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Abstract

Soybean [*Glycine max* (L.) Merr.], an economically important dicot legume, is a member of the family Fabaceae and belongs to the genus *Glycine* Willd. Based on classical and molecular taxonomy, the genus *Glycine* has been divided into two subgenera; the subgenus *Soja* (Moench) F.J. Hermann includes soybean and its wild annual progenitor *G. soja* Sieb. & Zucc. Both species contain $2n = 40$ chromosomes, are cross-compatible, produce fertile F_1 plants, and belong to the primary gene pool. The subgenus *Glycine* consists of 26 wild perennial species. Vegetative and reproductive morphology of soybean has been examined extensively. The cytogenetic knowledge of soybean lags far behind that of other model economically important crops (viz. rice, maize, wheat, tomato), because its somatic chromosomes are symmetrical and only one pair of satellite chromosomes can be identified. Molecular linkage maps have been associated with specific chromosomes, and soybean genome has been sequenced. The soybean breeders, worldwide, are confined to crossing within the primary gene pool; thus, genetic base of soybean is very narrow. Wild perennial *Glycine* species of the tertiary gene pool have been recently exploited to broaden the genetic base of modern soybean cultivars.

2.1 Introduction

The soybean [*Glycine max* (L.) Merr.; $2n = 40$] is an economically very important leguminous seed crop for feed and food products that is rich in seed protein (about 40%) and oil (about 20%). Taxonomy of the genus *Glycine* Willd. is well defined based on morphological features, cytogenetics,

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and molecular methods (Chung and Singh 2008). Vegetative (Lersten and Carlson 2004) and reproductive (Carlson and Lersten 2004) morphological features of soybean have been extensively described. However, soybean is not considered a model plant for cytogenetic studies because of the large number of chromosomes ($2n = 40$) (Karpechenko 1925; *Soja hispida*; syn. *G. max*), their small and similar chromosome size (1.42–2.84 μm) (Sen and Vidyabhusan 1960), and the lack of morphological distinguishing landmarks (Singh 2003). Using primarily restriction fragment length polymorphism (RFLP) and simple sequence repeat (SSR) loci, 20 molecular linkage groups (MLGs) have been developed (Song et al. 2004; Xia et al. 2007) but not all linkage groups have been associated with the respective chromosomes (Zou et al. 2003b). The cytogenetic knowledge of the soybean lags far behind many crops such as maize, barley, rice, wheat, tomato, brassicas, pea, and faba bean (Singh 2003; Singh et al. 2007a, b).

The genetic base (number of ancestral varieties that contributed to the development of modern commercial varieties) and diversity are narrow for public soybean cultivars being grown worldwide. Soybean breeders have been confined to improving soybeans using land races and occasionally used *G. soja* Sieb. & Zucc. Soybean breeders have not exploited wild perennial *Glycine* species for broadening the genetic base of soybean (Chung and Singh 2008). Despite the apparent limitation of having a narrow genetic base for world soybean production, soybean breeding has continued to make significant progress.

The objective of this chapter is to document brief information on vegetative and reproductive features (botany) of soybean and describes cytogenetics of the genus *Glycine*. Cytogenetics covers handling of soybean chromosomes, genomes of the *Glycine* species, origin of polyploid complexes, chromosomal aberrations and wide hybridization.

2.2 Botany

2.2.1 Taxonomy

The taxonomy of wild annual and cultivated soybean is as follows:

Order	Fabales
Family	Fabaceae (Leguminosae)
Subfamily	Papilionoideae
Tribe	Phaseoleae
Subtribe	Glycininae
Genus	<i>Glycine</i> Willd.
Subgenus	<i>Soja</i> (Moench) F.J. Herm.
Species	<i>Glycine soja</i> Sieb. & Zucc.
Species	<i>Glycine max</i> (L.) Merr.

The taxonomy of the genus *Glycine* to which soybean belongs has been revised many times. Hermann (1962) divided the genus *Glycine* into three subgenera (Tables 2.1 and 2.2): The subgenus *Leptocyamus* included six wild perennial species indigenous to Australia, South China, South Pacific Islands, Philippines, and Formosa (Taiwan). The subgenus *Glycine* contained two species (*G. petitiata* from Ethiopia and *G. javanica* from India and Malaya (Malaysia)). *Glycine javanica* included two subspecies: the subspecies *G. micrantha* with four varieties and subspecies *G. pseudojavanica* with one variety, and all were indigenous to Africa (Tables 2.1). He included cultigen soybean [*G. max* (L.) Merr.] and *G. ussuriensis* Regel and Maack. in the subgenus *Soja*.

Verdcourt (1966) questioned the validity of *G. javanica* L. since it has $2n = 22$ or 44 chromosomes and the chromosomes (morphology) are larger than those of other species of the genus *Glycine*. He kept the generic name and proposed *G. wightii* (R. Grah. Ex Wight and Arn.) Verdcourt as the species name. He changed the names of the genus *Glycine* L. assigned by Hermann (1962) to *Glycine* Willd., and the names of two of the subgenera of *Glycine*: subgenus *Leptocyamus* (Benth.) Hermann became

Table 2.1 Systematic classification of the *Glycine* L. (Hermann 1962)

Species	Distribution
Subgenus <i>Leptocyanus</i>	
1. <i>Glycine clandestina</i> Wendl	Australia; Formosa (Taiwan), Micronesia
1a. var. <i>sericea</i> Benth	Australia
2. <i>G. falcata</i> Benth	Australia
3. <i>G. latrobeana</i> (Meissn.) Benth	Australia
4. <i>G. canescens</i> F.J. Herm	Australia
5. <i>G. tabacina</i> (Labill.) Benth	Australia; S. China; S. Pacific Islands
6. <i>G. tomentella</i> Hayata	Australia; S. China; Philippines; Formosa (Taiwan)
Subgenus <i>Glycine</i>	
1. <i>G. petitiiana</i> (A. Rich.) Schweinf	Ethiopia
2. <i>G. javanica</i> L	India; Malaya (Malaysia)
2a. ssp. <i>micrantha</i> (Hochst.) F.J. Herm	Trop. Africa
2b. var. <i>claessensii</i> (De Wild.) Hauman	Uganda to Nyasaland (Republic of Malawi)
2c. var. <i>paniculata</i> Hauman	Belgian Congo (Democratic Republic of the Congo)
2d. var. <i>longicauda</i> (Schweinf.) Bak	Ethiopia to Angola
2e. var. <i>moniliformis</i> (hochst.) F.J. Herm	Ethiopia to Eritrea
2f. subsp. <i>pseudojavanica</i> (Taub.0 Hauman	Belgian Congo (Democratic Republic of the Congo) to Angola
2g. var. <i>laurentii</i> (De Wild.) hauman	Belgian Congo (Democratic Republic of the Congo)
Subgenus <i>Soja</i>	
1. <i>G. ussuriensis</i> Regal and Maack	Asia
2. <i>G. max</i> (L.) Merr	Cultigen

Table 2.2 Revision of the genus *Glycine* by Verdcourt (1966)

Genus <i>Glycine</i> Willd.
Subgenus <i>Glycine</i>
1. <i>Glycine clandestina</i> Wendl.
1a. var. <i>sericea</i> Benth
2. <i>G. falcata</i> Benth
3. <i>G. latrobeana</i> (Meissn.) Benth
4. <i>G. canescens</i> F.J. Herm
5. <i>G. tabacina</i> (Labill.) Benth
6. <i>G. tomentella</i> Hayata
Subgenus <i>Bracteata</i> Verdcourt
1. <i>G. wightii</i> (R. Grah. ex Wight and Arn.) Verdcourt
Subgenus <i>Soja</i>
1. <i>G. soja</i> Sieb. & Zucc
2. <i>G. max</i> (L.) Merr

a synonym of *Glycine* subgenus *Glycine*; subgenus *Soja* (Moench) Hermann was unchanged. Lackey (1977) (Table 2.2) later removed *G. wightii* (Arnott) Verdcourt from the genus *Glycine* and designated it *Neonotonia wightii* (Wight and Arn.) J.A. Lackey.

