivars adapted to several environmental conditions. This is particularly true for the institutions that breed soybean for the diverse latitude range of the Brazilian cropping regions.

Broadening the genetic base requires large scale crossing that will involve several parents sown at different dates for flowering to coincide. Performing all the planned crosses at only the best time of day such as those reported by Borém et al. (1999) is difficult, namely, that crosses made during late afternoon to obtain higher chances of successful pollination.

The present study was carried out to ascertain whether artificial soybean crosses can be successfully carried out at different time of day under controlled greenhouse conditions.

MATERIAL AND METHODS

The study was carried out at the Research Center of the Mato Grosso Foundation for Support to Agricultural Research located in Cambé, altitude 576 m and 23°18´34” latitude, in northern Paraná state, Brazil. The experiment was conducted in a Van der Hooven (12.4 x 18.6 m) double model greenhouse with an internal temperature ranging from 25°C to 30°C controlled by air flow through wall of moistened expanded clay bricks. The pots were placed on tables arranged in the central greenhouse area to increase the uniformity of the internal environmental conditions. Plastic 10kg pots were used, containing substrate of 6kg of local soil collected from the surface to a 20cm depth, 2kg of organic matter compost, 2kg of coarse washed sand and 10 grams of chemical 00-20-20 formula fertilizer.

Three soybean cultivars recommended for cropping in Paraná state were chosen. BR-16 and CD 201 were used as female parents and CD 206 as the male parent. These cultivars are early maturing and differ in marker gene characteristics such flower, pubescence and hilum colors. Table 1 shows the main characteristics of the parent cultivars (Destro and Montalván, 1999). The experiment was carried out in the 2000/2001 growing season.

The female and male parents were sown on ten dates. The first was 4th December, and the others every three days thereafter. For each period, fifteen pots of each female and five pots of each male parent were sown. Three plants were kept per pot, and insects and mites were controlled by weekly application of pesticides recommended for soybean cropping.

Artificial hybridization procedure was carried out by a single technician at the different times tested on each day, namely 8.30 am, 10.00 am, 11.30 am, 1.00 pm, 2:30 pm, 4.00 pm and 5.30 pm. Each time, pollen was collected from donors and emasculations and pollinations performed shortly afterwards. Fifteen flowers per combination were emasculated and pollinated, but they did not necessarily belong to a single plant or pot.

A randomized complete block design with six replications with treatments in split plots was used. The plots consisted of the seven day times and the subplots were the two crosses: BR-16 x CD 206 and CD201 x CD 206, which varied only in terms of female parent. The replications were carried out over time, that is, one replication, of all treatments on each day. The experimental unit consisted of the 15 pollinated flowers per combination between cross and time of day.

The following variables were assessed: number of pollinated flowers, number of pods resulting from the artificially pollinated flowers and percentage of successful pollination (number of hybrid pods produced)/(number of flowers crossed). Selfing was checked by sowing the supposedly F1 seeds collected from the pods derived from artificially pollinated flowers (pods without sepals). For this purpose, the color of the seedling hypocotyl, which demonstrates their hybrid or selfed seed origin, was assessed. The CD 206 male parent has purple flowers, controlled by a single dominant gene. The hypocotyl color is controlled by the same gene and allows early assessment, approximately a week after emergence. Plants with purple flowers present purple hypocotyls, while plants with white flowers present green hypocotyls, when developed under sunlight (Harwig and Hinson, 1962). Destro et al. (1990) and Destro and Montalván (1999) mentioned that this trait can be used as marker to ascertain whether there was cross among cultivars or selfing.

Table 1. Main characteristics of the soybean parent cultivars used in the crosses.

Parents	Cycle	Flower color	Pubescence color	Hilum color
BR-16	Early	White	Gray	Pale brown
CD 201	Early	White	Gray	Pale brown
CD 206	Early	Purple	Brown	Black

Analyses of variance were performed on the number of emasculated flowers and successful pollination percentage without and with transformation to arcsin root percentage.

