Paper 5

HYBRIDISATION OF SOYBEAN WITH ITS DIPLOID WILD PERENNIAL RELATIVES

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ABSTRACT

Morphological and cytological details of the first cross between soybean and its diploid wild perennial relatives are described. This hybridisation was made using a fertile hybrid (2n = 40) between two wild species, *Glycine argyrea* and *G. canescens,* as the female parent and the soybean cultivar Improved Pelican as the male parent. Colchicine doubling of this sterile hybrid resulted in almost complete chromosome pairing — an average of 38.08 bivalents out of a possible 40 at pollen mother cell meiosis. Unfortunately this did not result in pollen fertility. Backcrossing the doubled hybrid with soybean led to some pod initiation but mature seed was not produced.

KEYWORDS

Glycine argyrea, Glycine canescens, Glycine max, colchicine, tissue culture, backcrossing.

INTRODUCTION

Soybean, *Glycine max,* is the world's most important oilseed crop with major growing areas in the USA, Brazil, and Asia. The genus *Glycine* consists of two subgenera, *Glycine* and *Soja* (Table 1). Soybean, *G. max,* and its immediate progenitor, *G. soja* Sieb. and Zucc. are classified in the subgenus *Soja.* These species are annuals, have a chromosome number 2n = 40 and originate from China. The subgenus *Glycine* consists of nine described perennial species, *G. canescens, G. clandestina, G. falcata, G. latifolia, G. latrobeana, G. tabacina, G. tomentella, G. argyrea, and G. cyrtoloba.* All members of this subgenus are indigenous to Australia, all have chromosome number 2n = 40 except *G. tabacina* with 2n = 40,80, and *G. tomentella* with 2n = 38, 40, 78, 80. These two latter species also occur outside Australia. The wild perennial *Glycine* species encompass a broad range of morphological and cytological variability and represent a potentially valuable

Table 1. The genus Glycine.

Species	Chromosome no.	Distribution
A. Subgenus *Glycine* (perennials)		
G. canescens F.J. Herm	40	Dryland Australia
G. clandestina Wendl.	40	NE & SE Australia
G. latifolia (Benth.) Newell & Hymowitz	40	NE Australia
G. latrobeana (Meissn.) Benth.	40	SE Australia
G. falcata Benth.	40	Queensland, Northern Territory
G. cyrtoloba Tind.	40	NE Australia
G. argyrea Tind.	40	S. Queensland Coast
G. tabacina (Labill.) Benth.	40,80	NE & SE Australia, Taiwan, S. China, South Pacific Islands
G. tomentella Hayata	38,40,78,80	NE Australia, Papua New Guinea, Taiwan
B. Subgenus *Soja* (annuals)		
G. soja Sieb. & Zucc.	40	China, Taiwan, Japan, Korea, USSR
G. max (L.) Merr.	40	Cultigen

source of germplasm for soybean breeders. This germplasm has so far only been available from tissue culture because embryos of crosses between the subgenera inevitably abort, primarily because of endosperm failure. Using ovule and embryo culture techniques, the first hybrids between *G. max* and its wild perennial relatives were obtained using the wild species parent at the tetrapoloid chromosome level. Broué *et al.* (1982) used a synthetic amphiploid (2n = 78) between *G. tomentella* (2n = 38) and *G. canescens* (2n = 40), while the other reported hybrids (Newell and Hymowitz 1982, Singh and Hymowitz 1985) used *G. tomentella* (2n = 78, 80) as the wild species parent. All hybrids were sterile and colchicine doubling of the hybrids produced by Broué *et al.* (1982) failed to restore fertility.

Recently, success has been achieved in obtaining a hybrid with soybean at the diploid level. The female parent was a fertile hybrid between *G. argyrea* and *G. canescens* with the *G. max* cultivar Improved Pelican as male parent (Brown *et al.* 1985, Grant *et al.* 1986). *G. argyrea* Tind. is a newly described species which was first discovered in 1982. It hybridises and forms fertile interspecific hybrids more readily than the other perennial species. It was thought that *G. argyrea* may act as a bridge in obtaining diploid crosses with *G. max* and doubling such crosses might be more successful for restoring fertility. This paper describes the morphological and cytological characteristics of the hybrid (*G. argyrea* x *G. canescens*) x *G. max,* the results of colchicine doublng and attempts to backcross the colchicine doubled hybrid.

