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AN INTERSUBGENERIC HYBRID BETWEEN *GLYCINE TOMENTELLA* HAYATA AND THE SOYBEAN, *G. MAX* (L.) MERR.

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INDEX WORDS

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SUMMARY

The objective of the present paper is to provide information on the morphology and cytology of an intersubgeneric hybrid (2n = 59) between *Glycine tomentella* HAYATA (2n = 78) and *G. max* (L.) MERR. (2n = 40) obtained through in vitro immature seed culture. The hybrid plant was slow in vegetative growth and twinning like the female parent but morphologically was intermediate between both parents for several traits. At metaphase I, the average chromosome associations and ranges for 25 cells were 44.0 I (37-51) + 7.5 II (3-11). The plant was completely pollen and seed sterile. The present investigation suggests that wild perennial *Glycine* species can be exploited as either the male or female parent in wide hybridization programs with the soybean, *G. max*.

INTRODUCTION

The genus *Glycine* WILLD. consists of two subgenera, *Glycine* and *Soja* (MOENCH) F. J. HERM, (HYMOWITZ & NEWELL, 1981). Seven wild perennial species, *G. canescens* F. J. HERM., *G. clandestina* WENDL., *G. falcata* BENTH., G. latifolia (BENTH.) NEWELL & HYMOWITZ, *G. latrobeana* (MEISSN.) BENTH., *G. tabacina* (LABILL.) BENTH., and *G. tomentella* HAYATA belong to the subgenus *Glycine* and they are indigenous to Australia having chromosome numbers of 2n = 38, 40, 78 or 80. Many intraspecific and a few interspecific hybrids within the subgenus *Glycine* have been studied (NEWELL & HYMOWITZ, 1983; PALMER & HADLEY, 1968; PUTIEVSKY & BROUÉ, 1979; SEDOVA, 1982; SINGH & HYMOWITZ, 1985). The subgenus *Soja* includes the cultivated soybean, *G. max* (L.) MERR., and its nearest wild relative *G. soja* SIEB & ZUCC., both of which are annual and diploid (2n = 40). Hybridization between both species can be readily affected (AHMAD et al., 1977; BROICH, 1978; HADLEY & HYMOWITZ, 1973). However, until recently, hybridization between species at the subgeneric level was not successful. Failure in obtaining such hybrids was attributed to pod abortion after 10–20 days after pollination (LADIZINSKY et al., 1979; PALMER & HADLEY, 1968). NEWELL & HYMOWITZ (1982) reported hybrid plants between *G. max* cv. Altona (2n = 40) and *G. tomentalla* (2n = 78, 80). The hybrid plants carried, as expected, 2n = 59 or 60 chromosomes and were completely sterile. On the other hand, BROUÉ et al. (1982) used

a synthesized amphiploid (2n = 78) of *G. tomentella* (2n = 38) and *G. canescens* (2n = 40) as a female and *G. max* cv. Lincoln and Hark as male parents. They also obtained sterile hybrids with 2n = 59 chromosomes. In both cases, immature seeds were germinated through in vitro rescue procedures.

In our wide hybridization program, a successful hybrid was obtained through in vitro culture techniques, using *G. tomentella* (2n = 78) as the female and *G. max* cv. Clark 63 as a male. The purpose of the present paper is to furnish information on the morphological and cytological identification of the hybrid and its possible use in gene transfer will be discussed.

MATERIALS AND METHODS

Seeds of *G. tomentella* (P.I. 483224) used in this study were obtained from the late P. Broué, CSIRO, Canberra, Australia. They were collected from the Mountain View picnic area, 12.5 km NNW of Grafton, New South Wales, Australia. Seeds of *G. max* cv. Clark 63 were obtained from R. L. Bernard, USDA, Urbana, Illinois. Both parents were grown in the greenhouse. Young flower buds of *G. tomentella* were emasculated and immediately pollinated with the pollen of newly opened flowers of Clark 63. Two pods were harvested after 26 days of pollination. Each pod contained one seed. The two immature seeds were extracted from the surface sterilized pods and cultured on an artificial medium of NEWELL & HYMOWITZ (1982). Both seeds germinated after 9 months in culture and were transferred to the greenhouse for further growth.

Morphological identification were based on five measurements each of several traits of both parents and hybrid. For cytological identification, flower buds undergoing meiosis were fixed in a freshly prepared mixture of 3:1 absolute ethanol: propionic acid. Ferric chloride (1 g/100 ml fixative) was added to the fixative to intensify the chromosome staining. Buds were transferred to 70% ethanol after 48 hrs of fixation and stored under refrigeration. Anthers with meiotic stages were stained in 0.7% aceto-carmine for one week and squashes were made in 45% acetic acid. Observations on all meiotic stages were recorded.

RESULTS AND DISCUSSION

Of two seedlings, the growth of one was rapid and morphologically it resembled the female parent, *G. tomentella*. Cytological analysis revealed 2n = 78 chromosomes indicating that this plant was not a hybrid but rather a self. At the time of culture the size of the selfed seed was 2.0 mm.