Currently, the genus *Glycine* consists of two subgenera. The subgenus *Glycine* consists of 26 wild perennial species indigenous to Australia and various surrounding Pacific Islands. Of the 26 perennial species, *G. tomentella* Hayata constitutes four cytotypes ($2n = 38, 40, 78, 80$) and *G. hirticaulis* and *G. tabacina* have accessions with $2n = 40$ and 80 chromosomes (Table 2.3). *Glycine tomentella* accessions with $2n = 80$ chromosomes is distributed in Australia, Papua New Guinea, the Philippines, Indonesia, and Taiwan, while 80-chromosome *G. tabacina* (Labill.) Benth. has been collected from Australia, Tonga,

Table 2.3 Taxonomy of the Genus *Glycine* Willd

Species		2n	Genome symbol		PI-number	G number	Distribution	Species described since Table 2.2
Mol Group	N		C					
Subgenus <i>Glycine</i>								
1. <i>G. albicans</i> Tindale and Craven		40	I	A		2049	Aust.: WA	Tindale and Craven (1988)
2. <i>G. aphyonota</i> B. Pfeil		40	I ₃	A		2589	Aust.: WA	Pfeil and Craven (2002)
3. <i>G. arenaria</i> Tindale		40	H	A	505204	1305	Aust.: WA	Tindale (1986b)
4. <i>G. argyrea</i> Tindale		40	A ₂	A	505151	1420	Aust.: Q	Tindale (1984)
5. <i>G. canescens</i> F.J. Hermann		40	A	A	440932	1853	Aust.: Q, NSW, V, SA, NT, WA	
6. <i>G. clandestina</i> Wendl		40	A ₁	A	440958	1126	Aust.: Q, NSW, V, SA, T	
7. <i>G. curvata</i> Tindale		40	C ₁	C	505166	1849	Aust.: Q	Tindale (1986a)
8. <i>G. cyrotoloba</i> Tindale		40	C	C	440962	1184	Aust.: Q, NSW	Tindale (1984)
9. <i>G. falcata</i> Benth		40	F	A	505179	1155	Aust.: Q, NT, WA	
10. <i>G. gracei</i> B.E. Pfeil and Craven		40	?	?		3124	Aust.: NT	Pfeil et al. (2006)
11. <i>G. hirticaulis</i> Tindale and Craven		40	H ₁ , (??)	A, (A)	IL1246	2876	Aust.: NT	Tindale and Craven (1988)
		80			IL943	1956	Aust.: NT	
12. <i>G. lactovirens</i> Tindale and Craven		40	I ₁	A	IL1247	2720	Aust.: WA	Tindale and Craven (1988)
13. <i>G. latifolia</i> (Benth.) Newell and Hymowitz		40	B ₁	B	378709	1697	Aust.: Q, NSW	Newell and Hymowitz (1980)
14. <i>G. larrobeana</i> (Meissn.) Benth		40	A ₃	A	483196	1385	Aust.: V, SA, T	
15. <i>G. microphylla</i> (Benth.) Tindale		40	B	B	440956	1867	Aust.: Q, NSW, V, SA, T	Tindale (1986a, b)
16. <i>G. montis-douglas</i> B.E. Pfeil and Craven		40	?	?			Aust.: NT	Pfeil et al. (2006)
17. <i>G. peratosa</i> B.E. Pfeil and Tindale		40	A ₅	A		2916	Aust.: WA	Pfeil et al. (2001)
18. <i>G. pescadrensis</i> Hayata		80	AB ₁	A	440996	1433	Aust.: Q, NSW; Taiwan, Japan	Pfeil et al. (2006)
19. <i>G. pindanica</i> Tindale and Craven		40	H ₂	A	595818	2951	Aust.: WA	Tindale and Craven (1993)
20. <i>G. pullenii</i> B. Pfeil, Tindale and Craven		40	H ₃	A		2599	Aust.: WA	Pfeil and Craven, 2002
		40	A ₄	A	440954	1874	Aust.: NSW, SA, WA	Pfeil et al. (2002)

(continued)

Table 2.3 (continued)

Species	Mol	2n	Genome symbol		PI-number	G number	Distribution	Species described since Table 2.2
	Group		N	C				
21. <i>G. rubiginosa</i> Tindale and B.E. Pfeil								
22. <i>G. stenophita</i> B. Pfeil and Tindale		40	B ₃	B	378705	2600	Aust.: Q, NSW; (Japan ??)	Doyle et al. (2000)
23. <i>G. syndetika</i> B.E. Pfeil	D4	40	A ₆		441000	1300	Aust.: Q	Pfeil et al. (2006)
24. <i>G. dolichocarpa</i> Tateishi and Ohashi		80	D ₁ A	?			Taiwan	Tateishi and Ohashi (1992)
25. <i>G. tabacina</i> (Labill.) Benth		40	B ₂	B	373990	1317	Aust.: Q, NSW	
		80	BB ₁ ;BB ₂ ; B ₁ B ₂	B	373992	1314	Aust.: Q, NSW, V SA; West Central and South Pacific Islands	
26. <i>G. tomentella</i> Hayata	D1, D2	38	E	A	440998	1858	Aust.: Q	
	D3	40	D	A	505222	1749	Aust.: Q, WA, PNG	
	D5B	40	H ₂	A	505294	1943	Aust.: WA	
	D5A	40	D ₂	A	505203	1303	Aust.: WA, NT	
	T1	78	D ₃ E	A	441001	1133	Aust.: Q, NSW; PNG	
	T5	78	AE	A	509501	1487	Aust.: NSW	
	T6	78	E H ₂	A	505286	1945	Aust.: WA	
	T2	80	D A ₆	A	441005	1188	Aust.: Q; Taiwan	
	T3	80	D D ₂	A	483219	1927	Aust.: Q, NT, WA; PNG; Timor	
	T4	80	D H ₂	A	330961	1348	Aust.: Q, NT, WA; Philippines, Taiwan	
Subgenus <i>Soja</i> (Moench) F.J. Hermann								
<i>G. soja</i> Sieb. & Zucc.		40	G	G	51762		China, Japan, Russia, Korea, Taiwan	
<i>G. max</i> (L.) Merr		40	G ₁	G			Cultigen; worldwide	

N nuclear; *C* chloroplast; *PI* Plant introduction; *G* CSIRO (Commonwealth Scientific and Industrial Research Organization) number; *Aust* Australia; *IL* Illinois; *Q* Queensland; *NSW* New South Wales; *NT* Northern Territory; *SA* South Australia; *V* Victoria; *WA* Western Australia; *T* Tasmania; *PNG* Papua New Guinea

Vanuatu, Ryukyu Islands, and Taiwan. Tateishi and Ohashi (1992) examined *Glycine* of Taiwan and recognized four species: *G. dolichocarpa* Tateishi and Ohashi, *G. tomentella*, *G. tabacina*, and *G. max* subsp. *formosana* (Hosokawa Tateishi and Ohashi). *Glycine dolichocarpa* contains $2n = 80$ chromosomes, and *G. tomentella* and *G. tabacina* of Taiwan contain $2n = 80$ chromosomes (Chung and Singh 2008). It is likely that *G. dolichocarpa* is a variant of *G. tomentella*, possibly of Australian origin, and migratory birds may have played a major role in its dispersion (Hymowitz et al. 1990).

The newly described species since 1986 (Table 2.3) are distributed in a very restricted region of Australia. For example, *G. latrobeana* is listed nationally as rare, and in Tasmania, seven populations have been recorded (Lynch 1994). Only 19 wild perennial *Glycine* species are being maintained in the soybean collection at

Urbana, Illinois (http://www.ars-grin.gov/cgi-bin/npgs/html/site_holding.pl?SOY).

