

I- F Development of Hybrids between Wild Perennial Soybeans and *Glycine max* (L.) Merr. and Resistance to *Phakopsora pachyrhizi*

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The alien species relatives to cultivars are currently being utilized for increasing genetic variability particularly in cereals (20). However, the exploitation of 16 wild perennial species of the subgenus *Glycine* Willd, [*G. albicans* Tind.

and Craven, *G. arenaria* Tind., *G. argyrea* Tind., *G. canescens* F. J. Herm., *G. clandestina* Wendl., *G. curvata* Tind., *G. cyrtoloba* Tind., *G. falcata* Benth., *G. hirticaulis* Tind. and Craven, *G. lactovirens* Tind. and Craven, *G. latifolia* (Benth.) Newell and Hymowitz, *G. latrobeana* (Meissn.) Benth., *G. microphylla* Tind., *G. pindanica* Tind. and Craven, *G. tabacina* (Labill.) Benth., *G. tomentella* Hayata] to improve their cultivated counterpart, soybean [*G. max* (L.) Merrill], has been hampered due to an extremely low intersubgeneric crossability and the lack of efforts from the scientific community. Thus far, only a few intersubgeneric sterile F₁ hybrids have been produced (1, 2, 16, 17, 23, 24).

The genetic base of American public soybean cultivars is extremely narrow (4, 5, 11). The ancestors of 258 public cultivars released between 1947 to 1988 are traced back to 80 introductions and cultivars. Moreover, more than 50% of the genetic base for the North American soybean is contributed by only six ancestors and 94% of the genes in the Southern American cultivars are constituted by merely 17 ancestors (5). A majority of the introductions originated from the same geographical area (4). Because of narrow genetic base of soybean, an outbreak of pests and diseases for which no source of resistant soybean germ plasm is available could be catastrophic. Thus, soybean breeders should exploit the tertiary gene pool (GP-3) described by Harlan and de Wet (7), i.e. wild perennial species of the subgenus *Glycine*.

Wild perennial *Glycine* spp. harbor several useful genetic traits such as resistance to rust (3, 9, 19) and brown spot (14), tolerance to salt (12) and certain herbicides such as 2,4-D (8) and glyphosate (15), and regenerability of plants from cell and tissue cultures (6, 10, 18). These

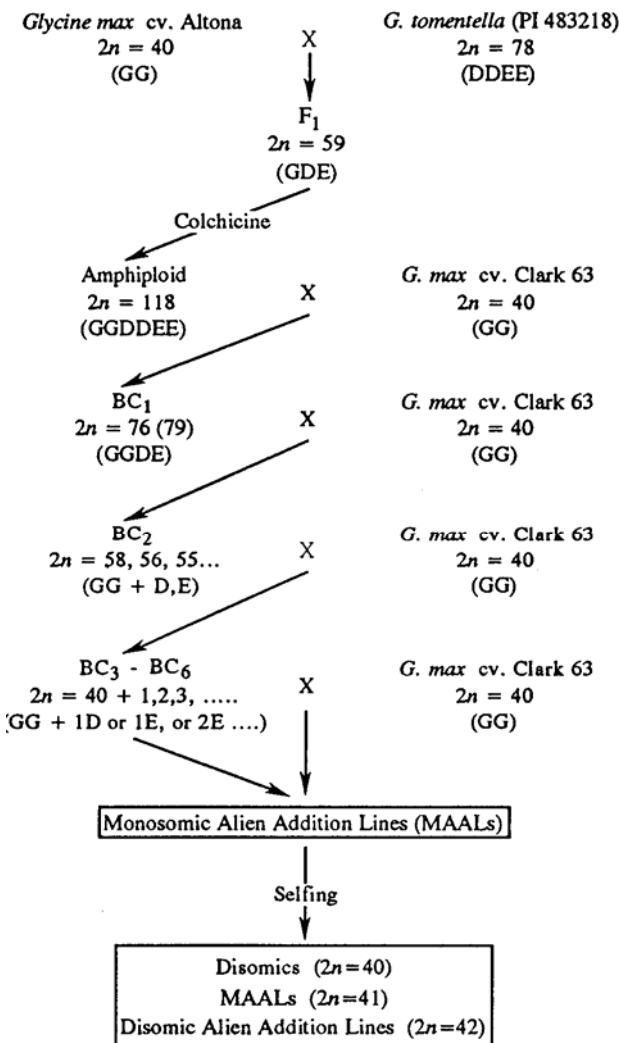


Figure 1. Diagram showing the scheme used to isolate plants containing complete chromosome complement (2n=40, GG) of the soybean cv. Clark 63 plus one or two chromosomes from *Glycine tomentella*.

economically valuable characteristics could be transferred to soybean germ plasm through intersubgeneric hybridization and backcross procedures.

Singh *et al.* (25, 26) produced, for the first time, fertile lines with 2n= 40, 41, 42, 43, 44 chromosomes from an amphidiploid (2n=118, genome GGDDEE) of *G. max* (2n=40, genome GG) x *G. tomentella* (2n=78, genome DDEE) (Figure 1). This study sets a stage for the feasibility of gene introgression from the wild perennial *Glycine* spp. to the soybean to broaden the soybean genetic base.

