CROP BREEDING AND APPLIED BIOTECHNOLOGY

ARTICLE

A simple and inexpensive procedure to more quickly obtain new varieties in soybean

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Abstract: *Soybean (*Glycine max *L.) is one of the four most important crops in the world. The creation of new commercial varieties is a long-term activity that requires from seven to eight years from the beginning of the cross design up to registration for commercialization of cultivars. Rapid generation advance (RGA) is a technique that consists of controlling external factors that affect plant growth with the aim of shortening the sowing to harvest cycle. In the present study, an optimized method is described that can accelerate soybean breeding by means of RGA using inexpensive facilities and that can be easily applied by breeders. Our breeding method uses a modified cold storage chamber fitted with fluorescent lamps delivering a 12/24 h light photoperiod, while temperature is set at 24 °C. This method allows development of up to 5 generations per year instead of the 1-2 generations currently possible under field or greenhouse conditions.*

Keywords: *Genetic gain, rapid generation advance, selection cycle, soybean*

INTRODUCTION

Soybean (*Glycine max* [L.] Merr.) is one of the four most important crops worldwide, which, along with corn, rice, and wheat, contribute up to two-thirds of the calories in the human diet (Tilman et al. 2011, Ray et al. 2013). This importance is achieved through the extensive areas dedicated to these crops and the availability of varieties adapted to local conditions. In the case of soybean, generation of new, better-adapted varieties (high and stable yield, disease resistance, etc.) is a process led mainly by breeding programs from the private sector (Heisey et al. 2001, Sakiyama et al. 2014). Nevertheless, opportunities exist for small public breeding programs that generate varieties adapted to specific environments. These programs must be efficient in increasing genetic gain without significant investment. Studies on other species have shown that decreasing the duration of the selection cycle generates the greatest impact on genetic gain in terms of the cost-benefit ratio (Bonnecarrere et al. 2019).

Soybean breeding programs worldwide implement different strategies to shorten normal breeding cycles (Cobb et al. 2019, Moreira et al. 2019). Most of them are focused on increasing the number of cycles per year, which is mainly feasible in tropical or subtropical regions due to temperature and light requirements. In temperate climate countries, other strategies must be implemented to increase the number of cycles per year. In this context, rapid generation advance (RGA) is a tool that enables speed breeding (Depauw and

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Clarke 1976, Collard et al. 2017). The RGA method consists of controlling the external factors that affect plant growth, such as temperature, light intensity and quality, photoperiod, nutrition, and growth promoters, with the objective of shortening the cycle from sowing to harvest. RGA accelerates the vegetative and reproductive phases of the crop, achieving fast growth and early induction of flowering (Depauw and Clarke 1976, Watson et al. 2018). Previous studies on soybean RGA reported the use of the embryo rescue technique, which requires expensive facilities and sterile materials, as well as skilled labor (Roumet and Morin 1997, Gieco et al. 2007). Recently, Nagatoshi and Fujita (2019) described a method that allows reduction in generational time without the use of the embryo rescue technique, though it uses expensive equipment and facilities with limited capacity to house plants. In these studies, it was stated that controlled dehydration of immature embryos on intact pods is crucial for preserving their germination capacity.

The objective of our study was to establish a method that accelerates the breeding of soybean by means of RGA using inexpensive facilities and that can be easily applied by breeders in small public breeding programs. Taking into consideration that soybean is a short-day crop (Cregan and Hartwig 1984), which means it needs long, uninterrupted periods of darkness for induction of flowering, two approaches were used to shorten the soybean cycle: 1) short-day growth conditions to induce flowering using affordable facilities and 2) application of plant growth regulators (PGR) that may induce flowering regardless of the length of the day. Different soybean genotypes were used to evaluate variations on cycle duration in relation to maturity groups. Finally, possible negative effects of applying RGA in successive generations were investigated.

MATERIAL AND METHODS

Plant material

Seven F7 soybean lines from the Soybean Breeding Program of the National Agricultural Research Institute (Instituto Nacional de Investigación Agropecuaria – INIA, Uruguay) were used. Lines were selected to include differences in crop cycles or maturity groups (MG), from late IV to late VI. The varieties chosen included 1 genotype belonging to the late IV group, 2 genotypes to the late V, 1 genotype to the early VI, and 3 genotypes to the late VI. For additional simplification and attribution of field cycle length, based on Fassio et al. (2017), all lines were classified into three main groups: early maturity (EM) (MG < 5.4), medium maturity (MM) (5.4 ≤ MG ≤ 6.2), and late maturity (LM) (MG > 6.2). The phenological model developed by Fassio et al. (2017) was used to attribute the duration of phenological stages to all the lines (Table 1).

