

EFFICIENCY OF ARTIFICIAL HYBRIDIZATION IN SOYBEAN DURING THE SUMMER DEPENDING ON TEMPERATURE AND RELATIVE HUMIDITY

EFICIÊNCIA DA HIBRIDAÇÃO ARTIFICIAL EM SOJA DURANTE O VERÃO DEPENDE DA TEMPERATURA E UMIDADE RELATIVA

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ABSTRACT: Hybridization is an important improvement method used in the soybean culture. However, there is little information on the recommended relative moisture and air temperature degree for artificial pollination. Therefore, this study aimed to determine the efficiency of artificial hybridization between soybean parents according to different periods of the day. Artificial pollination of 14 hybrid combinations occurred in greenhouse in three periods of the day. The parents were: TMG 801, TMG 803, BRSGO 7560, BRS Valiosa RR, Agua-Marinha RR and NK 7059 RR. The studied variables were: relative moisture, air temperature, number of days to flowering, performed artificial pollination, pods without sepal, produced seeds, germinated seeds, hybrid plants and percentage of pods without sepals. Data were submitted to normality and homogeneity of variance test, analysis of variance, Tukey, Scheffé and χ^2 tests, and correlation analysis. Six hundred and seventy-two artificial pollinations were performed. From which were obtained 436 pods without sepals and approximately 90% of produced seeds was hybrid. The results indicated that artificial pollinations performed in January, with parent used in this study, were more efficient in the period from 10:00 a.m. to 12:00 a.m., with mean relative moisture of 34.1% and mean temperature of 38.5 °C and 2:00 a.m. to 4:00 p.m. with 30.7% and 41.6 °C respectively for relative moisture and means of temperature.

KEYWORDS: *Glycine max.* Improvement. Genetic variability.

INTRODUCTION

Higher production and grain yield in the soybean (*Glycine max* (L.) Merr.) culture has been observed the last years. In the 1976/1977 growing season, 6.95 thousand hectares were cultivated, where 12.15 thousand tons of soybean were produced, resulting in 1,748 kg mean yield ha⁻¹ (CONAB, 2011). Thirty-seven years later, 81.5 million tons of soybean were produced in 27.7 million hectares in the 2012/2013 growing season, resulting in 2,933 kg mean yield ha⁻¹ (CONAB, 2013).

The considerable increase in soybean production and yield is a result of the persistent role of genetic improvement programs in developing and recommending cultivars, of farmers and researchers in several correlated areas with technology and production. Breeders have used hybridization in the development of new soybean cultivars (MIYASAKA; SILVA, 1958; SEDIYAMA et al., 2005). With hybridization between genetically different parents, populations with higher genetic

variability can be developed, which allows the application of appropriate evaluation methods and selection of genotypes with superior phenotypes (BORÉM et al., 2009). The main aspects that must be considered to optimize hybridization and hybrid seed production process are: operator skill, environment, pests, diseases, flowering synchronization of progenitors and plant vigor (SEDIYAMA et al., 2005; BORÉM et al., 2009). The ideal environment to perform artificial hybridization in soybean plants may vary depending on the region and work conditions available to the breeder (SEDIYAMA et al., 2005). In other words, the environmental conditions can determine the hour of the day for pollen collection and highest and lowest efficiency in hybridizations (SEDIYAMA et al., 1981; SEDIYAMA et al., 2005; BORÉM et al., 2009). High or low relative air moisture and temperature at night, among several factors, may reduce the crossing rate (JOHNSON; BERNARD, 1963). Moreover, direct sunstroke or high relative moisture may impair the success of hybridizations (SEDIYAMA et al., 1985). In the literature, there is

limited information on the magnitude of relative moisture and air temperature for achievement of higher numbers of pods and hybrid seeds. Thus, the purpose of this study was to determine the efficiency of soybean artificial hybridization according to different periods of the day.

MATERIAL AND METHODS

Artificial hybridization activities were performed in greenhouse of the Soybean Genetic

Improvement Program of the Department of Plant Science, at the Universidade Federal de Viçosa, Viçosa, Minas Gerais State, Brazil (20°45'14" S; 42°52'54" W; 640 m altitude), in the 2009/2010 growing season (Seed sowing: Dec. 8th, 2009 to Jan. 9th, 2010; Artificial pollinations: Jan. 29th, 2010 to Feb. 19th, 2010). The parents were: TMG 801, TMG 803, BRSGO 7560, BRS Valiosa RR, Água-Marinha RR and NK 7059 RR (Table 1).