MATERIALS AND METHODS

Figure 1 describes schematic diagram for isolating disomic (2n=40), monosomic alien addition lines (2n= 41; MAALs), and disomic alien addition lines (2n=42; DAALs) from an intersubgeneric hybrid of soybean (2n=40) and *G. tomentella*, PI 483218, 0 1978, IL 428 (2n=78). A similar approach could be followed to obtain several other intersubgeneric hybrids involving wild perennial *Glycine* species carrying gene(s) for rust resistance or for other valuable traits. Fl 483218 carries a moderately resistant gene for rust resistance (9). However, Schoen *et al.* (19) found T1 group tomentellas which

includes PI 483218 carry a single dominant gene for resistance to all three Australian isolates of rust.

All plants were grown in the greenhouse. Young flower buds were emasculated and pollinated simultaneously with the pollen of newly opened flowers. If crosses were successful, pods started to grow within 3-5 days and matured within 4-5 weeks. However, pod abortion was a rule in the intersubgeneric crosses. In order to enhance pod retention, pollinated gynoecea were sprayed with a growth hormone mixture (100 mg OA +25 mg NAA + 5 mg Kinetin IL distilled water) once a day for 17-21 days. Pods were harvested 19-21 days after pollination (DAP) and immature seeds were cultured in vitro (24).

Hybrids and their derived lines were identified morphologically and cytologically. For cytological analysis, mitotic chromosomes were studied according to Feulgen procedure. Meiotic chromosomes were examined based on procedure described by Singh and Hymowitz (22).

RESULTS AND DISCUSSION

Intersubgeneric

crossability. Intersubgeneric crossability rate in *Glycine* has been formidable due to an extremely low crossability and an early pod abortion (13). Pod abortion is a post-fertilization problem (23). Concerted efforts to obtain wide hybrids have resulted in only a few sterile F₁ hybrids (Table 1). Of the 16 wild perennial *Glycine* spp., currently identified taxonomically, only four species [*G. argyrea*, *G. canescens*, *G. clandestina*, *G. tomentella* (2n=78,80)] have been hybridized successfully- with soybean.

Origin and identification of amphidiploid plants. In order to restore seed fertility, chromosome numbers of sterile intersubgeneric F₁ plants were doubled by 0.1% colchicine treatment. Amphidiploid plants (2n= 118, genome GODDEE) of *G. max* cultivar Altona (2n=40, genome GG) x *G. tomentella* (2n=78, genome DDEE), PI 483218, set a few one-seeded selfed pods (25). On the other hand, amphidiploid plants (2n=80, genome A₁A₁GG)

Table 1. Intersubgeneric hybrids in the genus *Glycine* Willd.

Hybrids ¹	Literature Citation
(TOM,2n=38xCAN,2n=40)2 x MAX,2n=40 =59	1
Max x TOM,2n78 =59	17
Max x TOM,2n=80 =60	17
TOM,2n78 x MAX =59	21,22
(ARGxCAN) x MAX =40	2
MAX x CLA =40	24
CANxMAX40	16
MAX x TOM,2n=78 =59	16
TOM,2n78 x MAX =59	16

¹Abbreviations: TOM, *G. tomentella*; CAN, *G. canescens*; MAX, *G. max*; ARC, *G. argyrea*; CLA, *G. clandestina*.

of *G. max* cv. Altona and *G. clandestina* (2n=40, genome A₁A₁), PI 440948, did not set pods, although meiotic chromosome pairing was completely normal (24). The accession PI 440948 is highly amenable to regeneration in cell and tissue culture systems (10),

Amphidiploid, H213-2a (2n=118), of *G. max* cv Altona and *G. tomentella*, PI483218, bred true and was morphologically easily distinguished from H213 (F₁ designation) because the H213-2a plants were vigorous with larger leaves and the seeds were considerably smaller than those of soybean but larger than those of PI 483218. After a considerable effort, Singh *et al.* (25) succeeded in producing backcross-derived fertile lines while the other scientists failed. It needed scientific skills, persistency and motivation.

Crossability between amphidiploid (2n=118) and soybean. The initial effort to produce BC₁ was a total failure despite a large number of flowers were hybridized (Table 2). Pollinated gynoecea aborted 3-5 DAP. However,

a mixture of GA₃, NAA and Kinetin facilitated pod retention (Table 2). Pod set was recorded in all the cross combinations. Although treated pods remained green, it was essential to culture immature seeds in an artificial medium 19-2 1 DAP. BC₁ plants were recovered only in H213-2aF₃ x Clark 63 and H213-2aF₃ x Essex (Table 2). Thus, the frequency of plant recovery was extremely low.

Morphology, cytology and breeding behavior of BC₁ (H562(H213-2aF₃ x Clark 63)) plants. A total of 15 regenerants, recovered from three cultured immature seeds, were transferred to the greenhouse, and only 12 survived. Based on morphological features and growth habits, eight plants resembled the soybean and the other four plants expressed more traits of *G. tomentella*.