Table 1. Duration of field cycle according to the crop maturity group using a theoretical phenology model. EM: early maturity, MM: medium maturity, and LM: late maturity. S: sowing, V: vegetative stage, R: reproductive stage, TCL: total cycle length. The numbers represent the number of days it takes for the plant to reach the stages indicated

Genotype	S-V4	V4-R1	R1-R3	R3-R5	R5-R7	R7-R8	TCL
EM	26	31	7	15	45	18	142
MM	26	41	21	32	29	14	163
LM	28	48	25	32	26	6	165

Treatments for flowering induction

In relation to photoperiod, two treatments were evaluated: long-day condition (LDC) and short-day condition (SDC). For induction of flowering in LDC, gibberellic acid (GA_3) (Sigma-Aldrich, St. Louis, MO, USA), salicylic acid (SA) (Sigma-Aldrich, St. Louis, MO, USA), and 6-benzylaminopurine (BAP) (Sigma-Aldrich, St. Louis, MO, USA) were evaluated by two foliar applications at 7 and 14 days after sowing. The following concentrations were tested: 1) GA_3: 20 and 100 mg L^{-1}; 2) SA: 50 mg L^{-1}, and 3) BAP: 1 mM. Lines were grown in greenhouse facilities under a 14-h natural light regime, with temperature ranging from 30 °C during the day to 20 °C at night. Relative humidity was 60-80%.

For SDC plant growth, a modified 3 m × 4 m cold storage chamber was used as a growth chamber (able to house 150 plants). It was fitted with Sylvania cool white f72t12/cw/h 160W fluorescent tube bulbs, which provided a light intensity of 350 µmoL m^{-2} s^{-1} at the canopy level (Figure 1), and programmed for a photoperiod of 12 h of light at a temperature of 24 °C.

Figure 1. Growth chamber adapted for soybean rapid generation advance (RGA). A) The current capacity is 150 plants. The use of ceiling lights mounted on pulleys allows easy adjustment of the amount of light supplied during the growth cycle. B) Soybean plants of the genotypes studied growing under the conditions described.

Together with SDC, the following treatments were evaluated: a) controlled dehydration of immature seeds + conventional germinators (CDCG), b) controlled dehydration of immature seeds + rescue of embryos (CDRE), and c) rescue of embryos from non-dehydrated seeds (RE). In all cases, the plants were grown until they reached the late R5 stage. For the CDCG and CDRE treatments, pods were harvested and dried for 5-8 days at 28 °C (RH~70%). For CDRE, immature embryos were rescued from harvested seeds and germinated in media described by Lippman and Lippmann (1993). The growth regulators were indole-3-acetic acid (IAA) (0 and 0.1 µM) and meta-topolin (Mt) (0 and 0.1 µM) (Sigma-Aldrich, St. Louis, MO, USA), used in their four possible combinations (Gieco et al. 2007). Conventional germinators used in CDCG consisted of Petri dishes containing filter paper moistened with a 1% PPM solution (Plant Preservative Mixture, 1823 Jefferson Pl. NW, Washington, D.C., USA). After 3 days, the germinated seeds were sown in the detailed pots. The CDCG protocols were repeated for 5 generations to assess the effect of RGA on plant vigor and viability.

In all cases, in both the LDC and SDC, plants were grown in 600-mL pots containing a 3:1 peat/perlite mixture.

Experimental design and data analysis

A completely random design was applied for each assay, with three replications and plot of a pot. For the SDC treatment, 20 plants per line were grown under the conditions described above. After each reproductive cycle, all harvested seeds were pooled together, and 20 of them were germinated for the next generation. The number of seeds obtained in each cycle was recorded and compared to assess changes in vigor. The number of days necessary to reach the R1 stage was recorded for each plant. Differences between the main effects were compared using the ANOVA.

Germination experiments were conducted on all the genotypes analyzed, with an average of 50 embryos each. Three replications were used for each treatment and germination percentages were compared. Differences between the main effects were compared using the ANOVA.