Table 1. Some phenotypes of the agromorphological traits parents used in crossing block, Viçosa-MG, 2009/2010

Parents	Characteristics			
	Relative Maturity	Flower color	Pubescence color	Glyphosate Response
NK 7059 RR	6.6	White	Grey	Resistant
BRSGO 7560	7.5	Purple	Tawny	Susceptible
BRS Valiosa RR	8.1	Purple	Tawny	Resistant
TMG 801	8.2	Purple	Tawny	Susceptible
TMG 803	8.7	White	Tawny	Susceptible
Água-Marinha RR	8.7	White	Tawny	Resistant

Sources: Pierozzi et al. (2008), Fundação MT (2011) and Syngenta Seeds (2011).

Aiming at flowering synchronization of plants in the crossing block, parent seed sowing was performed at different times in pots containing 3 dm³ soil, containing 1/3 of organic matter. Sowing began on Dec. 8th, 2009 and ended on Jan. 9th, 2010, with a two-day interval (totalizing 17 seasons). Cultivar BRSGO 7560 was sown in the three last planting only (Jan. 5th, 2010, Jan. 7th, 2010 and Jan. 9th, 2010), due to unavailability of seeds at installation of crossing block. In each season, at development stage V1 (FEHR; CAVINESS, 1977), thinning to two plants per pot was carried out. At stage V4 (FEHR; CAVINESS, 1977), the apical

meristem was removed, aiming to reduce the necessity of plant tutoring (TANCREDI et al., 2004). Subsequently, plants of the crossing block were grown according to culture recommendations (EMBRAPA, 2008).

Pollinations of 14 hybrid combinations (Table 2) were performed in three periods of the day (H1: 8:00 – 10:00 a.m., H2: 10:00 – 12:00 a.m. and H3: 2:00 – 4:00 p.m.), according to the hybridization process mentioned by Sedyama et al. (2005) and Borém et al. (2009).

Table 2. Hybrid combinations used in crossing block, Viçosa-MG, 2009/2010

Hybrid combinations	Hybrid combinations
TMG 801 x BRS Valiosa RR	TMG 803 x BRSGO 7560
TMG 801 x Água-Marinha RR	BRSGO 7560 x BRS Valiosa RR
TMG 801 x NK 7059 RR	BRSGO 7560 x Água-Marinha RR
TMG 803 x BRS Valiosa RR	BRSGO 7560 x NK 7059 RR
TMG 803 x Água-Marinha RR	Água-Marinha RR x BRS Valiosa RR
TMG 803 x NK 7059 RR	NK 7059 RR x BRS Valiosa RR
TMG 803 x TMG 801	NK 7059 RR x Água-Marinha RR

Aiming at identifying hybrid plants, parents presenting dominant phenotypic pattern over the feminine ones were used as masculine parents. The phenotypic patterns used as markers were: hypocotyl color, flower color, pubescence color and/or glyphosate tolerance. Sepal absence was used as phenotypic marker of pods originating from a flower submitted to artificial pollination process.

Magnitude of relative moisture and air temperature was recorded in all periods and days in which artificial pollination was carried out. Other analysed variables were: number of days to flowering, number of pollinations performed, number of pods without sepals, number of produced seeds, number of germinated seeds, number of hybrid plants and percentage of pods without sepals $\{PPWS\% = [(Number\ of\ pods\ without\ sepals / Number\ of\ performed\ pollinations) * 100]\}$. The percentage of pods without sepals was obtained in each period and hybrid combination.

After harvest and attainment of the number produced seeds, were planted at least of the 50% seeds produced in trays with sand (trays with 39 cm length x 26 cm width x 5 cm depth) was carried out, separated according to hybrid combination and period of day in which artificial pollination was performed. Determination of number of hybrid plants was carried out based on hypocotyl color, pubescence color and/or glyphosate tolerance.