The subgenus *Soja* still contains *G. soja* (a wild progenitor) and *G. max* (cultigen). Both species contain $2n = 40$ chromosomes, are cross-compatible, and produce vigorous fertile F_1 hybrids, and gene exchange between them is possible. Broich and Palmer (1980) used cluster analysis techniques to examine phenotypic variation among *G. max*, *G. soja*, and *G. gracilis*. *Glycine max* and *G. soja* were found to be morphologically distinct, and *G. gracilis* was found to be conspecific with *G. max*. Thseng et al. (1999) identified a new species *G. formosana* Hosokawa from Taiwan based on pod morphology, allozyme, and DNA polymorphisms and concluded that the newly defined species is different from *G. soja* though they did not hybridize both species. It is likely that *G. formosana* is a variant of *G. soja*. Thus, we have not included *G. formosana* in Table 2.3.

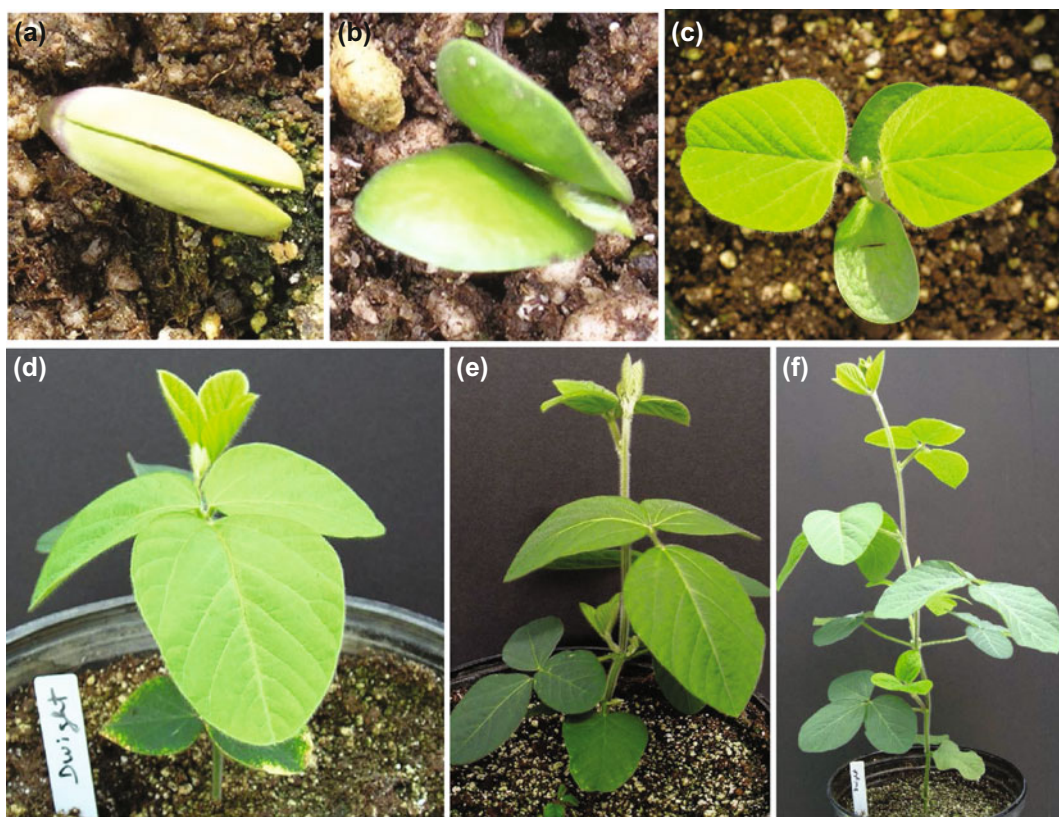


Fig. 2.1 Germination of Dwight soybean: **a** epigeal germination with two cotyledons leaves, **b** cotyledon leaves with emerging primary leaves, **c** simple primary leaves, **d** trifoliolate leaf, **e** three trifoliolate leaves, **f** six trifoliolate leaves

Table 2.4 Soybean vegetative and reproductive stages

Vegetative growth stages	Description
VE (emergence)	Cotyledons have been pulled through the soil surface
VC (unifoliolate leaves)	Unifoliolate leaves expand, one node
V1 (first trifoliolate)	One set of trifoliolate leaves completely unfolded, two nodes
V2 (second trifoliolate)	Two sets of trifoliolate leaves unfolded, three nodes
V4 (four trifoliolate)	Four trifoliolate leaves have unfolded
V(n) (nth trifoliolate)	V stages continue with the unfolding of trifoliolate leaves; the final number of trifoliolate depends on the soybean variety and the environmental conditions

2.2.2 Morphology

Soybean is an annual plant. It exhibits taproot growth initially, followed later by the development of a large number of secondary roots. The roots establish a symbiotic relationship with the nitrogen fixing bacterium, *Bradyrhizobium japonicum*, through the formation of root nodules. Soybean has four different types of leaves: the seed leaves (first pair of simple cotyledons; VE stage) (Fig. 2.1a, b; epigeal germination;

Table 2.4), simple primary leaves (Fig. 2.1c; VC stage; Table 2.4), pinnately trifoliolate leaves (Fig. 2.1d; V1 stage; Table 2.4), and the prophylls (a pair of 1 mm long simple leaves at the base of each lateral branch) [Lersten and Carlson (2004)]. Two sets of trifoliolate (V2) and four sets of trifoliolate (V4; Fig. 2.1e) leaves and continues to produce trifoliolate leaves (Vn; Fig. 2.1f) depending upon the environmental conditions. The stem type may be determinate, semi-determinate, or indeterminate.

Fig. 2.2 Reproductive organs (identified) of Dwight soybean: **a** complete mature axillary flowers, **b** terminal flowers, **c** a determinate Dwight soybean plant with developing pods (arrow)

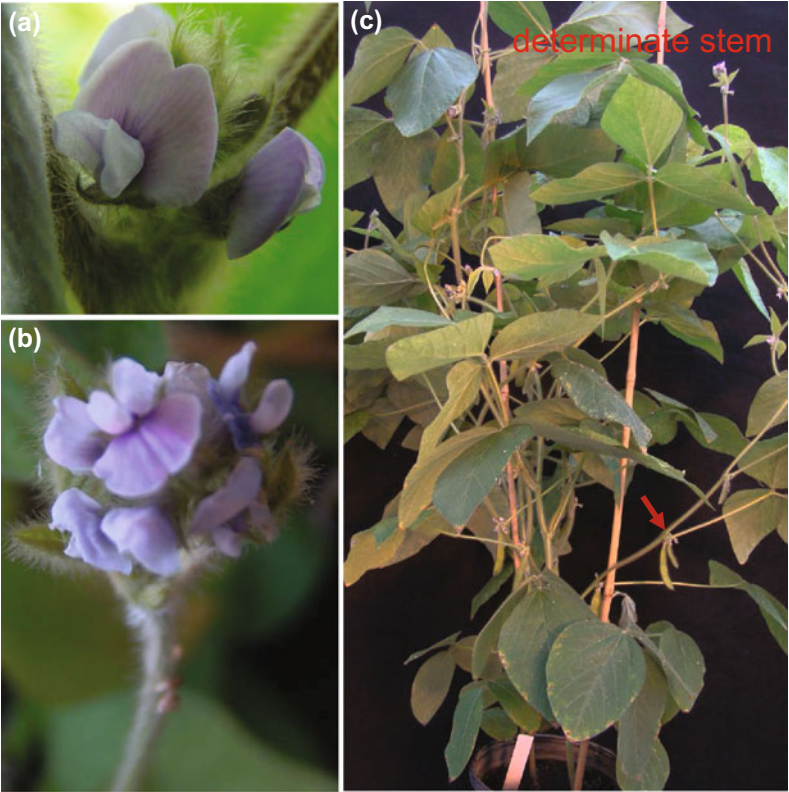
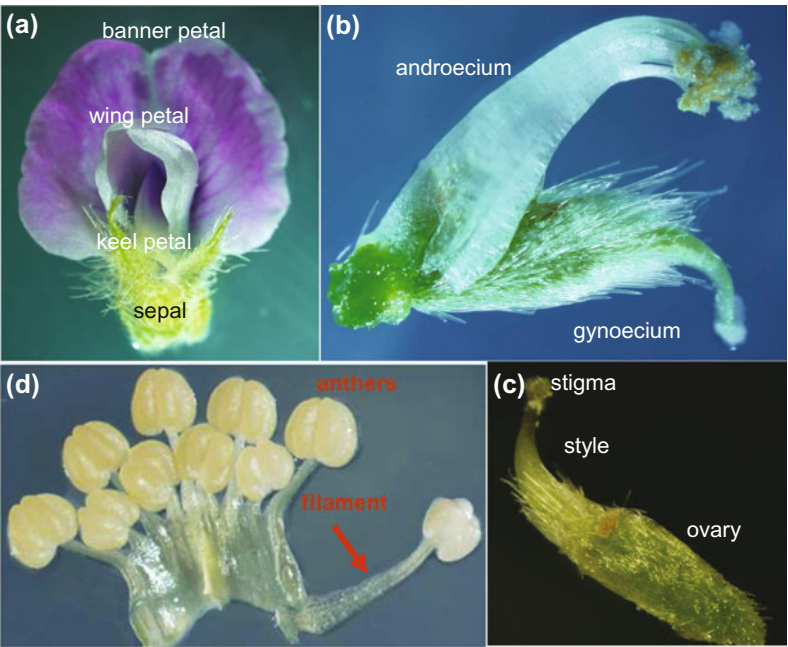