For the LDC flowering experiments, 10 plants of each line were used for each treatment. Plants were examined daily for the presence of flowers and other possible effects. Differences between means corresponding to the number of days necessary to reach the R1 stage were compared using the ANOVA.

RESULTS AND DISCUSSION

Cycle length under the short-day condition treatment

Immature seeds without dehydration did not germinate (0% gemination) on all the lines tested using the in vitro rescue technique or conventional germinators. In contrast, germination percentage sharply increased to 50% after the dehydration treatment. Using the in vitro embryo rescue technique did not lead to significant differences compared

to the use of conventional germinators; the risk of embryo loss even increased, due to the presence of contamination, since embryo rescue requires sterile growth conditions. No significant differences were observed among genotypes (Table 2), which makes dehydration treatment a critical step in the RGA process.

The duration of the growing cycle was significantly reduced in relation to field conditions. Sowing to the R1 stage (S-R1) took only 28 days for all the soybean lines, and no significant differences were observed among them (Table 3). As was mentioned above, S-R1 in the field takes 57 days for EM, 67 days for MM, and 76 days for LM. Hence, there was a reduction of 29, 39, and 48 days for EM, MM, and LM, respectively. Furthermore, in all cases, it took an additional 24 days to reach stage R5 (stage R1-R5) under SDC. Considering the field duration of 40 days for EM, 53 for MM, and 57 for LM, there was a reduction of 29 for MM and 33 for LM, while there was an increase of 2 days in this stage for EM (Figure 2). The duration of stages from R5-R8 were not evaluated under SDC since once the R5 stage was reached, the pods were harvested and evaluated for their germination capacity.

Table 2. ANOVA for germination capacity depending on the treatment and soybean line

Sources of variation	**df**	**SS**	**MS**	**F**	**Pr** (>F)
Genotype (G)	6	0.80	0.13	0.25	0.95
Dehydration treatment (DT)	3	12,556.50	4,185.50	7,990.81	<2e-16
G x DT	18	6.20	0.34	0.66	0.83
Error	56	29.30	0.52		
Total	83	12,592.80			

Table 3. ANOVA for days from sowing to the R1 stage for all the lines tested, using the SDC approach

Sources of variation	**df**	**SS**	**MS**	**F**	**Pr** (>F)
Cultivar	6	0.23	0.03	0.23	0.96
Error	698	115.30	0.16		
Total	704	115.53			

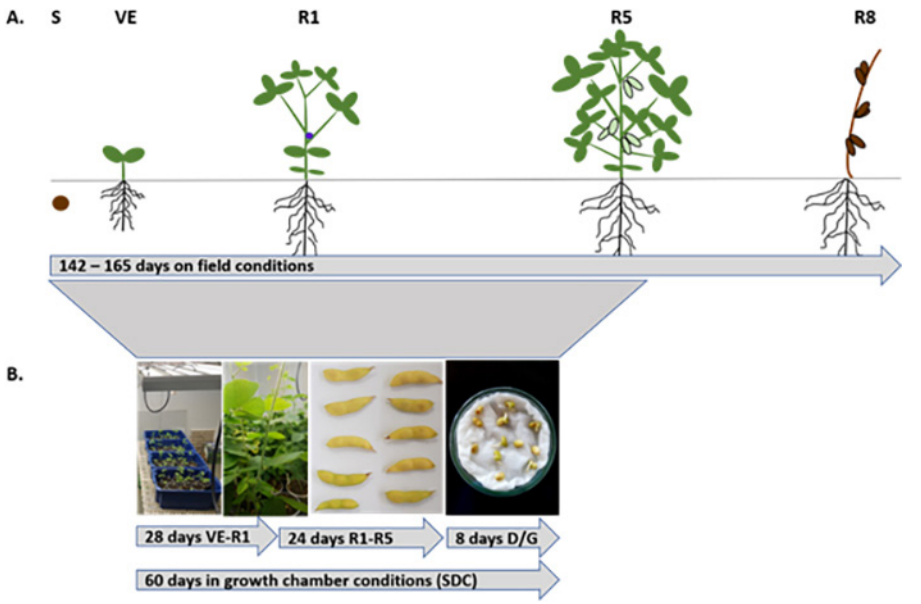

Figure 2. The soybean cycle is reduced by implementation of RGA. **A.** Schematic representation of soybean cycle stages: S, sowing; VE, vegetative; R1: R1 stage, R5: R5 stage, R8: R8 stage. The duration of the total crop cycle under field conditions is indicated below the plant cycle scheme. **B**. Photographs showing the RGA process (from left to right): plants in VE growing in the growth chamber under short-day conditions (SDC); D/G: controlled dehydration and germination. The duration of the cycle under SDC conditions is indicated below the scheme.