At the time of culture the second seed measured 1.0 mm. Its growth was very slow requiring 8 months to reach maturity. The plant exhibited the twinning trait of the female parent and carried several morphological characteristics intermediate between both parents (Table 1, Fig. 1). The true nature of hybridity was established from chromosome counts which showed 2n = 59. The plant was designated as H 261.

A total of 25 metaphase I sporocytes were analyzed from H 261. Average chromosome associations and (ranges) for 25 cells were 44.0 I (37–51) + 7.5 II (3–11). At metaphase I, bivalents were observed at the equatorial plate while univalent either already reached the poles or were scattered in the cytoplasm (Figs. 2a and 2b). The

Table 1. Vegetative and inflorescens morphology of *Glycine tomentella* (P.I. 483224), hybrid and *G. max* cv. Clark 63.

Morphological traits	P.I. 483224	F_1 Hybrid	Clark 63
Upper term. leaflet shape	elliptical	lanceolate	elliptic-oval
Stipule shape	ovate	lanceolate	lanceolate-deltoid
Upper term. leaf length (mm)	29.0	77.8	82.6
Upper term. leaf width (mm)	14.2	39.6	56.6
Lower term. leaflet shape	oval	elliptical	elliptic-oval
Raceme length (mm)	16.6	9.0	11.4
Calyx pubescence density	medium	dense	dense
Calyx pubescence type	spreading	erect	spreading-appressed

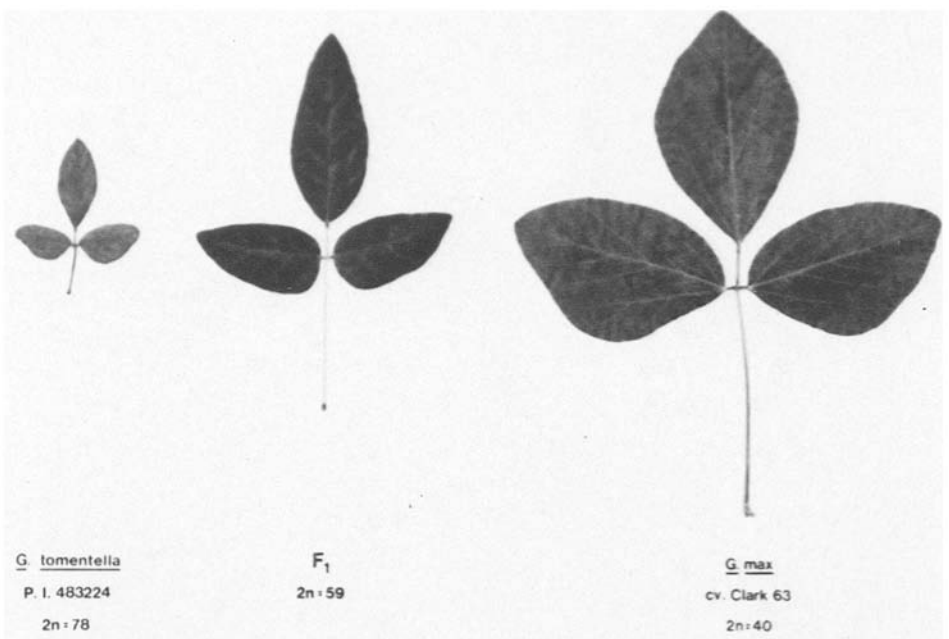
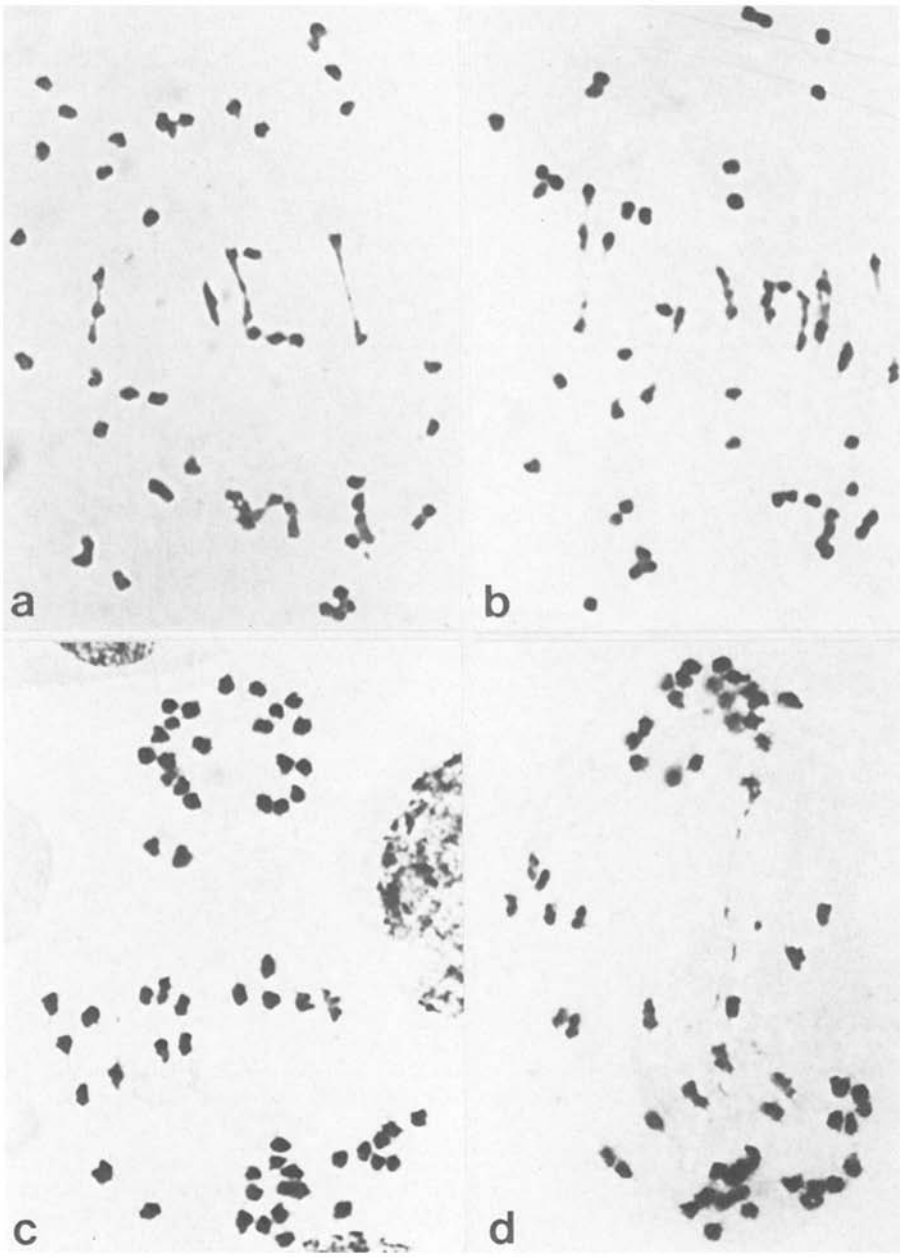