Relative moisture and air temperature data were analysed based on descriptive statistics: number of days which artificial pollination was carried out, mean, minimum and maximum values, coefficient of variation, variance, standard deviation and distribution with normality test carried out according to the Lilliefors methodology ($p \leq 0.05$). Data from number of days to flowering were initially submitted to analysis of variance considering the completely randomized design

unbalanced (seventeen replications for NK 7059 RR, BRS Valiosa RR, TMG 801, TMG 803 and Água-Marinha RR and three replication for BRSGO 7560), each experimental unit was represented by the average of two plants. Next, Tukey test considering unequal number of replications were applied for comparison of means at 5% probability. The homogeneity of values of number of performed pollinations, number of pods without sepal, number of produced seeds, number of germinated seeds and number of hybrid plants among the three pollination periods were tested by Chi-square test (χ^2), at 1 and 5% probability. To analyse the three pollination periods according to means of the 14 hybrid combinations were initially carried out the Lilliefors normality test and Cochran homogeneity of variance test, at 5% probability. Next, analysis of variance (considering the completely randomized design with fourteen replications and each experimental unit was represented by a hybrid combination) and Scheffé test were applied for comparison of mean contrast at 5% probability. The analyses were carried out in the Assistat Program (SILVA, 2002) and in the Genes Program (CRUZ, 2013).

RESULTS AND DISCUSSION

The results of the descriptive analysis for temperature and relative air moisture (Table 3) indicated normal distribution for two variables according to three pollination periods by Lilliefors test ($p \leq 0.05$). It was noticed that means for air temperatures in periods H1, H2 and H3 were 32.8 °C, 38.5 °C and 41.6 °C, respectively, and the magnitude of means for relative moisture in periods H1, H2 and H3 were 45.7%, 34.1% and 30.7%, respectively. These results indicated tendency of temperature increase and relative air moisture reduction along the day.

Table 3. Descriptive statistic for temperature (TA), in °C, and relative air moisture (RM), in %, according to three hybridization periods (H1, H2 and H3), in greenhouse, Viçosa-MG, 2009/2010

TA or RM / Periods ¹	Number of days ²	Mean	Min.	Max.	Coefficient of variation	Variance	Standard deviation	D ³ and 4
TA – H1	11	32.8	30.0	37.0	7.31	5.7636	2.4008	0.1788 ^{ns}
TA – H2	19	38.5	33.0	42.0	5.58	4.6359	2.1531	0.0928 ^{ns}
TA – H3	15	41.6	38.0	43.5	3.38	1.9881	1.4099	0.1934 ^{ns}
RM – H1	11	45.7	39.0	56.5	11.62	28.2682	5.3168	0.1728 ^{ns}
RM – H2	19	34.1	29.0	45.0	12.47	18.0994	4.2543	0.1356 ^{ns}
RM – H3	15	30.7	27.5	35.5	6.61	4.1381	2.0342	0.1531 ^{ns}

¹ H1: 8:00 – 10:00 a.m., H2: 10:00 – 12:00 a.m. and H3: 2:00 – 4:00 p.m.; ² Number of days which artificial pollination was carried out; ³ Statistic D of Lilliefors test; ⁴ ns: non-significant at 5% probability.

The normality (Lilliefors) and homogeneity of variance (Cochran) tests indicated that it is possible to analyse data of number of days to flowering considering normal distribution and with homogeneity of variance, respectively. The parents: NK 7059 RR and BRSGO 7560 presented lower values of number of days to flowering, whereas, TMG 803 showed the highest mean (Table 4). This is due to the agronomic characteristic of each parents, mainly according to the region of adaptation. This difference was evidenced because they are from different maturity groups and they were sown under the same environmental conditions

(planting date and latitude). According to Tancredi et al. (2006), the number of days to flowering is not influenced by removal of the apical meristem when the number of plants per pot is maintained constant. This situation is valid to the present study.

The number of days to flowering in crossing blocks is important to guide studies of plant breeding. Alternative strategies to remedy the group maturity difference in crossing blocks, would be the use of artificial photoperiod control or the use of grafting (SEDIYAMA et al., 1985, LUGÃO, 1988; BORÉM et al., 2009).

Table 4. Summary of analysis of variance and comparisons of means test for number of days from planting to flowering of six soybean parents, in greenhouse, Viçosa-MG, 2009/2010

Variation sources	Df	Days from planting to flowering
		QM (F test) ¹
Parents	5	701.5472 (63.57)**
Residue	82	11.03
Parents		Comparison of means – Tukey Test ²
NK 7059 RR		37.3 c
BRSGO 7560		37.7 c
TMG 801		45.6 b
BRS Valiosa RR		46.3 b
Água-Marinha RR		47.6 b
TMG 803		56.8 a
Mean		46.3
CV (%)		7.1

¹ ** Significant at 1% probability by F-test; ² Means followed by same letter do not differ statistically by Tukey test at 5% probability.