Fig. 2.3 Reproductive organs of Dwight soybean: **a** complete mature flower, **b** mature androecium and gynoecium, **c** a mature gynoecium with stigma, style, and ovary, **d** mature anthers with five anthers on longer filament (outer whorl), four anthers on shorter filament (inner whorl), and one free anther (*arrow*) always below the stigma



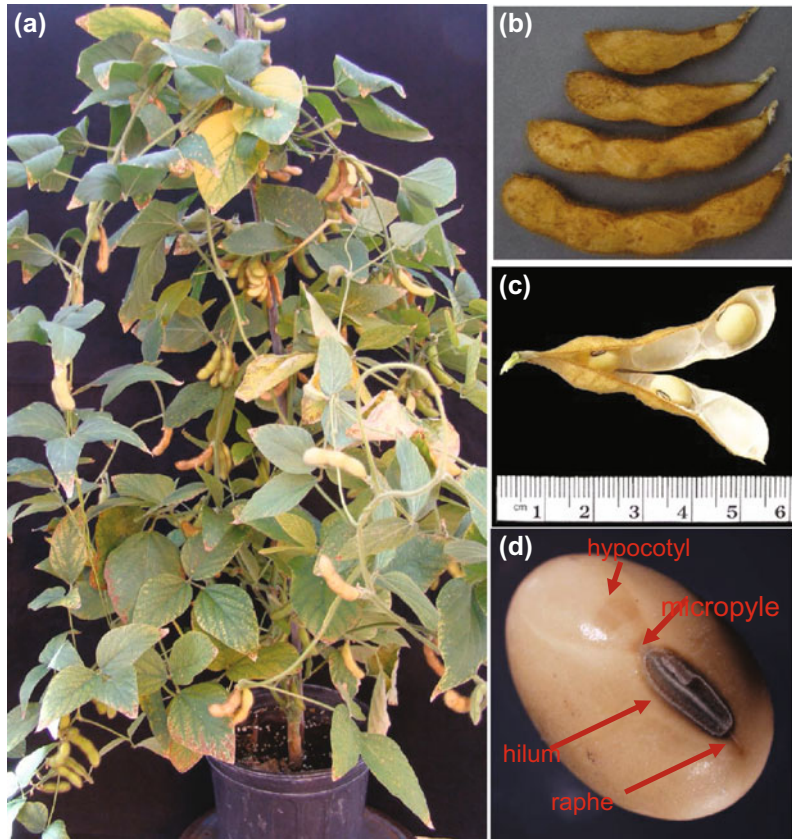
Soybean plants enter into reproductive stages following vegetative growth (Table 2.5). Axillary buds develop (Fig. 2.2a) into clusters of flowers (Fig. 2.2b). From 20 to 80% of the flowers, developed early, abscise (Carlson and Lersten 2004) and produce initially few pods (Fig. 2.2c). Generally, the earliest and latest flowers produced abort most often. Soybean has a typical papilionaceous flower with a tubular calyx of five unequal sepals and a five-part corolla. The corolla consists of a standard (posterior banner petal), two lateral wings and two anterior keel petals contacting with each other, but not fused (Fig. 2.3a). The stamens are clustered around the stigma, ensuring self-pollination

(Fig. 2.3b). The gynoecium consists of an ovary, style, and stigma (Fig. 2.3c). As many as four ovules develop in the ovary. Nine stamens are arranged in two whorls; the outer whorl contains five stamens, and inner whorl contains four stamens (Fig. 2.3d). The two whorls of nine anthers align themselves into a single whorl on a staminal tube. The larger and older anthers alternate with the smaller and younger anthers in sequence around the developing gynoecium. The single free stamen (the 10th) the last to appear (Fig. 2.3d). Soybean is highly self-pollinated with natural crossing usually below 1% because the stamens are elevated so that the anthers form a ring around the stigma. Thus, pollen is shed

Table 2.5 Soybean reproductive growth stages

R1	Beginning to bloom, at least one flower is present on the main stem
R2	Full bloom, flowers are found on any of the top two nodes
R3	Beginning of pod set, pods are 4.8 mm long on one of the top four nodes
R4	Full pod, pods are 19 mm long on one of the top four nodes
R5	Beginning seeds, seeds are 3.2 mm long on one of the top four nodes
R6	Full seeds, pods are completely filled by seeds on one of the top four nodes
R7	Beginning of maturity, one mature pod found on plant
R8	Full maturity, 95% pods have reached mature pod color

Fig. 2.4 A mature Dwight soybean plant: **a** a physiological mature Dwight plant showing tan and partial green pods and leaves are near to senescence, **b** four mature pods of Dwight soybean with one, two, three, and four seeds (*top to bottom*), **c** a Dwight soybean three-seeded open pod, **d** a mature Dwight soybean seed observed through dissecting microscope; organs are identified



directly onto the stigma surface ensuring self-pollination (Carlson and Lersten 2004).

When the pollen grains are shed onto the stigma, they germinate and the pollen tubes travel through style and enter into the filiform apparatus; perhaps as many as 90% of the tubes atrophy and die before reaching the distal end of the ovary (Carlson and Lersten 2004). Before pollen tube reaches toward ovule, the generative cell divides and forms two male gametes (sperm nuclei). Finally, the pollen tube grows through the micropyle of the ovule and enters the filiform apparatus of the degenerated synergid. The pollen tube tip bursts and releases two sperm nuclei. One sperm nucleus fuses with the egg nucleus and forms a diploid zygote, while the second sperm unites with the secondary nucleus forming the primary endosperm nucleus. The time

from pollination to fertilization takes about 8–10 h (Carlson and Lersten 2004).

The inflorescence of each node of the soybean plant may develop into one to more than 20 pods (Fig. 2.4a). The soybean pod is similar to that of other legumes. A pod usually contains 1–3 seeds and rarely 4 seeds (Fig. 2.4b), except for plants that have the *na* allele that produces narrow leaflets and a much high proportion of 4-seeded pods. Separation of the two halves of the pod is preceded by the appearance of clefts through the parenchyma of the dorsal and ventral sutures. After separation, the halves twist spirally around the axis (Fig. 2.4c; Carlson and Lersten 2004).

