LONG-TERM COLD SEEDLING STORAGE AS A TOOL IN SOYBEAN BREEDING[1]

CARLOS ROBERTO SPEHAR[2]

ABSTRACT - Breeding soybeans for long-juvenile trait has greatly contributed to expand Cerrado (Brazilian Savannah) cultivation. However, most of the recommended varieties are susceptible to diseases and mineral stresses, and subject of breeding programmes for the crop. Laboratory techniques for screening progenies by non-destructive evaluation can be used in both cases, when large populations are needed. Testing is quite laborious and the transplant of selected seedlings may not be feasible, if data are not analyzed prior to selection. The method described allows storage of selected individuals in cold temperature until all families from crosses have been tested and analyzed. Stored seedlings were kept for two and four weeks, at 5 °C and 95% relative moisture, before transplanted and evaluated at maturity. It has been effective to recover normal plants from long-term cold stored seedlings, and may be successfully used as a tool in soybean breeding.

Index terms: *Glycine max*, variety, cross, population, stress, tolerance, resistance.

ARMAZENAMENTO DE PLÂNTULAS POR LONGO PRAZO COMO INSTRUMENTO NO MELHORAMENTO DA SOJA

RESUMO - O melhoramento da soja para período juvenil longo tem contribuído enormemente para expandir o cultivo nos Cerrados. Contudo, grande parte das variedades recomendadas apresenta as limitações de suscetibilidade a doenças e a estresses minerais, as quais estão incluídas nos programas de melhoramento. Normalmente, no deselvolvimento varietal usam-se técnicas para a triagem de progênies por métodos não destrutivos, mediante o uso de grandes populações. A rotina pode não ser exequível no transplante das plântulas selecionadas, quando os dados necessitam ser analisados antes da tomada de decisão. O método presentemente descrito consiste no armazenamento dos indivíduos selecionados em câmaras frias até que todas as famílias por cruzamentos tenham sido testadas e analisadas. A armazenagem, entre duas a quatro semanas, a 5 °C e 95% de umidade relativa, mostrou não afetar a obtenção de plantas normais e pode ser utilizada como instrumento no melhoramento da soja.

Termos para indexação: *Glycine max*, variedade, cruzamento, população, estresse, tolerância, resistência.

INTRODUCTION

Cultivated soybeans [*Glycine max* (L.) Merrill] has greatly contributed for profitable cultivation of the Brazilian Savannahs (Cerrados). The breeding for long-juvenile trait has resulted in high yielding varieties adapted to the low latitudes of the tropics (Kiihl & Garcia, 1989). Their performance is comparable to the one obtained in the temperate zones of the world (Spehar, 1994c). In addition, breeding for other important traits such as disease resistance and mineral stress tolerance has contributed to the full adaptation of the crop (Spehar, 1994c).

Aluminium tolerance and efficiency in calcium utilization are two desirable characters in soybeans. They are responsible for deeper rooting of soybeans when cultivated in the acidic cerrado soils, and results in better exploitation of water and nutrients, leading to yield stability. Therefore, selection for these traits is necessary in crop improvement (Spehar, 1994b).

[1] Accepted for publication on July 18, 1995

[2] Eng. Agr., Ph.D., EMBRAPA - Centro de Pesquisa Agropecuária dos Cerrados (CPAC), Caixa Postal 08223, CEP 73301-970, Planaltina, DF, Brazil.

Mineral disorders are effectively studied in laboratory, under controlled environment. The advantages of using the technique are: i) lack of confoundment with other environmental factors and ii) possibility of advancing selected individuals for progeny test (Foy et al., 1993; Spehar, 1994a). Similar approach is effective for disease resistance (e.g., *Diaporthe phaseolorum* f. sp. *meridionalis*).

To select for desirable traits, testing of large populations may be necessary and hybrid seeds can be obtained by clonal propagation of F_1's on soybeans (Spehar & Galwey, 1990). Normally, the tests are quite laborious and the transplant of selected seedlings may not be feasible to generate desirable progenies for further evaluation in breeding programmes. Moreover, time may be spent on data analysis before selection can be made on individually tested plant.

The present study aimed to assess the length of time seedlings can be kept viable in cold storage before being transplanted to generate new progenies from selected plants in large populations.

MATERIAL AND METHODS

Seedlings of three soybean breeding lines, namely, BR 86-7485, BR 86-5947 and BR 86-7458, previously tested for root growth in high aluminium and in low calcium nutrient solution experiments, were stored in a dark and cold chamber, in the dark, at 5 °C and 95% R.H. Two lots of seedlings were kept for two and four weeks, respectively, in these storage conditions. Seedlings obtained by germinating seeds of the same lines, without any testing and not exposed to cold storage, were also used as a control. To prevent plants from early flowering, the day length was artificially prolonged to 16 h during 30 days by maintaining 200 W incandescent light. Under this light regime, vegetative phase can be kept indefinitely on tropical selected soybeans (Spehar & Galwey, 1990).