Six hundred and seventy-two artificial pollinations were performed in fourteen combinations, in three periods of the day, resulting in approximately 47 pollinations per day. Four hundred and thirty-six pods were obtained without sepals, which correspond to 64.8% of the total performed pollination.

In the analysis of the fourteen hybrid combinations, it was noticed heterogeneity between the pollination periods to number of performed pollination, pods without sepals and produced seeds, in 10, 8 and 12 hybrid combinations, respectively (Table 5). The number of pods from self-fertilization may be included in the number of pods without sepals. This occurs because, although pods were harvested only without sepals, at the moment that the artificial pollination was carried out, self-fertilization might have already taken place.

In the hybrid combinations TMG 803 x NK 7059 RR and BRSGO 7560 x NK 7059 RR were observed reduced number of performance pollination, pods without sepals, produced and

germinated seeds, hybrid plants between periods of the days (Tables 5). In the hybrid combination TMG 803 x NK 7059 RR the group relative maturity among genitors is very different (see Table 1). So, in the conditions in which the crossing block was conducted, the period of simultaneous flowering between two genitors was coincident in only one day. In BRSGO 7560 x NK 7059 RR, although two parents presented similarity regarding number of days to flowering, the reduced number of pollination occurred due to unavailability of seeds of BRSGO 7560 in the beginning of crossing block installation, resulting in planting only in the three last dates of the crossing block.

Considering the fourteen hybrid combinations and the three pollination periods, seed germination was superior at 84%. In the analysis of hybrid combinations, it was noticed heterogeneity for number of seeds germinated and plants confirmed as hybrid in 62.3% of hybrid combinations between the pollination periods.

Table 5. Number of performed pollinations (Pol), number of pods without sepals (Pods) number of produced seeds (Seeds), number of germinated seeds (G) and number of plants confirmed as hybrid (H) in 14 combinations, in three periods, Viçosa-MG, 2009/2010^{1 and 2}

	Periods ²			χ^2 test	Periods			χ^2 test
	H1	H2	H3		H1	H2	H3	
TMG 801 x BRS Valiosa RR				TMG 803 x BRSGO 7560				
Pol.	17	51	45	17.48**	2	9	4	5.20 ^{ns}
Pods	7	37	30	19.97**	1	6	1	6.25*
Seeds	18	90	79	48.26**	2	12	1	14.80**
G	9	45	38	23.76**	2	8	1	7.81*
H	6	42	34	26.14**	2	7	1	6.19*
TMG 801 x Água-Marinha RR				Água-Marinha RR x BRS Valiosa RR				
Pol.	16	66	56	30.43**	18	50	44	15.50**
Pods	9	51	38	28.30**	4	29	24	18.42**
Seeds	23	119	97	63.49**	9	51	46	29.79**
G	12	60	45	30.92**	3	51	46	41.78**
H	12	57	44	28.47**	1	49	41	43.60**
TMG 801 x NK 7059 RR				NK 7059 RR x BRS Valiosa RR				
Pol.	3	14	14	7.80**	5	38	40	27.92**
Pods	2	9	10	5.42 ^{ns}	0	29	30	29.52**
Seeds	4	23	27	16.77**	0	74	71	72.59**
G	2	12	14	8.85*	0	74	69	71.76**
H	2	12	13	8.22*	0	61	65	63.19**
TMG 803 x TMG 801				NK 7059 RR x Água-Marinha RR				
Pol.	5	14	6	5.84 ^{ns}	0	20	25	23.33**
Pods	3	9	5	3.29 ^{ns}	0	12	15	14.00**
Seeds	6	22	10	10.94**	0	30	21	27.88**
G	3	14	7	7.75*	0	28	18	26.26**
H	2	11	7	6.10*	0	27	16	25.20**
TMG 803 x BRS Valiosa RR				BRSGO 7560 x BRS Valiosa RR				
Pol.	4	15	7	7.46*	2	5	12	8.31*
Pods	2	8	5	3.60 ^{ns}	2	4	10	6.50*
Seeds	4	15	9	6.49*	4	8	16	8.00*
G	2	8	5	3.6 ^{ns}	2	4	8	4.00 ^{ns}
H	2	8	3	4.76 ^{ns}	2	4	7	2.92 ^{ns}
TMG 803 x Água-Marinha RR				BRSGO 7560 x Água-Marinha RR				
Pol.	4	18	10	9.25**	2	9	16	10.88**
Pods	3	12	5	6.70*	2	8	8	3.99 ^{ns}
Seeds	4	24	10	16.63**	5	19	17	8.39*
G	2	12	5	8.31*	3	10	9	3.90 ^{ns}
H	2	7	4	2.92 ^{ns}	3	9	9	3.42 ^{ns}
TMG 803 x NK 7059 RR				BRSGO 7560 x NK 7059 RR				
Pol.	0	2	2	2.00 ^{ns}	0	1	1	1.00 ^{ns}
Pods	0	2	2	2.00 ^{ns}	0	1	1	1.00 ^{ns}
Seeds	0	3	3	3.00 ^{ns}	0	2	2	2.00 ^{ns}
G	0	3	3	3.00 ^{ns}	0	2	2	2.00 ^{ns}
H	0	3	0	6.00*	0	2	2	2.00 ^{ns}