The seeds mature about 50–80 days after fertilization depending upon the variety and environmental factors. The soybean seed is devoid of

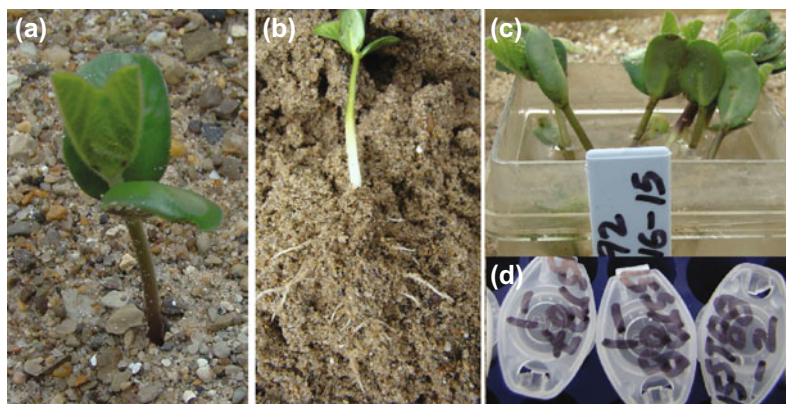


Fig. 2.5 Harvesting of roots for mitosis from seedlings grown in a sand bench of greenhouse: **a** a correct seedling stage; about 6 days old, **b** a seedling carefully uprooted covered with loose sand showing whitish-cream color

roots, **c** seedlings in a container with cold tap water, **d** labeled Eppendorf tube (1.5 ml) ready to harvest the roots

endosperm and contains two large fleshy cotyledons, hypocotyl, micropyle, hilum with a central fissure and a raphe (Fig. 2.4d; Carlson and Lersten 2004).

2.3 Cytology

2.3.1 Handling of Soybean Chromosomes

Mitotic and meiotic chromosomes of soybean are difficult to count because of the small size and the presence of cytoplasm stains in the simple aceto-carmin staining method. The ice-cold water pretreatment routinely used for the cereals fails to arrest a large number of cells at metaphase stage (Singh 2003). Palmer and Heer (1973) developed a root tip squash technique for soybean chromosomes: pretreating the roots with para-dichloro-benzene at 15 °C for 1½ to 2 h. The protocol is time-consuming with many steps which are not feasible when a large number of plants must be identified cytologically. The following method is simple, repeatable, and reproducible and produces excellent chromosome spreads at prometaphase and metaphase stages of mitosis (Singh 2003).

2.3.1.1 Mitotic Chromosomes

1. Germinate soybean seed in sand bench.
2. Select actively growing secondary roots from 1 week old seedlings (Fig. 2.5a).
3. Make sure not to break the actively dividing roots (Fig. 2.5b).
4. Wash off sand or soil in clean water (Fig. 2.5c) and cut 1–2 mm long root tips using forceps, transfer root tips into 1.5-ml Eppendorf tubes containing clean water (Fig. 2.5d).
5. Bring Eppendorf tubes to the laboratory, remove water, and add 0.05% (w/v) 8-hydroxyquinoline.
6. Place tubes at 16 °C heating block in a refrigerator and pretreat roots for 2–3 h.
7. Fix roots in a freshly prepared fixative consisting of 3 parts absolute ethyl alcohol (200 proof): 1 part glacial acetic acid or propionic acid for 24 h at room temperature. Store roots at –20 °C.
8. Remove fixative, wash roots once with distilled water, hydrolyze in 1 N HCl at 60 °C for 12 min, remove 1 N HCl, and wash roots once with distilled water.
9. Stain roots in Feulgen stain for 45–60 min at room temperature.

10. Remove Feulgen stain, add cold distilled water, and store in a refrigerator (4 °C).
11. Cut purple colored tip, place on a clean slide, add a drop of Carbol Fuchsin, and prepare slide using a root tip squash method. Soybean chromosomes stain very well with Carbol Fuchsin, leaving the cytoplasm clear; better than with aceto-carmine or propiono-carmine stains.
12. An alternative is to stain the roots in Carbol Fuchsin overnight in a refrigerator (4 °C), remove the stain, wash root tips with cold distilled water 3–4 times, and store the roots in distilled water in a refrigerator (4 °C). Cut tip by a sharp razor blade, apply a drop of 45% (v/v) acetic acid, and prepare chromosome spread slide by the squash method.

Some useful tips are: (1) Try to collect roots with creamy yellow tips from young seedlings; (2) make sure roots do not dry; (3) remove water completely from the Eppendorf tubes, as water will dilute the concentration of 8-hydroxyquinoline; (4) place only dark purple tips on the slide, add 1–2 drops of Carbol Fuchsin, apply cover glass slowly, heat slide but do not boil, tap slowly three to four times, heat again, and squeeze out excess stain by applying light pressure with the thumb; make sure not to move the cover glass otherwise the cells will be rolled.

The fluorescence in situ hybridization (FISH) technique has been used to identify a primary trisomic containing a nucleolus organizer chromosome (Skorupska et al. 1989; Griffor et al. 1991) and to determine the distribution of rDNA loci in the genus *Glycine* (Singh et al. 2001). Findley et al. (2010) used a fluorescence in situ hybridization system for karyotyping soybean chromosomes. The protocol was published by Singh (2003) and can be modified by researchers depending upon materials, preferences, and priorities.

2.3.1.2 Meiotic Chromosomes

1. Fix soybean buds undergoing meiosis in a proportion of 1 part propionic acid (propiono) + 3 parts 100% (v/v) ethanol + 1

g/100 ml fixative ferric chloride. Propionic acid is preferred over glacial acetic acid because propionic acid produces a clear cytoplasm, whereas the cytoplasm becomes stained when glacial acidic acid is used.

2. Fix buds for 24 h at room temperature; wash in 70% (v/v) ethanol 2 times; and store in 70% (v/v) ethanol in a refrigerator (4 °C).
3. Stain anthers with desired meiotic stages in 1% (w/v) propiono-carmine for 1 week in a refrigerator (4 °C) and prepare chromosome slides using the squash method (Singh 2003).

Some useful tips are:

1. Identify the meiotic stage using one anther and transfer the remaining 9 anthers into 1% propiono-carmine. Meiosis in soybean anthers is asynchronous, but prechecking helps in finding suitable stages.
2. Collect very young (how young? depends upon experience) soybean buds between 9 and 10 AM when the greenhouse is still cool; if greenhouse is hot or buds are collected after 10 AM, the anthers contain cells at pachynema to tetrad.
3. Place one-to-two one-week stained anthers on a clean slide, apply a drop of 45% acetic acid, and cover with a cover glass; heat but *do not boil*, tap 1 or 2 times, and squeeze out excess 45% (v/v) acetic acid by thumb pressure.

2.3.1.3 Karyotype Analysis

Karpechenko (1925) determined the $2n = 40$ as the chromosome number of *S. hispida* Mönch (now known as *G. max*), and it was verified by Fukuda (1933), Veatch (1934), and Sakai (1951). Fukuda (1933) measured chromosome diameter of *G. max*, *G. gracilis*, and *G. soja*, and arranged chromosomes 1–20 in order of diameter. The range was 1.038 μm (chromosome 1) to 0.650 μm (chromosome 19 and 20), and there was no great difference in the size of chromosomes among three species. Veatch (1934) showed a photomicrograph of cross section of metaphase-I cell with 20 bivalents.

Mitotic metaphase chromosomes of the *Glycine* species are symmetrical and lack

Fig. 2.6 A mitotic metaphase cell of soybean with $2n = 40$ chromosomes. One pair of chromosomes containing a nucleolus organizer region (NOR) can be distinguished (arrows), while 38 chromosomes are almost similar



morphological landmarks. Only a pair of nucleolus organizer chromosomes is occasionally visible (Fig. 2.6), and this has been verified by FISH (Skorupska et al. 1989; Griffor et al. 1991; Singh et al. 2001; Krishnan et al. 2001). *Glycine cyrtoloba* (C-genome) and *G. curvata* (C₁-genome) produce only curved pods, a distinct morphological trait which distinguishes these species from other *Glycine* species (Tindale 1984). These species also express 2 pairs of nucleolus organizer chromosomes at mitotic metaphase which are visible by Feulgen staining and 4 signals by FISH, while other species of the genus *Glycine* express only one pair. It is feasible that the second pair either is silent or has lost its NOR activity.