RESULTS AND DISCUSSION

The analysis of variance indicated significant differences only between crosses, which was caused by the effect of the female parent genotype (Table 2). Although the genotype x day time interaction was not significant by the F test, this source of variation was partitioned because the conventional analysis of variance emphasizes the mean effect of the interaction, but in genetic breeding it is convenient to check what happened to each genotype and how they reacted to each test environment. The partitioning showed that there was no significant difference among the genotypes except at the 1.00 p.m. and 4.00 p.m. times. It was also detected that there was no 'time day' effect of the different day times on any of the female parents (Table 2).

Transformation of the original data in arcsin root percentage did not change the significance of the different sources of variation, but lowered the coefficients of variation from 18.44% to 11. 15%.

Table 3 shows the mean percentage of successful pollination depicting a significant difference between the female parents. The BR-16 and CD 201 cultivars used as female parents presented mean percentages of successful pollination of 35.9% and 44.9%, respectively.

The results can be better observed in the graph presented in Figure 1, where it is clear that CD 201 always reached higher values than BR-16. The latter did not show consistent alteration with hybridization time, while CD 201 presented a decreasing tendency. The analysis of variance in Table 2 also detected significant genotype effects. Further, Table 3 showed that the CD 201 cultivar is superior to BR 16 in successful pollination percentages.

Similar results were obtained by Bonetti and Beskow (1974), who reported that the highest successful pollination percentages under field conditions occurred between 9.00 am and 4.00 pm during the conventional soybean growing season, in Cruz Alta, Rio Grande do Sul state.

CONCLUSIONS

The efficiency of the artificial hybridization in the present study depended on the female parent genotype.

The CD 201 cultivar presented greater percentage of successful pollination in artificially pollinated flowers than BR-16.

There was no time of day effect on the hybridization efficiency. This justifies using most of the day, at least from 8.30 am to 5.30 pm, for hybridizations under greenhouse conditions.

ACKNOWLEDGEMENTS

We thank the Mato Grosso Foundation for Support to Agricultural Research for the space given in the greenhouse at the Cambé Research Center in Paraná state. We also thank the technician Sr. Sebastião Gonçalves de Godoy for performing the crosses.

RESUMO

Interação cultivares x período do dia na eficiência de cruzamentos artificiais em soja

Avaliou-se a eficiência de cruzamentos artificiais envolvendo os cultivares de soja BR-16, CD 201, e CD 206 (BR-16 x CD 206 e CD 201 x CD 206),

Table 2. Partitioned analysis of variance of successful pollination percentage in artificial soybean crosses. Original and transformed data, indicating the degrees of freedom (GL), mean squares (QM) and F test significance (SIG) at the 5% (1/) and 1% (2/)levels of probability.

Source of variation	GL	% Successful pollination		% Transf. pollination	
		QM	SIG.	QM	SIG.
Blocks	5	74.20		25.45	
Times of day	6	72.86		25.17	
Error (A)	30	38.63		13.27	
Genotypes	1	1719.95	2/	596.53	2/
Times of day x genótipos	6	45.06		15.85	
Genotypes in time of day = 08:30	1	448.96	2/	154.40	2/
Genotypes in time of day = 10:00	1	369.63	1/	128.79	1/
Genotypes in time of day = 11:30	1	299.00	1/	104.64	1/
Genotypes in time of day = 13:00	1	182.52		62.45	
Genotypes in time of day = 14:30	1	448.96	2/	156.90	2/
Genotypes in time of day = 16:00	1	3.63		1.12	
Genotypes in time of day = 17:30	1	237.63	1/	83.31	1/
Times of day in genotype = br-16	6	48.68		17.40	
Times of day in genotype= Cd 201	6	69.24		23.61	
Residue	35	55.50		19.29	
Total	83				
General mean		40.39		39.39	
Coefficient of variation (%)		18.44		11.15	

Table 3. Mean percentage of successful pollinations in artificial soybean crosses involving the BR-16 and CD 201 soybean cultivars as female parents and the CD 206 cultivar as male parent, at different day times.