¹ **, * and ns Significant at 1%, 5% and non-significant, respectively, by χ^2 test; ² H1: 8:00 – 10:00 a.m., H2: 10:00 – 12:00 a.m. and H3: 2:00 – 4:00 p.m.

In the three pollination periods and within these periods in fourteen hybrid combinations the normality test (Lilliefors) showed not reasonable to study the data by normal distribution for the number of performed pollinations, pods without sepals, produced seeds, germinated seeds, plants confirmed as hybrid and percentage of pods without sepals. Therefore, the original data were transformed in

$\log(x+0.5)$. Posteriorly, by Lilliefors and Cochran tests, both at 5% probability, indicated possible to analyse data considering normal distribution and with homogeneity of variance, respectively.

Non-significant result was observed ($p>0.05$) by Scheffé test in contrasts H2-H3 for variables: number of performed pollinations, pods without sepals, produced seeds, germinated seeds

and plants confirmed as hybrid; whereas, for contrasts H1-H2 and H1-H3 the result was significant (Table 6). This allows affirming that periods H2 and H3 presented similar means, however, higher when compared with pollination period H1.

The reduced number of pods without sepals originating from the hybridization in H1 can be explained due to the fact that in this period the pollen is probably in massulae, and thus, not

powdered. In addition to this fact, the stigma probably presents higher receptivity in the H2 and H3 periods, increasing the success of cross-pollinations. This agrees with Sedyama et al. (1981), Sedyama et al. (2005), Young and Gravitz (2002) and Borém et al. (2009) that reported higher success rates of pollination performance in the afternoon due to pollen liberation and stigma receptivity.

Table 6. Means of groups H1, H2 and H3 and different contrasts (Contrast) according to number of performed pollinations (Pol), number of pods without sepals (Pods), number of produced seeds (Seeds), number of germinated seeds (Germinated), number of seedling confirmed as hybrid (Hybrids) and percentage of pods without sepals (PPWS), Viçosa-MG, 2009/2010

Variables	Times ^{1 e 2}			Contrasts ²		
	H1	H2	H3	H1-H2	H1-H3	H2-H3
Pol.	0.5250	1.1561	1.1060	-0.6311*	-0.5811*	0.0500 ^{ns}
Pods	0.2970	1.0196	0.9340	-0.7226*	-0.6371*	0.0855 ^{ns}
Seeds	0.4983	1.3331	1.1987	-0.8348*	-0.7004*	0.1344 ^{ns}
Germinated	0.3209	1.1649	1.0402	-0.8440*	-0.7193*	0.1248 ^{ns}
Hybrids	0.2724	1.1229	0.9405	-0.8505*	-0.6681*	0.1825 ^{ns}
PPWS	1.1727	1.8612	1.8170	-0.6884*	-0.6443*	0.0441 ^{ns}

¹ H1: 8:00 – 10:00 a.m., H2: 10:00 – 12:00 a.m. and H3: 2:00 – 4:00 p.m.; ² Original values transformed in $\log(x+0.5)$; ³ ns and *: Non-significant and significant, respectively, at 5% probability by Scheffé Test.