Few attempts using mitotic metaphase chromosomes of soybean (*G. max*) to construct chromosome map were not successful. The length of the mitotic chromosomes of soybean ranged from 1.42 to 2.84 μm (Sen and Vidyabhusan 1960); they grouped the chromosomes into 2 long pairs (2.61–2.84 μm), 4 short pairs (1.42–1.85 μm), and 14 medium-sized pairs (1.97–2.37 μm). They suggested that all the chromosomes contain a median or nearly median kinetochore, and they could not identify satellite chromosomes. Ladizinsky et al. (1979a) karyotyped Giemsa-stained soybean chromosomes and recorded a single band in the majority of the soybean chromosomes. They concluded that this technique has limited value for identifying individual chromosomes. Yanagisawa et al. (1991) separated 40 soybean mitotic metaphase chromosomes into 5 groups (A, B, C, D, and E) containing 7 categories, by using the

chromosome image analyzing system (CHIAS). They also used the N-banding technique to identify cytological chromosome markers such as satellite chromosomes. Ohmido et al. (2007) supported idiograms of soybean developed by Yanagisawa et al. (1991) and reported a pair of nucleolus organizer chromosomes by conventional staining and FISH technique. They did not observe condensation patterns for chromosome 14.

Recently, Clarindo et al. (2007) developed a method based on using DNA synthesis inhibiting, anti-drying techniques, and digital image analysis of prometaphase and metaphase chromosomes. Chromosome lengths ranged from 1.99 to 1.26 μm for metaphase chromosomes, and the range was 3.35–1.84 μm for prometaphase chromosomes. They identified six metacentric (1, 2, 9, 10, 17, and 19) and fourteen submetacentric (3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16, 18, and 20) chromosomes. Furthermore, they grouped 40 chromosomes into 14 groups (1–2, 3–4, 5–6, 7, 8, 9–10, 11–12, 13, 14, 15–16, 17, 18, 19, 20). Based on these karyotypes, they proposed that soybean is of tetraploid origin. Findley et al. (2010) karyotyped *G. max* and *G. soja* metaphase chromosomes by using fluorescence in situ hybridization. They used genetically anchored bacterial artificial chromosome (BAC) clones to identify all 20 chromosomes.

Ahmad et al. (1983) utilized a quantitative method (scatter diagram) and grouped soybean chromosomes as 12 metacentric, 7 submetacentric, and 1 subtelocentric and chromosome length ranged from 2.8 μm to <1.6 μm . In *G. soja*,

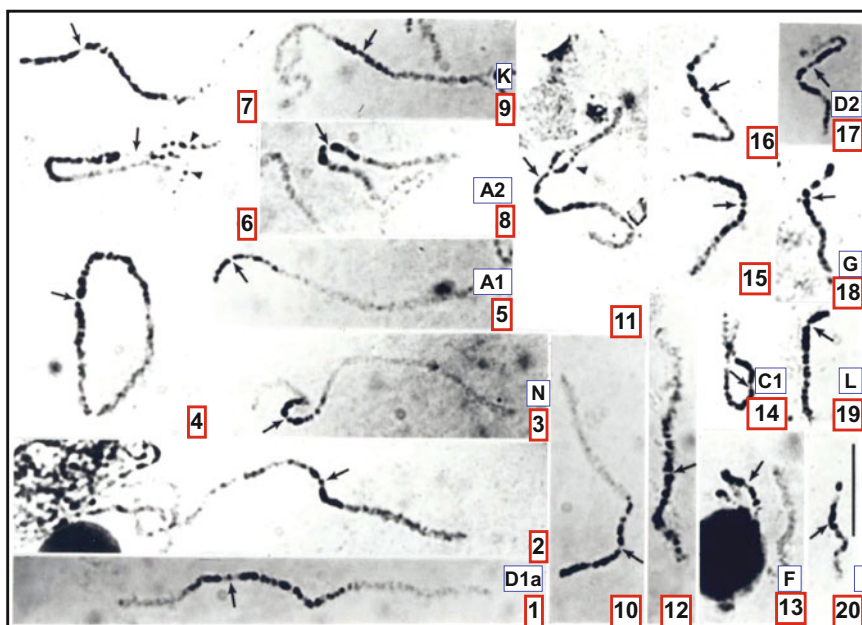


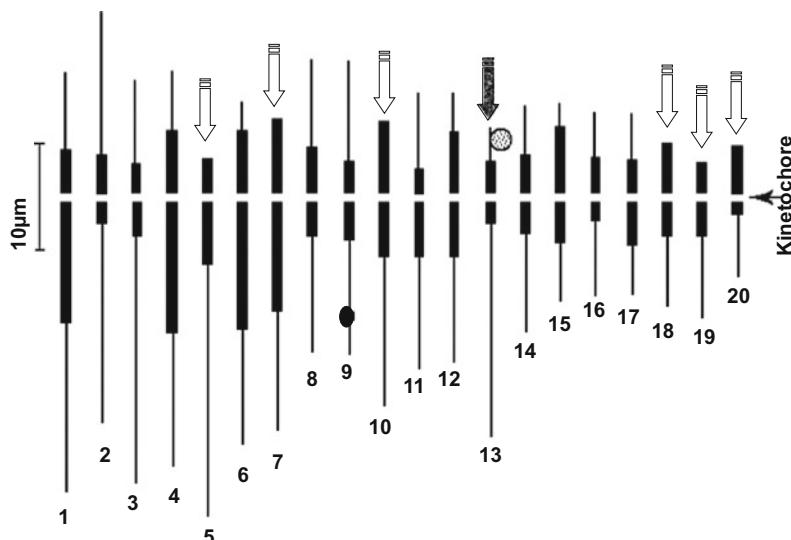
Fig. 2.7 Photomicrographs of the pachynema chromosome complement of *G. max* $\times$ *G. soja* F₁ hybrid. Each figure shows a different chromosome, 1–20. Arrows

indicate centromere location. The letter above the number represents the molecular linkage group (MLG)

Ahmad et al. (1984) reported 12 metacentric and 2 submetacentric chromosomes, but no subtelocentric chromosomes. The *G. soja* chromosome complement was found to be about 6–7% smaller than that of *G. max*. In contrast, pachynema chromosomes of an F₁ hybrid of *G. max* and *G. soja*, described below, do not support this result. Singh and Hymowitz (1988) identified individual soybean pachynema chromosomes on the basis of length, euchromatin and heterochromatin distribution, and association of a chromosome with the nucleolus. The heterochromatin was distributed proximal to and on either side of the kinetochore on the long and short arms. It is interesting to note that 35.84% of the soybean chromosomes (genome) are composed of heterochromatin (gene-poor regions) and 64.16% chromosomes (genome) are euchromatic (gene-rich regions). Chromosomes of *G. max* and *G. soja* paired perfectly, with minor structural differences (Fig. 2.7). One reciprocal translocation differentiated materials used in this study, and one of the chromosomes involved in the interchange was a satellite chromosome.

Pachynema chromosomes were arranged from 1 to 20 based on total lengths. The range was 39.79 μ m (chromosome 1) to 10.63 μ m (chromosome 20). Each pair was unique with characteristic landmark features: (1) Seven short arms (chromosome 5, 7, 10, 13, 18, 19, 20) are heterochromatic; (2) chromosome 2 contains the least (14.22%) heterochromatin, chromosome 5 contains the shortest heterochromatic short arm and was easy to identify from other chromosomes, chromosome 7 consists of 59.44% heterochromatin, and chromosomes 18 and 19 were difficult to distinguish, but these chromosomes were very easy to separate from the clump of pachynema chromosomes; (3) chromosome 13 was difficult to isolate although it was expected to separate from the other groups. The satellite was completely imbedded in the nucleolus, and the short arm was completely heterochromatic; (4) it should be noted that a standard chromosome (cytological) idiogram of soybean was constructed based on pachynema chromosomes (Figs. 2.7 and 2.8) (Singh and Hymowitz 1988).