Plant vigor after RGA cycles

The seed germination rate and plant vigor were not affected through the five cycles of RGA using the SDC approach. As shown in Figure 3, the number of seeds produced was not affected through the 5 cycles of RGA, and the germination rate was also not affected (data not shown).

Plant growth regulators for inducing early flowering in the plant cycle

The use of different treatments with hormones did not induce early flowering at levels comparable to those obtained using a growth chamber under SDC. Significant differences were observed among genotypes but there were no significant differences in the days necessary to reach the R1 stage between the treated plants and the control plants (Table 4). Nevertheless, different effects on plant development were observed (Figure 4). The BAP treatment did not induce any change in growth or number of flowers (Figure 4A). The GA3 treatment stimulated elongation of the internodes and the number of flowers (Figure 4B). Foliar application of SA led to more robust and bigger plants (Figure 4C).

The application of RGA in breeding programs has proven to be one of the most efficient tools in terms of cost-benefit ratio to increase genetic gain (Bonnecarrere et al. 2019) and accelerate the process of obtaining varieties. Here, we present a method for accelerating breeding of soybean in an inexpensive growth chamber, which greatly reduces the time for each generation and facilitates rapid breeding and research projects. The use of SDC, together with controlled dehydration of immature seeds on intact pods, reduced duration of the total cycle to 60 days, compared to a minimum of 140 days under field conditions, with no effect on plant vigor. Considering this reduction of time and independence

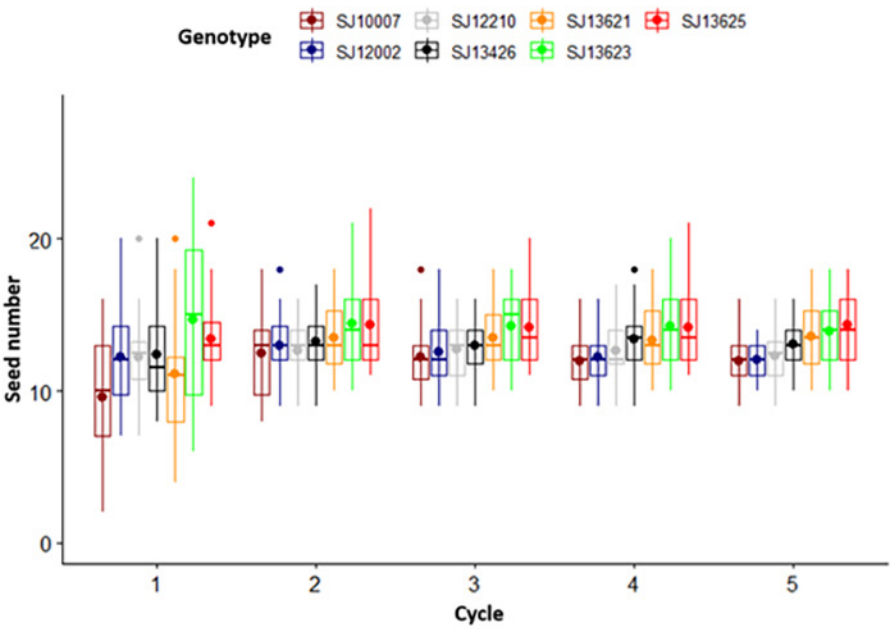

Figure 3. Average number of seeds obtained for each genotype in each RGA cycle (n = 20 for each genotype). Error bar = standard deviation. Different colors represent soybean genotypes.

Table 4. ANOVA for days from sowing to R1 for all the lines tested using the LDC approach

Sources of variation	df	SS	MS	F	Pr (>F)
Genotype (G)	6	534.00	89.00	49.20	< 2e-16
Treatment (T)	3	14.14	4.71	2.54	0.05
G x T	18	20.55	1.14	0.61	0.89
Error	256	474.80	1.85		
Total	283	1043.49			

Figure 4. Soybean plants treated with different plant growth regulators. A. BAP; B. GA; C. SA; D. No treatment.

from atmospheric conditions, the use of RGA makes it possible to carry out up to 5 annual cycles, instead of the 1-2 generations currently possible under field and greenhouse conditions.