The percentage of pods without sepals mean was superior to identified by Volmann et al. (1992). These authors identified pods originating from artificial pollination based on sepal absence and, subsequently, carried out planting of seeds of these pods to quantify the number of hybrid plants for hypocotyl color analysis. Approximately 64% of pods containing hybrid seeds were obtained when female flowers were emasculated and pollinated, and only 18% of efficiency when pollination was carried out with the naked eye and without emasculation.

High temperatures are frequent and important causes of abortion of fertilized ovule and consequently not development of pods, i.e. at 40 °C, from 57% to 71% of pods can be aborted (MÜLLER, 1981). According to Rosolen (2006), the best temperature for ovule fertilization and development of pods is 30 °C during the day and 20 °C at night.

In periods H2 and H3 good environmental conditions (relative moisture and air temperature) were observed, both for plant development and for the operator, collaborating for the efficiency of artificial pollination. In other words, pollen is powdered and it is easier to identify feminine flowers in appropriate stage, associated to better work conditions. According to Sedyama et al. (1981), even when the best methodologies are employed by experienced technicians, the success of

artificial pollination varies greatly according to the environmental conditions.

An experienced, careful and skillful operator can obtain over 90% success percentage in the performed pollinations (BORÉM et al., 2009). The factor operator skill, in this study, was of great importance in obtaining the hybrid seeds. High efficiency of artificial pollination was observed both in hybrid combinations that originated many seeds and in those which originated few seeds. Hence, the correlation between the total number of pods without sepals and the total number of hybrid seeds was 0.9229 ($p < 0.01$, by t test and Mantel test with 5000 simulation). If the operator is very skilled, it is possible to obtain hybrid seeds even when there is a reduced number of genitor plants and when pollination is performed in inappropriate times of the day.

CONCLUSIONS

Artificial pollinations performed in January, with parent used in this study, were more efficient in the period from 10:00 to 12:00 a.m., with mean relative moisture of 34.1% and mean temperature of 38.5 °C and 2:00 to 4:00 p.m. with 30.7% and 41.6 °C respectively for relative moisture and means of temperature.

Best hybrid combinations were: TMG 801 x BRS Valiosa RR, TMG 803 x BRS GO 7560, TMG

801 x Água-Marinha RR, BRSGO 7560 x BRS Valiosa RR, TMG 801 x NK 7059 RR, NK 7059 RR x BRS Valiosa RR, TMG 803 x BRS Valiosa RR and NK 7059 RR x Água-Marinha RR.

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RESUMO: A hibridação é um importante método de melhoramento utilizado na cultura da soja. No entanto, são poucas as informações sobre a magnitude da umidade relativa e da temperatura do ar recomendadas para a atividade de polinização artificial. Portanto, objetivou-se determinar a eficiência da hibridação artificial entre genitores de soja em função de diferentes períodos do dia. Efetuaram-se, em casa de vegetação, polinizações artificiais em 14 combinações híbridas em três períodos do dia. Os genitores utilizados foram: TMG 801, TMG 803, BRSGO 7560, BRS Valiosa RR, Água-Marinha RR e NK 7059 RR. As variáveis estudadas foram: umidade relativa, temperatura do ar, número de dias para o florescimento, polinizações artificiais efetuadas, vagens sem sépala, sementes produzidas, sementes germinadas, plantas híbridas e porcentagem de vagens sem sépalas. Os dados foram submetidos ao teste de normalidade e homogeneidade de variância, análise de variância, testes de Tukey, de Scheffé e χ^2 e análise de correlação. Foram efetuadas 672 polinizações artificiais, nas quais se obtiveram 436 vagens sem sépala. Aproximadamente, 90% das sementes produzidas foram híbridas. Os resultados indicaram que as polinizações artificiais realizadas em janeiro, com os genitores do presente estudo, foram mais eficientes no período das 10:00 h às 12:00 h, com umidade relativa média de 34,1% e temperatura média de 38,5 °C e das 14:00h às 16:00h com 30,7% e 41,6 °C, respectivamente para umidade relativa e temperatura média.

PALAVRAS-CHAVE: *Glycine max.* Melhoramento. Variabilidade genética.

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