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Intersubgeneric hybridization of soybeans with a wild perennial species, *Glycine clandestina* Wendl

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Summary. The exploitation of wild perennial species of subgenus *Glycine* has been formidable in soybean breeding programs because of extremely poor crossability and an early pod abortion. The combination of gibberellic acid application to hybridized gynoecia and improved seed culture media formulations resulted in a new intersubgeneric hybrid between *Glycine max* (L.) Merr. ($2n=40$) and *G. clandestina* Wendt. ($2n=40$). Of the 31 immature seeds cultured, 1 regenerated 21 plants through organogenesis while the remaining 30 failed to germinate. All the regenerated plants were similar morphologically, carried expected $2n=40$, possessed hybrid isozyme patterns and were completely sterile. Complete absence of chromosome pairing was observed in 40.9% sporocytes. The occurrence of 1 to 6 loosely paired rod bivalents suggests some possibilities of allosyndetic pairing. Hybrid plants set aborted pods after backcrossing to *G. max*.

Key words: *Glycine max* – *G. clandestina* – Wide hybridization – Seed culture

Introduction

The exploitation of wild perennial species of subgenus *Glycine* has been formidable in soybean breeding programs because of extremely poor crossability and an early pod abortion (Palmer and Hadley 1968; Ladizinsky et al. 1979). Therefore, incorporation of useful alien traits from wild perennials to soybean has not been feasible so far, compared to other crop species (see Harlan 1976; Hadley and Openshaw 1980; Stalker 1980). Wide hybridizations in the genus *Glycine* are sporadic and have been limited to a few sterile hybrid combinations (Broué et al. 1982; Newell and Hymowitz 1982; Singh and Hymowitz 1985a; Brown et al. 1985; Newell et al. 1986). Repeated attempts to generate BC1 progeny by crossing colchicine induced amphiploid ($2n=118$) (*G. max* (L.) Merr. ($2n=40$) × *G. tomentella* Hayata ($2n=78$)) with soybeans have been unsuccessful (Singh and Hymowitz, unpublished results).

Pod abortion in wide crosses of *Glycine* is a post-fertilization problem and pod retention can be improved with the application of growth hormones to the hybridized gynoecia (Singh and Hymowitz 1987). Furthermore, an improved seed rescue procedure over Newell and Hymowitz (1982), reported in this study, facilitated the recovery of several new intersubgeneric hybrids not reported before. In this paper we report the origin, morphology, cytology, and breeding behavior of one of the hybrids, *G. max* ($2n=40$) × *G. clandestina* ($2n=40$).

Materials and methods

Six soybean (*Glycine max*) cultivars 'Altona', 'Bonus', 'Clark 63', 'Essex', 'Williams', 'Wye', and one breeding line T31 were utilized in this study as females and a wild perennial species *G. clandestina*, P.I. 440948, was the male parent. Plant introduction 440948 regenerates plants from hypocotyl, cotyledons, and leaf explants through organogenesis (Hymowitz et al. 1986). This particular line was selected with the objective of transferring it's plant regenerability trait to soybeans. Plant growing conditions, crossing procedure, and cytological techniques described by Singh and Hymowitz (1985b) were used. Putative hybrid gynoecia were sprayed with 70 ppm gibberellic acid (Singh and Hymowitz 1987).

All the crossed pods aborted 14–29 days after pollination (DAP). Immature seeds were extracted under sterile conditions and cultured. The media used for immature seed rescue

Table 1. Composition of the media used for immature hybrid seed rescue, all in mg/l