Fig. 2.8 Proposed ideogram, based on Figs. 14 and 15, of the pachynema chromosomes of soybean. *Dotted arrows* show totally heterochromatic short arm. Chromosome 13 is a nucleolus organizer chromosome. Chromosome 9 has a heterochromatic knob on the long arm



2.4 Cytogenetics

2.4.1 Genomes of the *Glycine* Species

2.4.1.1 Classical Taxonomy

Classical taxonomy and extensive plant exploration in Australia and surrounding Islands have played a major role in the identification and nomenclature of new species in the subgenus *Glycine* (Tables 2.1, 2.2, and 2.3). *Glycine clandestina* ($2n = 40$) has been observed to be a morphologically highly variable species (Hermann 1962). Newell and Hymowitz (1980) revised the subgenus *Glycine* by proposing a new species, *Glycine latifolia* (Benth.) Newell and Hymowitz. Stems of wild perennial species are twining, climbing, or procumbent and exhibit distinct morphological traits. Short pod *G. clandestina* (Singh and Hymowitz 1985b) was removed and named *G. microphylla* (Benth.) Tind. (Tindale 1986b), and curved pod *G. clandestina* were classified as *G. cyrtoloba* (Tindale 1984) and *G. curvata* (Tindale 1986a). Costanza and Hymowitz (1987) observed the presence of adventitious roots in B-genome species (Table 2.3) that includes *G. microphylla*, *G. latifolia*, and *G. tabacina*. This morphologically distinguishing trait is absent in other *Glycine* species. Tindale and Craven (1988) described

three new species (*Glycine albicans* Tind. and Craven, *Glycine lactovirens* Tind. and Craven, and *Glycine hirticaulis* Tind. and Craven). *Glycine hirticaulis* contains accessions with $2n = 40$ and 80 chromosomes. Newly described species have restricted geographical habitats in Australia, and they have proven difficult to maintain under greenhouse conditions in Urbana, Illinois.

Diploid ($2n = 40$) *G. tomentella* accessions have been separated into five groups [D3 (A, B, C.), D4, D5, D6, and D7] based on isozyme similarities (Doyle and Brown 1985; Doyle et al. 1986). Later, accessions of the D6 group from Western Australia were classified as *G. arenaria* Tind. (Tindale 1986b). Singh and Hymowitz (1985b) studied meiotic chromosome pairing between D4 group *G. tomentella* (PI441000) and *G. clandestina* ($2n = 40$; PI440948; A₁-genome) and observed 9–18II at metaphase-I. The PI441001 accession contains long pods and narrow leaves, and its meiotic pairing suggests that it is closer to A-genome species (Singh et al. 1992a; Kollipara et al. 1995). Pfeil et al. (2006) removed PI441000 from *G. tomentella* and named it *G. syndetica*. Singh et al. (2007b) assigned to it genome symbol A₆. Currently, we have 26 classified wild perennial *Glycine* species because of extensive plant exploration and taxonomic and molecular studies (Table 2.3).

Glycine tomentella with $2n = 38$ chromosomes is morphologically indistinguishable from 40-, 78-, and 80-chromosome *G. tomentella*. However, it differs genomically from 40-chromosome *G. tomentella* (Kollipara et al. 1993). Taxonomists should closely observe 38-chromosome *G. tomentella* and determine whether this warrants specific species recognition.

Crossing Affinity

Intra- and interspecific crossability is an excellent method to establish the species relationships. Interspecific crosses involving parental species with similar genomes usually set normal pods and F_1 seeds, and the hybrids are fertile, while in crosses between genomically dissimilar species, seed abortion is common, and hybrids if obtained are sterile (Newell and Hymowitz 1983; Grant et al. 1984b; Singh and Hymowitz 1985b, c; 1987, 1988, 1989, 1992a, b; Kollipara et al. 1993). Normal pod set and fertile F_1 hybrids are expected from crosses between morphologically and genomically similar species. However, this is not always true. For example, *G. cyrtoloba* and *G. curvata* carry curved pods and their morphological features are nearly identical (Tindale 1984, 1986a). In one study involving a *G. cyrtoloba* and *G. curvata* cross, a total of 748 flowers were pollinated, but all of the gynoeceia died 2–3 days after pollination (DAP) and no pod set was recorded (Singh et al. 1992a). Pod abortion was recorded in a cross between 38- and 40-chromosome *G. tomentella* (Singh et al. 1988) even though both cytotypes were morphologically similar.

2.4.1.2 Meiotic Chromosome Pairing

The degree of chromosome pairing in interspecific hybrids provides an important cytogenetic context for interpreting phylogenetic relationships among diploid species, enhances our understanding of the evolution of the genus, and provides information about the ancestral species. Generally, species with similar genomes exhibit complete or almost complete chromosome pairing (intragenomic chromosome pairing) in their hybrids (Fig. 2.9a). Chromosome migration to anaphase-I poles is normal, and sometimes,

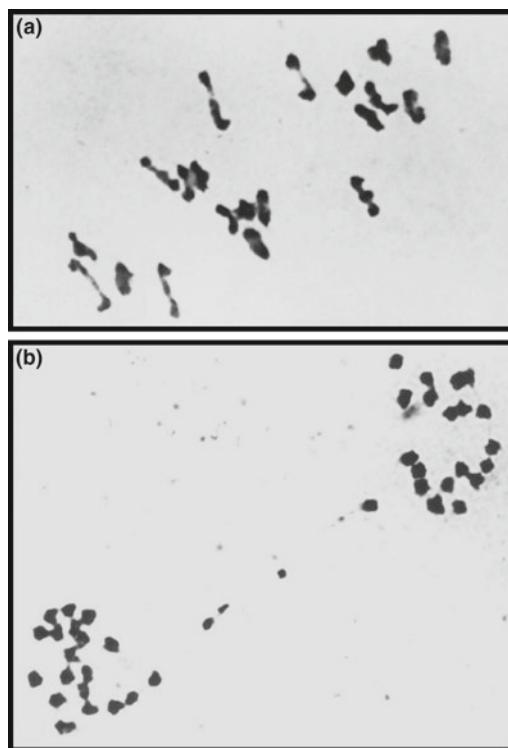


Fig. 2.9 Meiosis in intragenomic F_1 hybrid of *G. latifolia* (B_1B_1 ; $2n = 40$) $\times$ *G. microphylla* (BB ; $2n = 40$). **a** Metaphase-I, showing 20 bivalents, (From Singh et al. 2007b); **b** Anaphase-I, showing a chromatin bridge and an acentric fragment (paracentric inversion) in an interspecific hybrid of *G. clandestina* (A_1A_1 ; $2n = 40$) $\times$ *G. canescens* (AA ; $2n = 40$)

species differ only by chromosomal interchanges or by paracentric inversions (Fig. 2.9b).

Putievsky and Broué (1979) laid the foundation of genomic relationships in the genus *Glycine*. They produced 19 intraspecific and 30 interspecific F_1 hybrids among *G. canescens*, *G. clandestina*, *G. tomentella* ($2n = 78, 80$), *G. falcata*, and *G. tabacina* ($2n = 40, 80$). In the genus *Glycine*, all F_1 hybrids from crosses among A-genome (*G. canescens*, *G. argyrea*, *G. clandestina*, and *G. syndetika*) and B-genome (*G. microphylla*, *G. latifolia*, and *G. tabacina*) species displayed 20 bivalents at metaphase-I in the majority of the sporocytes (Putievsky and Broué 1979; Newell and Hymowitz 1983; Grant et al. 1984a, b; Singh and Hymowitz 1985b, c; Singh et al. 1988, 1992a, b; Fig. 2.10).