Field evaluations were carried out, during the dry season and supplemented with irrigation, on a dark red latosol (a variety of oxisol classified as typic Haplustox, fine, kaolinitic, isohyperthermic by the U.S. soil taxonomy), at the Centro de Pesquisa Agropecuária dos Cerrados (CPAC), Planaltina, Brazil, which is located 15°36' S and 47°12' W at an elevation of 1,000 m a.s.l. The area was previously limed and fertilized. The chemical analysis, after the experiment, indicated pH (H_20 1:1) 5.9; Al 0.05 cmol/kg; Ca+Mg 6.58 cmol/kg; P 9.5 mg/kg; K 54 mg/kg. Nitrogen was mostly supplied to the plants through symbiotic association with *Bradyrhizobium japonicum* bacteria strains selected for cerrado soils. The following characteristics were evaluated for both stored and non-stored seedlings: plant height, number of nodes, pods and grains. Analysis of variance was carried out to assess if there were differences due to cold storage treatment.

RESULTS AND DISCUSSION

Plant height and number of nodes, pods and grains on individual plants originated from four-week cold-stored seedlings for the three genotypes are shown in Fig. 1.

There were no significant differences in the agronomic characters between plants originated from seeds and both cold stored treatments and between the two periods of cold storage. Under short-day condition (dry season), the plants were shorter in height and cycle, indicating that they were responsive

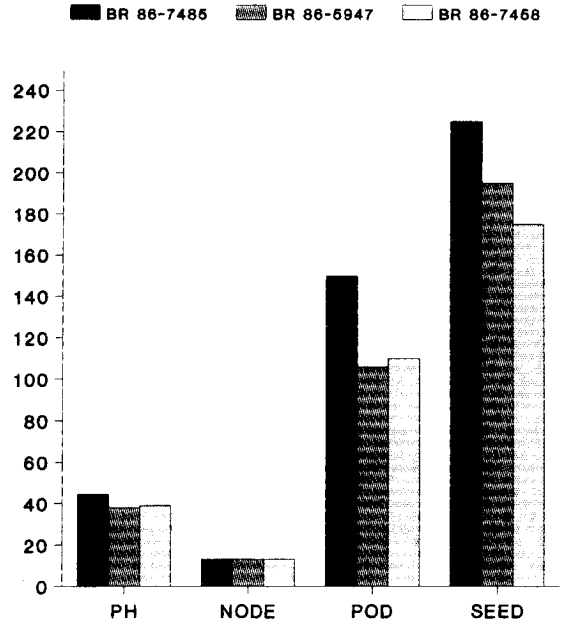

FIG. 1. Plant height (PH) in cm and number of nodes, pods and seeds of plants from three soybean breeding lines recovered from four-week cold-stored seedlings tested for mineral stress.

to the short day length. The breeding lines did not seem to be affected by the cold stress storage at this early stage of plant development; the number of seeds per plant was sufficiently large for successive tests in the next generations. It is expected that appropriate light supply to extend day length will further delay flowering and a larger number of seeds shall be produced.

This technique can be successfully employed in breeding programmes when large numbers of seedlings are tested at once and superior individuals cannot be identified promptly, such as in mineral stress tests (Spehar, 1994a). Also, this technique will make possible selected seedlings for tolerance to mineral stresses be evaluated to disease reaction or other traits to increase the efficiency of selection (tandem selection). It should be pointed out that the treatment for cold storage during four weeks resulted in minor etiolation of the first node that did not affect plant growth. About 15% of the transplanted seedlings died. To reduce the rate of failure, it is suggested that cold stressed seedlings be hardened by exposing them to increasing temperatures and gradually reducing moisture. It is also suggested that further testing be carried out by extending the length of storage to verify the limit at which seedlings would tolerate cold storage and still being recovered normal plants for progeny testing.

CONCLUSION

Soybean seedling cold storage for long periods is a feasible method to use in screening for mineral stresses and disease resistance and may turn into a useful technique in breeding programmes that use early progeny testing.

ACKNOWLEDGEMENTS

The author thanks the CNPq (Brazilian National Research Council) for providing financial support to research, and scholarship to Mr. L. A. C. Souza, who efficiently helped conducting the experiment, collecting and analyzing the data.

REFERENCES

FOY, C.D.; CARTER, T.E.; DUKE, J.A.; DEVINE, T.E. Correlations of shoot and root growth and its role in selecting for aluminium tolerance in soybean. **Journal of Plant Nutrition**, v.16, p.305-325, 1993.

KIIHL, R.A.S.; GARCIA, A. The use of long-juvenile trait in breeding soybean cultivars. In: WORLD SOYBEAN RESEARCH CONFERENCE, 4., 1989, Buenos Aires. **Proceedings**. Buenos Aires, Argentina: AASOJA. 1989. v.2, p.994-1000.

SPEHAR, C.R. Aluminium tolerance of soya bean genotypes in short term experiments. **Euphytica**, v.76,n.1/2,p.73-80, 1994a.

SPEHAR, C.R. Breeding soybeans to the low latitudes of the Brazilian cerrados (Savannas). **Pesquisa Agropecuária Brasileira**, Brasilia, v.29,n.8,p.1167--1180, 1994c.

SPEHAR, C.R. Screening soybean germplasm for aluminium tolerance using cluster analysis. **Pesquisa Agropecuária Brasileira**, Brasília, v.29, n.1, p.113-122, 1994b.

SPEHAR, C.R.; GALWEY, N.W. Clonal propagation of F1 hybrids as a tool in genetic studies of the soya bean [*Glycine max* (L.) Merrill]. **Euphytica**, v.47, p.21-23, 1990.