In addition, the use of growth chambers, such as the one described in this study, allows significant reduction in costs, since a chamber of these dimensions, which can house up to 150 plants, has the same cost as a single growth chamber used by Nagatoshi and Fujita (2019), which has a capacity of only 12 plants, along with additional costs due to CO_2 supplementation. The use of controlled dehydration along with gemination in conventional germinators means even greater savings on facilities and personnel since the in vitro culture technique for embryo rescue described by Gieco et al. (2007) is not necessary. To further reduce costs, we implemented a LED lighting system that allowed us to achieve energy savings of 88% and to avoid the maintenance costs inherent to the use of light obtained from fluorescent sources.

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REFERENCES

Bonnecarrere V, Rosas J and Ferraro B (2019) Economic impact of marker-assisted selection and rapid generation advance on breeding programs. **Euphytica 215**: 197.

Cobb JN, Juma RU, Biswas PS, Arbelaez JD, Rutkoski J, Atlin G, Hagen T, Quinn M and Eng Hwa Ng (2019) Enhancing the rate of genetic gain in public-sector plant breeding programs: lessons from the breeder's equation. **Theoretical and Applied Genetics 132**: 627-645.

Collard BCY, Beredo JC, Lenaerts B, Mendoza R, Santelices R, Lopena V, Verdeprado H, Raghavan C, Gregorio GB, Vial L, Demont M, Biswas PS, Iftekharuddaula KM, Rahman MA, Cobb JN and Islam MR (2017) Revisiting rice breeding methods – evaluating the use of rapid generation advance (RGA) for routine rice breeding. **Plant Production Science 20**: 337-352.

Cregan PB and Hartwig EE (1984) Characterization of flowering response to photoperiod in diverse soybean genotypes. **Crop Science 24**: 659-662.

Depauw RM and Clarke JM (1976) Acceleration of generation advancement in spring wheat. **Euphytica 25**: 415-418.

Fassio A, Ibañez W, Rodriguez M, Ceretta S, Pérez O, Rabaza C, Vergara G, Cesán A and Restaino E (2017) Predicción de estados fenológicos de soja y otros cultivos de verano. Available at http://www.inia.uy/gras/Alertas-y-herramientas/Utilidades. Accessed on July 25, 2017.

Gieco LC, Caviglia OP and Lassaga SL (2007) Rescate in vitro de embriones inmaduros de soja como técnica para programas de mejoramiento genético. **Revista Científica Agropecuaria 11**: 87-93.

Heisey P, Srinivasan CS and Colin T (2001) Public sector plant breeding in a privatizing world. United States Department of Agriculture. Economic Research Service. Agricultural Information (**Bulletin, 772)**.

Lippmann B and Lippmann G (1993) Soybean embryo: factors influencing plant recovery from isolated embryos. **Plant Cell, Tissue and Organ Culture 32**: 83-90.

Moreira FF, Hearst AA and Cherkauer KA (2019) Improving the efficiency of soybean breeding with high-throughput canopy phenotyping**. Plant Methods 15**: 139.

Nagatoshi Y and Fujita Y (2019) Accelerating soybean breeding in a CO_2-supplemented growth chamber. **Plant and Cell Physiology, 60**: 7784.

Ray DK, Mueller ND, West PC and Foley JA (2013) Yield trends are insufficient to double global crop production by 2050. **Plos One 8**: 66428.

Roumet P and Morin F (1997) Germination of immature soybean seeds to shorten reproductive cycle duration. **Crop science 37**: 521-525.

Sakiyama NS, Ramos HC, Caixeta ET and Pereira MG (2014) Plant breeding with marker-assisted selection in Brazil. **Crop Breeding and Applied Biotechnology 14:** 54-60.

Tilman D, Balzer C, Hill J and Befort BL (2011) Global food demand and the sustainable intensification of agriculture. **Proceedings of the National Academy of Sciences 108**: 20260-20264.

Watson A, Ghosh S, Williams MJ Cuddy WS, Simmonds J and Rey MD (2018) Speed breeding is a powerful tool to accelerate crop research and breeding. **Nature Plants 4**: 23-29.