Constituent	Medium A	Medium B	Medium C	Medium D
KNO_3	3,000	2,500	2,000	80
$(NH_4)_2SO_4$	134	134	–	–
NH_4NO_3	1,650	–	1,000	–
$NaH_2PO_4 \cdot H_2O$	150	150	100	16.5
KH_2PO_4	170	–	300	–
$MgSO_4 \cdot 7H_2O$	250	250	375	–
$MgSO_4 \cdot H_2O$	–	–	–	576
Na_2SO_4	–	–	–	200
$CaCl_2 \cdot 2H_2O$	150	150	600	–
$Ca(NO_3)_2$	–	–	–	432
H_3BO_3	3.0	3.0	3.0	1.5
$MnSO_4 \cdot H_2O$	10.0	10.0	10.0	7.0
$ZnSO_4 \cdot 7H_2O$	2.0	2.0	2.0	3.0
KI	0.75	0.75	0.75	0.75
$CuSO_4 \cdot 5H_2O$	0.025	0.025	0.025	0.1
$Na_2MoO_4 \cdot 2H_2O$	0.25	0.25	0.25	0.01
$COCl_2 \cdot 6H_2O$	0.025	0.025	0.025	–
KCl	–	–	–	65.0
EDTA	–	–	26.1	–
$Na_2 \cdot$ EDTA	37.3	–	–	–
Fe · NaEDTA	–	73.4	–	73.4
$FeSO_4 \cdot 7H_2O$	27.8	–	24.9	–
KOH	–	–	15.035	–
Inositol	100	100	250	100
Nicotinic acid	1.0	1.0	1.0	1.0
Pyridoxine. HCl	1.0	1.0	1.0	1.0
Thiamine. HCl	10	10	10	1.0
Glycine	2.0	2.0	2.0	4.0
Ascorbic acid	100	50	–	–
L-Glutamine	7,300	–	–	–
Coumarin	–	–	–	9.0
IAA*	0.2	0.3	–	1.0
NAA*	2.0	–	–	–
Kinetin	1.28	0.75	–	–
BAP*	0.5	–	0.25	–
Sucrose	100,000	30,000	25,000	10,000
Bacto-agar	8,000	8,000	6,000	8,000
pH	5.8	5.8	5.6	6.0

A = B-5 medium of Gamborg et al. (1968) as modified by Newell and Hymowitz (1982);
B = B-5 medium of Gamborg et al. (1968);
C = PC-L2 medium of Phillips and Collins (1979) as modified by Meyer (person commun);
D = Medium of White (1963) as modified by Hymowitz et al. (1986)
* IAA, indole-3-acetic acid; NAA, 1-naphthalene acetic acid; BAP, 6-benzylaminopurine

were derived from the modification of several media formulations (Table 1). All cultures were incubated at 25 ± 1 °C, with a 16 h photoperiod under cool white fluorescent tubes (Ca 45 $\mu EM^{-2} s^{-1}$) in a Percival model LVL incubater. Furthermore, seeds were transferred to fresh medium every 2 weeks. Depending upon hybrid combinations, immature seeds were kept on maturation medium (designated A) for 4–10 weeks. During this period, they either began germination or callused. Both types of seeds were transferred to germination medium (designated B) for 4–6 weeks. When the shoot in a germinating seed was completely developed, it was transferred to rooting medium (designated D) (Table 1). When multiple shoot bud differentiation was recorded in calluses, they were moved to shoot proliferation medium (designated C) (Table 1). When these shoots were completely developed, they were excised and transferred to D-medium (Table 1). Plantlets were transplanted to small pots containing sterile vermiculite and peat moss (3:1), and were fertilized twice a week with half strength Hoagland's Solution (Kartha 1975). Parents and hybrid plants were also examined for phosphoglucose mutase (PGM) banding patterns. Protein was extracted from the healthy leaves and isozyme gel electrophoresis was performed according to Cardy and Beversdorf (1984).