There were no apparent meiotic differences among the 10 H562 plants examined. The average chromosome association (range) at metaphase-I in H562-1 was 21.61 (12 to 34)+27.2II (21 to 32). The observed ranges of bivalents could represent 20 bivalents from GG and 1-12 bivalents from homoeologous chromosome pairing between D and E genomes.

Chromosome pairing among G, D and E genomes is also a possibility. Due to a high frequency of bivalents, chromosomes in telophase-I nuclei ranged from 31-41. This suggests that there was an inclusion of a few univalents in dyads which resulted in a wide range (31-45) of chromosome constitution in tetrads. The H562 plants were

Table 2. Effect of growth hormone treatment on pod set in crosses with the H213-2a F₃ plants (female) of the amphidiploid *Glycine max* x *G. tomentella* and soybean (male) (25).

No growth- hormone treatment			Growth- hormone treatment			
Pollen donors culture	Flowers pollinated	Pod set	Flowers pollinated	Pod set	Seeds cultured	Plants
No.						
Altona	158	0	14	2	7	0
Bonus	158	0	151	30	98	0
Clark 63	519	0	391	46	130	15 ¹
Essex	1,610	0	510	83	82	5 ²
Williams	238	3	215	23	58	0
Wye	238	0	86	10	26	0
Total (%)	3,417	3 (0.09)	1,367	194 (14.19)	401	

¹Plants from three-cultured embryos.

²Plants from one-cultured embryo.

pollen and seed sterile. A low frequency of aborted pod set was observed in H562 x soybean cultivars crosses (Table 3) and a few F₃BC₂ plants were obtained.

Production of BC₂ plants. A total of 1,017 flowers of H562 were pollinated by soy bean cultivar Clark 63. Nine aborted pods with 12 immature seeds were cultured. Three BC₂ plants with 2n=58 (H622-1), 2n=56 (H628-1), and 2n=SS (H638-1) were recovered. All BC₂ plants generally resembled the soybean.

Table 3. Crossability rate in H562 BC₁ plants (female) of the amphiploid ((*Glycine max* x *G. tomentella*) x *G. max* cv. Clark 63) x soybean cultivars (male) (25).

Soybean cultivar	Flowers pollinated	Aborted pod set ¹	Seeds cultured
No.			
Bonus	646	3	4
Clark 63	1,017	9	12
Essex	459	1	1
Williams	269	1	1
Wye	37	0	0
Total (%)	2,428	14 (0.58)	18

¹Received growth hormone treatment for 18 to 21 days.

Table 4. Number of plants with 2n=40 to 46 isolated from *Glycine max* x *Glycine tomentella* from BC₄ to BC₆ generations.

Lines*	Number of plants with 2n=						
	40	41	42	43	44	45	46
H	131	191	34	12	4	1	6
MT	77	96	21	5	0	0	0
Total	208	197	55	17	4	1	6

*Abbreviations: H = hybrid; MT = max x tomentella

Meiosis and breeding behavior of BC₂ plants. Generally soybean chromosomes formed bivalents while *G. tomentella* remained univalents at meiotic metaphase-I. The BC plants did not produce selfed seed. The crossability rates of three BC plants with Clark 63 are shown (Table 4). The H622-1 and H638-1 plants were not used extensively in the backcross program because both plants died prematurely. Two pods, containing one seed each, were harvested from the crosses H622-1 x Clark 63 and H638-1 x Clark 63. In contrast, 21 pods matured from the cross H628-1 x Clark 63.

Isolation of fertile plants. A total of 16 BC₃ plants with 2n=42 to 2n=51 chromosomes were obtained. All plants were sterile, but produced normal mature pods after pollinating by cultivar Clark 63. The progenies of BC₄ contained plants with chromosome number ranging from 2n=40 to 64. All disomic (2n=40) plants showed normal pod set and seed fertility. The MAALs (2n=41) exhibited total male sterility to normal fertility. Male sterile MAALs set seeds after pollinating by cultivar Clark 63. Male sterility trait was always associated with an extra *G. tomentella* chromosome. Based on morphological traits (growth habit, leaf size and shape, color of stem, leaves, pods, and seeds, maturity, male sterility, number of seeds per pod), 30 morphological distinct MAALs (2n=41) have been identified and designated (MT-I to MT-XXX). Additional MAALs will be isolated and all lines will be identified based on cytological, biochemical and molecular methods. Furthermore, plants with 2n=42 to 46 will throw many more MAALs that are morphologically not grouped so far. Table 4 shows the number of plants with the 2n chromosome number.

Screening of fertile (2n=40, 41) lines for rust resistance. The ultimate aim of this study is to establish a methodology by which the tertiary gene pool (GP-3) of soybean could be more accessible than those of previous attempts. Fertile disomics and MAALs are being distributed to scientists through the Material

Transfer Agreement from the UIUC. A total of 78 disomic lines and 47 MAALs have been sent to Dr. A. H. D. Brown, CSIRO, Australia, to screen for the rust resistance. The screened disomic plants were found to be susceptible to the rust.

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