Genotype	Successful pollination
BR-16	35.9 B
CD 201	44.9 A
Mean	40.4

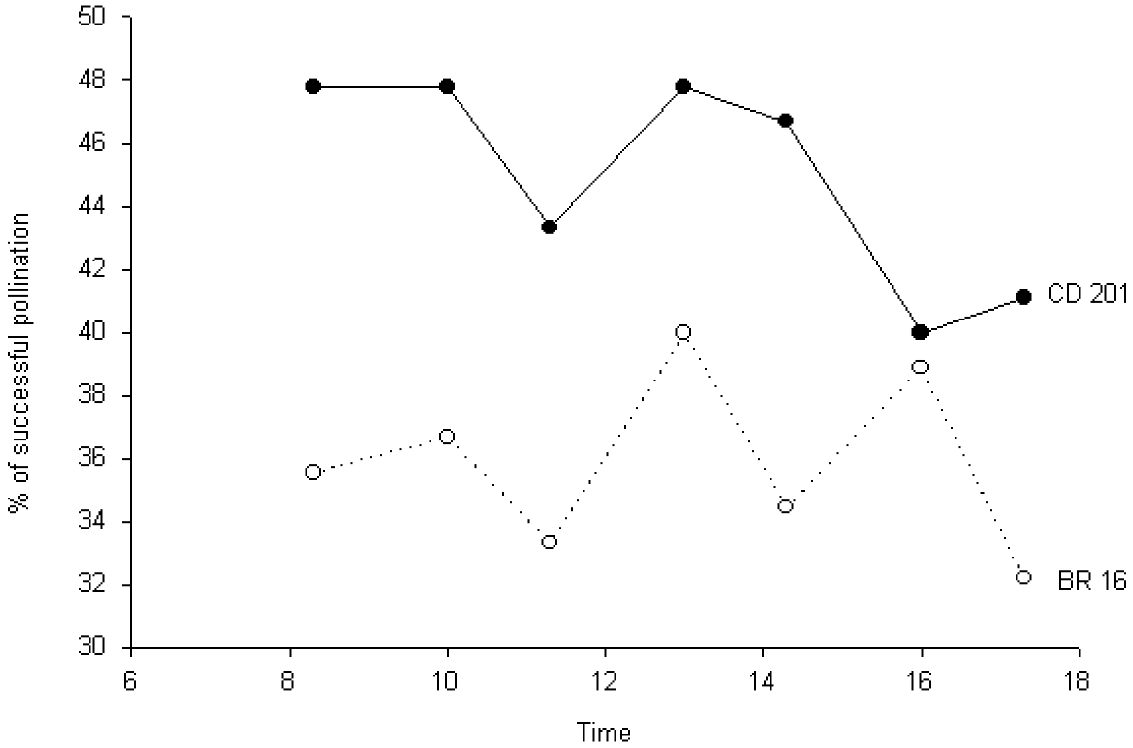

Figure 1. Variation in the percentage of successful pollination in soybean crosses, in function of time of day, from hybridization and the female parents.

indicados para cultivo no Estado do Paraná. As hibridações foram realizadas em sete diferentes horários: 8:30, 10:00, 11:30, 13:00, 14:30, 16:00 e 17:30h em condições controladas de casa de vegetação, no município de Cambe, norte do Estado do Paraná. Utilizou-se o delineamento de blocos casualizados com seis repetições, esquema de parcelas subdivididas. Os resultados indicaram que a eficiência na porcentagem de pegamento nas flores polinizadas nos dois cruzamentos dependeu exclusivamente do genótipo do parental feminino. O cultivar BR-16 necessita maiores cuidados durante a hibridação artificial, visto que a porcentagem de pegamento foi consistentemente inferior a CD 201. Os dados confirmam a possibilidade de se efetuar hibridações artificiais em soja durante todo o dia, em condições controladas em casa de vegetação, aumentando a capacidade efetiva de realização de grande número de combinações híbridas.

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Received: March 25, 2002;

Accepted: October 17, 2002.