Results and discussion

Early pod abortion in intersubgeneric crosses of the genus *Glycine* is attributed to post-fertilization incom-

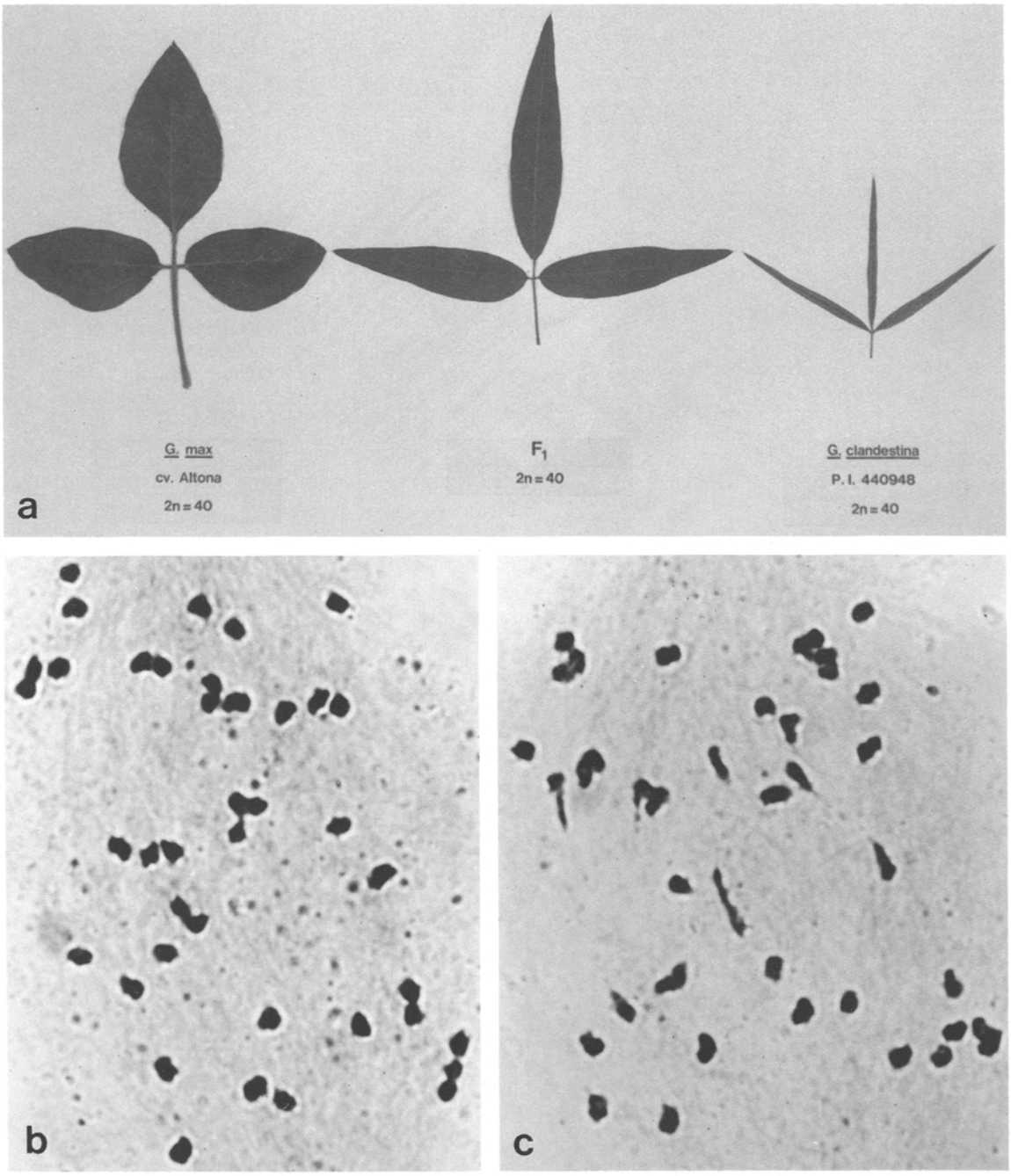

Fig. 1a. Herbarium leaf specimen of intersubgeneric hybrid of *Glycine max* cv Altona female (left), *G. clandestina* (P.I. 440948) male (right) and the hybrid (middle). **b, c** Meiotic metaphase I chromosome of F_1 hybrid, *G. max* ($2n = 40$) × *G. clandestina* ($2n = 40$) showing **b** 40I and **c** 34I + 3II