MATERIALS AND METHODS

The accession of *G. argyrea* used was G1420 collected from Rainbow Beach in Queensland. Its herbarium specimen is held at the Australian National Herbarium (CANB. No. 309110). *G. canescens,* G1232 was collected at Cobham Lake in New South Wales (CANB. No. 264073). Cytological, tissue culture and colchicine doubling methods are as described in Grant *et al.* (1986).

For grafting, soybean plants were grown until the first trifoliate leaves expanded in a temperature regulated glasshouse (24°C day/15°C night). These young plants were used as stocks and were grafted by oblique cleft grafting using a 3 cm drinking straw slit down one side and sealed with masking tape to hold the graft together. The scion were growing tips of the doubled hybrid which were 8-16

Table 2. Some morpological comparisons between diploid perennial Glycine, soybean and their hybrid.

	G. arg.[1] x *G. can.*[2] G1420 x G1232	(*G. arg.* x *G. can.*) x *G. max* (G1420 x G1232) x Improved Pelican	*G. max* cv. Improved Pelican
Growth habit	twining, perennial	twining, perennial	erect, annual
Terminal leaflet shape	narrowly oblong	lanceolate	ovate
Stipules			
— shape	triangular	triangular	triangular
— length mm	2.0	3.5	6.0
— indumentum	hairs white, appressed and ascendant	hairs white, ascendant	hairs white, ascendant
Indumentum			
— lower leaf	sericeous, hairs white, appressed, moderately dense, c. 0.5 mm long	hirsute, hairs white, appressed, moderately dense, c. 0.8 cm long	hirsute, hairs white appressed, moderately dense, c. 1.0 mm long
— midrib of lower leaf	hairs white and pale brown, appressed	hairs white and pale brown, ascendant	hairs white, erect
— margins	hairs white and pale brown, appressed	hairs pale brown, appressed and ascendant	hairs pale brown, appressed and ascendant
— stem	retrorse hairs white and pale brown, appressed	retrorse hairs white, ascendant	retrorse hairs pale brown, suberect
— calyx	hairs white and brown, ascendant	hairs white and pale brown, ascendant	hairs white, suberect

[1] *G. arg.* — *Glycine argyrea.*
[2] *G. can.* — *Glycine canescens.*

cm long. The pots of grafted plants were covered with a plastic bag for two to three weeks and uncovered on a cloudy day.

RESULTS

The F_1 hybrid between (*G. argyrea* x *G. canescens*) x *G. max* is a very vigorous twining perennial. Some morphological comparisons of the hybrid and each of its parents are given in Table 2. The hybrid shows some intermediate characters, e.g. leaf shape, stipule and hair length; some distinctly wild species characters, e.g. the twining growth habit; and some soybean characters, e.g. indumentum type. As might be expected the plant is sterile and pollen mother cell meiosis on 33 cells showed an average of 2.61 bivalents (range 1-6) and 34.79 univalents. Cuttings of this cross were doubled using colchicine and pollen mother cell meiosis of the resulting plant showed an average of 38.08 bivalents and 3.84 univalents. The chiasmata appeared to terminalise. In spite of this high bivalent formation, no stainable pollen was observed. Grafting the doubled hybrid as scions onto soybean stocks, using cultivars Improved Pelican and Ross, was successful but resulted in no change in pollen fertility. However, backcrossing using soybean pollen led to pod initiation in four out of 100 crosses. The resulting ovules were extremely small and could not be successfully cultured.

DISCUSSION

The accessibility of the useful agronomic traits in the perennial *Glycine* will be limited until fertility problems associated with this wide hybridisation are overcome. At this stage there are several possible explanations. One is that development may be environmentally determined on both the male and female side, as in some *Arachis* interspecific hybridisation (Singh and Moss 1984). Therefore changing the environment will change the fertility. Secondly, incompatibility may be due to the genotypes of species involved which produce an array of hybrids with differing fertility. Thirdly, there may be some genic/genomic incompatibility. The incomplete pairing of the doubled hybrid may indicate some chromosomal sterility (Stebbins, 1958) although interspecies hybrids in the genus *Glycine* showing similar pairing patterns would produce seed. Therefore, it appears more likely that the fertility problem is genic and could be associated with one, two, or all of the species involved in this cross. Nuclear male sterility genes have been recognised in a number of *Glycine max* cultivars but not yet in the wild species. It therefore appears that a range of hybrid material from reciprocal crosses could provide further information on reasons for infertility.

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