Fig. 1. Herbarium leaf specimen of intersubgeneric hybrid of *Glycine tomentella* (P.I. 483224) female (left), *G. max* cv. Clark 63 (right) and the hybrid (middle). Note the difference in the leaf shape and size in F_1 hybrid and compare with the parents.

majority of the bivalents were rod shaped with terminalized chiasma. Similar results were found by NEWELL & HYMOWITZ (1982) although they recorded low frequencies of trivalents (0–2) and quadrivalents (0–1). The observation of expected chromosome pairing at metaphase I (3–11 II) suggests two possibilities: chromosome pairing between two tomentella genomes, as tetraploid tomentella are allopolyploids (SINGH & HYMOWITZ, 1985) or chromosome pairing is taking place between *G. tomentella* and *G. max* chromosomes. The former possibility appears to be the most logical because loose chromosome pairing at the diploid level has been observed in interspecific



Figs. 2a–d. Meiosis in intersubgeneric hybrid of *G. tomentella* (P.I. 483224) × *G. max* cv. Clark 63.
Fig. 2a. Metaphase I showing 4 II + 51 I.
Fig. 2b. Metaphase I showing 9 II + 41 I.
Fig. 2c. Anaphase I showing 23-15-21 chromosome separation.
Fig. 2d. Anaphase I showing a chromatin bridge and an acentric fragment.

hybrids in wild *Glycine*. However, chromosome pairing in amphiploids of *Glycine* appears to be under genic control because hexaploid (2n = 6x = 118) plants obtained by chromosome doubling with colchicine of triploids (2n = 3x = 59) (HYMOWITZ & SINGH, 1984) showed 59 II in the majority of sporocytes analyzed which is not expected based on chromosome pairing of triploids (SINGH & HYMOWITZ, unpub. data).

The precocious movement of univalents resulted in anaphase I cells with unexpected chromosome separations (Fig. 2c). A total of 10 anaphase cells were analyzed. Average chromosome separations and (ranges) were 19.9 (17–23): 18.7 (14–22): 20.4 (19–24). These data suggest that the majority of univalents will be included in the daughter nuclei; but they did have a higher frequency of laggards leading to the formation of unbalanced gametes. A chromatin bridge with an acentric fragment also was observed at anaphase I (Fig. 2d) suggesting a paracentric inversion was involved. Paracentric inversions have played a major role in the speciation of several wild *Glycine* (SINGH & HYMOWITZ, 1985). The disturbed meiotic stages resulted in shrivelled anthers and the absence of stainable pollen. The hybrid plant was completely sterile and set no seed. Attempts are being made to double the chromosome number of H 261 by colchicine treatment.

The present study and the report by NEWELL & HYMOWITZ (1982) demonstrate that it is possible to hybridize wild perennial *Glycine* with *G. max* in both directions. Efforts have been made to hybridize the other wild *Glycine* with soybean cultivars and we hope to germinate immature seed through in vitro procedures. The results presented here suggest that gene transfer between the two subgenera *Glycine* and *Soja* is feasible and gene exchange is possible at the tertiary gene pool level of HARLAN & DE WET (1971).

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