patibility. However, the application of growth hormones to hybridized gynoecia controls pod abortion apparently by inhibiting the formation of the abscission layer (Singh and Hymowitz 1987). Pod-set in the crosses between soybean (*G. max*) cultivars female × *G. clandestina,* P.I. 440948 male ranged from 0.0% to 30.8% (Table 2). A total of 15 aborted pods yielded 31 seeds. One seed generated 21 plants through organogenesis while the remaining thirty seeds became necrotic. Seed inviability, seedling lethality and vegetative lethality are rather common in wide hybridizations (Stebbins 1958; Hadley and Openshaw 1980).

An extremely low frequency of hybrid seed rescue through in vitro technique prompted us to re-examine

Table 2. Crossability and seed germination, in vitro between soybean cultivars female × *G. clandestina* (P.I. 440948) male

Soybean cultivars used as female	No. florets pollinated	Pot-set (%)	No. seeds cultured	Germination
Altona	54	9 (16.7)	20	1
Clark 63	11	0 (0.0)	0	0
Essex	4	0 (0.0)	0	0
Wye	13	4 (30.8)	8	0
Williams	18	1 (5.6)	2	0
Bonus	19	0 (0.0)	0	0
T31	7	1 (14.3)	1	0
Total	126	15 (9.6)	31	1

Table 3. Effect of two media on germination of immature seeds of four species of genus *Glycine*

Species[a]	No. seeds cultured	Germination (%)	
		N and H[b] medium	Present medium
CAN ($2n=40$) P.I. 505159	40[c]	30	100
MIC ($2n=40$) P.I. 440956	40	15	55 + 15[d]
TOM ($2n=80$) P.I. 446956	40	35 + 5[e]	75 + 5[e]
MAX ($2n=40$) cv. Bonus	40	5	65 + 20[e]

[a] CAN = *G. canescens*, MIC = *G. microphylla*, TOM = *G. tomentella*, MAX = *G. max*
[b] N and H medium = Newell and Hymowitz (1982)
[c] 20 seeds per medium
[d] Seeds devoid of embryo
[e] Callused seeds

the efficacy of the media formulations utilized in this study (Table 1) and also compare it with the media of Newell and Hymowitz (1982) utilized in previous investigations (Singh and Hymowitz 1985a, b, c). The response of immature seeds (21 DAP) of three wild perennial species and one soybean cultivar 'Bonus' to two media formulations is presented in Table 3. The immature seeds responded better in the media compositions listed in Table 1 than to the media of Newell and Hymowitz (1982); the majority of seeds became necrotic in the latter. The two media differ for the composition of organics. Newell and Hymowitz's seed maturation medium does not contain ascorbic acid, glutamine, IAA, NAA, and is a liquid medium. Differences in immature seed germination in vitro among species observed in this study may be ascribed to physiological and developmental maturity of the seeds; for example, 15% of seeds had no embryo in *G. microphylla* Tind. (Table 3). Therefore, it is reasonable to assume that some of the intersubgeneric hybrid seeds lost germinability in culture may have lacked an embryo. Recently, McCoy and Smith (1986) also reported a low recovery of plants in interspecific crosses of *Medicago*.

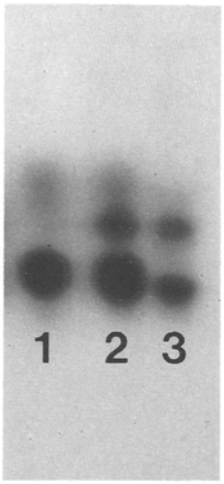

Fig. 2. Phosphoglucosemutase (PGM) banding pattern for *G. max* cv Altona female (lane 1), F_1 hybrid (lane 2) and *G. clandestina* (P.I. 440948) male (lane 3)

The *G. max* × *G. clandestina* F_1 hybrids were identified morphologically (Fig. 1a, Table 4), cytologically (Fig. 1b, c) and by isozyme phenotype (Fig. 2). Morphologically, all 21 regenerated hybrid plants were similar, displayed the twining features of the male parent, carrying flowers at the axillary nodes like the female parent, and with shorter raceme with fewer flowers than those observed in *G. clandestina*, P.I. 440948. They were intermediate between both parents for several other morphological traits (Fig. 1a, Table 3).

All the regenerated plants carried the expected $2n=40$. Chromosome configurations at metaphase I in 15 of the 21 plants analyzed varied from plant to plant (Table 5). The mean pairing frequencies (%) of 15 plants revealed complete absence of chromosome synapsis (range) in 40.9% (14.3–70.8) sporocytes (Fig. 1b). The observed 1 to 6 loosely paired rod bivalents in varying frequencies suggests some possibilities of allosyndetic pairing (Fig. 1c). However, this cannot be substantiated because chromosomes of both species are indistinguishable.

Since chromosomes remained asynaptic in majority of sporocytes of *G. max* × *G. clandestina* F_1 hybrid, it was anticipated that fertility would be restored due to unreduced functional gamete formation (Harlan and de Wet 1975). The pod initiation, elongation and retention in hybrid plants after selfing and back crossing to *G. max* were observed, but seeds were small. Immature seeds are being cultured out. Furthermore, we are attempting to restore fertility by doubling the chromosomes with colchicine.

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Table 4. Vegetative morphology of *Glycine max* cv Altona, F_1 hybrid and *G. clandestina* (P.I. 440948), measurements in mm

Morphological traits	Altona	F_1 hybrid	P.I. 440948
Upper term. leaflet shape	lanceolate	elleptic-lanceolate	linear
Upper term. leaflet length	89	88	72
Upper term. leaflet width	48	18	5
Upper lateral leaflet length	73	66	61
Upper lateral leaflet width	48	16	6
Leaves	pinnate	pinnate	digitate
Rachis length	11	4	0
Petiole length	77	32	18
Stipule length	3	2	2
Upper internode length	175	95	64

Table 5. Average chromosome configuration (%) in regenerated plants ($2n=40$) of *G. max* ($2n=40$) and *G. clandestina* ($2n=40$) hybrid

Plant no.	Chromosome configurations (%)							Total PMC
	I	38I + 1II	36I + 2II	34I + 3II	32I + 4II	30I + 5II	28I + 6II	
1	40.6	32.8	15.6	9.4	0.0	1.6	0.0	64
2	70.8	20.8	8.3	0.0	0.0	0.0	0.0	24
3	42.4	24.2	18.2	6.1	3.0	3.0	3.0	33
4	40.0	40.0	12.0	4.0	4.0	0.0	0.0	25
5	46.7	46.7	6.7	0.0	0.0	0.0	0.0	15
6	51.3	17.9	7.7	23.0	0.0	0.0	0.0	39
7	50.0	34.0	12.0	0.0	0.0	4.0	0.0	50
8	35.0	20.0	30.0	15.0	0.0	0.0	0.0	20
9	44.0	20.0	20.0	4.0	12.0	0.0	0.0	25
10	20.0	27.5	30.0	10.0	7.5	0.0	5.0	40
11	14.3	35.7	23.8	9.5	9.5	7.2	0.0	42
12	50.0	15.0	25.0	10.0	0.0	0.0	0.0	20
13	26.7	26.7	20.0	20.0	0.0	6.6	0.0	15
14	45.0	40.0	10.0	5.0	0.0	0.0	0.0	20
15	36.4	54.5	0.0	9.1	0.0	0.0	0.0	22
Average	40.9	30.4	16.0	8.3	2.4	1.5	0.5	454

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