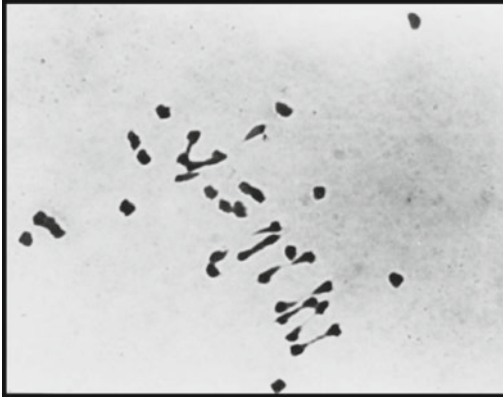


Fig. 2.10 Meiosis in intergenomic F_1 hybrid of *G. latifolia* (B_1B_1 ; $2n = 40$) $\times$ *G. canescens* (AA; $2n = 40$); metaphase-I, showing 20 univalents + 10 bivalents

The extent of chromosome association with the hybrids of genomically dissimilar species elucidates structural homology in the parental chromosomes and hence furnishes evidence regarding the progenitor species (Singh 2003; Singh 2017; Singh and Hymowitz 1985b). Usually, the F_1 hybrids generated from genomically unlike parents (different biological species) are germinated through in vitro techniques. Hybrid seed inviability, seedling lethality, and vegetative lethality are common occurrence in intergenomic crosses (Newell and Hymowitz 1983; Singh et al. 1988, 1992a). In general, such hybrids are weak, slow in vegetative and reproductive growth, and sterile. In the subgenus *Glycine*, A- and B-genome species hybrids show an average chromosome association of $19.7I + 10.2II$ ($A_3 B_1$) and $20.9I + 9.5II$ ($A \times B_1$) (Singh et al. 1988). This suggests strongly that one genome is common in the A- and B-genome species and that it may be the common progenitor species with $2n = 20$ chromosomes. However, since *Glycine* species with $2n = 20$ chromosomes have not been identified, we cannot identify the species.

Variable (semi-homologous–homoeologous) and minimal chromosome pairing are common in intergenomic F_1 hybrids. An erroneous conclusion may be drawn if genome designations of species are based on classical taxonomy only. For example, aneuploid ($2n = 38$) *G. tomentella* is

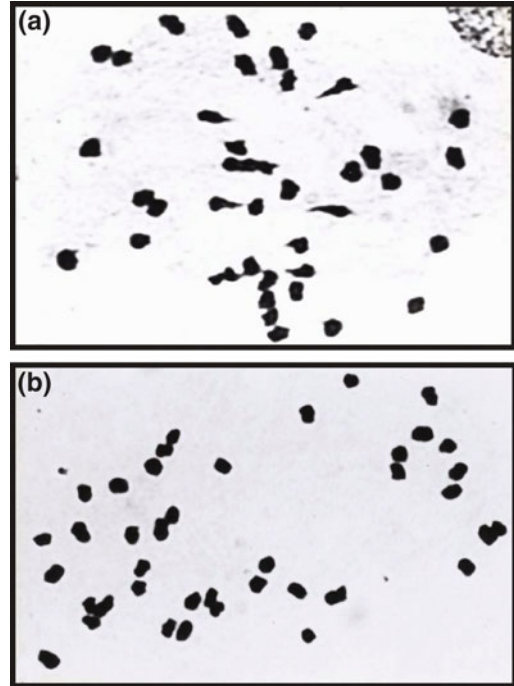


Fig. 2.11 Meiosis in interspecific *Glycine* hybrids. **a** Metaphase-I, showing 31 univalents + 4 loosely associated rod-shape bivalents in *G. tomentella* (EE; $2n = 38$) $\times$ *G. canescens* (AA; $2n = 40$). **b** Metaphase-I, showing 40 univalents in [*G. clandestina* (A_1A_1 ; $2n = 40$) $\times$ *G. canescens* (AA; $2n = 40$)] $\times$ *G. falcata* (FF; $2n = 40$)

morphologically similar to 40, 78, and 80 chromosome tomentellas. In contrast, limited chromosome pairing was observed between 38- and 40-chromosome *G. tomentella* (D_3) and aneuploid *G. tomentella* (E-genome) (Singh et al. 1998) and *G. canescens* (A-genome) (Fig. 2.11a). *Glycine falcata* is morphologically distinct among 26 wild perennial species of the subgenus *Glycine* and 2 species of subgenus *Soja*. Chromosome pairing results ($B_1 \times F$, $37.8I + 1.1II$; $A \times F$, $38.7I + 0.6II$) support the uniqueness of genome (F) of *G. falcata* because it showed minimal chromosome synapsis with A- and B-genomes (Fig. 2.11b). Putievsky and Broué (1979) reported distant relationship between *G. falcata* (F) and *G. clandestina* (genome A_1) with an average chromosome association of $36.1I + 1.85II + 0.05III$. Newell and Hymowitz (1983) obtained non-viable hybrids in *G. falcata* $\times$ *G. canescens* and *G. falcata* $\times$ *G. tomentella* ($2n = 40$). Cytogenetic

studies demonstrate that *G. falcata* does not have a common progenitor present in A, B, C, D, and E-genome species of the subgenus *Glycine* and the origin of this species may be independent or the genomes are completely differentiated.

Based on classical taxonomy, *G. soja* and *G. max* are different species (Hermann 1962). Both species carry $2n = 40$ chromosomes, hybridize readily, produce viable, vigorous, and fertile hybrids, and some lines differ by a reciprocal translocations (Karasawa 1936; Palmer et al. 1987; Singh and Hymowitz 1988) or by paracentric inversions (Ahmad et al. 1977, 1979). Pachynema chromosome pairing was completely normal all along the lengths of the long and short arms with the exception of chromosomes 6 and 11 (Singh and Hymowitz 1988). Therefore, *G. soja* and *G. max* have now been assigned genome symbols G and G₁, respectively (Singh et al. 2007b).

2.4.1.3 Genomic Affinity by Molecular Techniques

During the past two decades, the literature on genomic relationships (plant phylogenetic relationships) has been dominated by molecular studies, including nuclear [seed protein electrophoresis, isozyme variation, restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplification of polymorphic DNA (RAPD), simple sequence repeat (SSR), sequences variation in the gene such as ITS region of rDNA], extra-nuclear (chloroplast and mitochondrial) DNA variation, and genomic in situ hybridization (GISH) by multicolor FISH. Molecular techniques are extremely powerful tool especially for determining species relationships where production of interspecific or intergeneric hybrids is not feasible by conventional methods (Singh 2003). Molecular tools verified the cytogenetically based conclusion that *G. max* and *G. soja* are genomically similar (Doyle and Beachy 1985; Doyle 1988; Kollipara et al. 1995, 1997; Zhu et al. 1995). *Glycine max* and *G. soja* appeared to be identical

by Doyle (1988), and the sequence divergence for internal transcribed spacer (ITS) region of rDNA was 0.2% (Kollipara et al. 1997).

Broué et al. (1977) were the first to use isozyme technique to establish genomic relationships among *G. canescens*, *G. clandestina*, and *G. tomentella*. Kollipara et al. (1997) determined phylogenetic relationships among 16 species of the subgenus *Glycine* and two species of the subgenus *Soja* from nucleotide sequence variation in the ITS region of nuclear ribosomal DNA. This study helped to assign genome symbols to five species: H to *G. arenaria*, H₁ to *G. hirticaulis*, H₂ to *G. pindanica*, I to *G. albicans*, and (I₁) to *G. lactovirens*. Cytogenetic relationships among these five species have not been determined because only a few accessions are available and they are difficult to grow in the greenhouse. These genome designations have been verified by histone H3-D gene sequences, and genomes were also assigned to *G. aphyonota* (I₃), *G. peratosa* (A₅), *G. pullenii* (H₃), and *G. stenophita* (B₃) (Brown et al. 2002; Doyle et al. 2002). The ITS region (nrDNA) is a multigene family. However, in the soybean, the nrDNA has been mapped to a single locus on the short arm of chromosome 13 based on the location of the nucleolus organizer region by pachynema chromosome analysis (Singh and Hymowitz 1988) and also by FISH (Singh et al. 2001).

Of the 26 wild perennial *Glycine* species, *G. tomentella* is a unique species because it consists of four cytotypes ($2n = 38, 40, 78, 80$). Aneuploid ($2n = 38$) *G. tomentella* is distributed in a restricted region of Queensland, Australia. The diploid ($2n = 40$) cytotype is distributed widely in Australia (Queensland, Northern Territory, Western Australia) and Papua New Guinea. Isozyme banding patterns grouped the aneuploids into two isozyme groups (D1 and D2), and the diploids form six isozyme (D3A, D3B, D3C, D4, D5, D6) groups (Doyle and Brown 1985). Cytogenetics revealed that D1 and D2 isozyme groups carry a similar genome and are distinct from other isozyme groups (Fig. 2.12). Singh

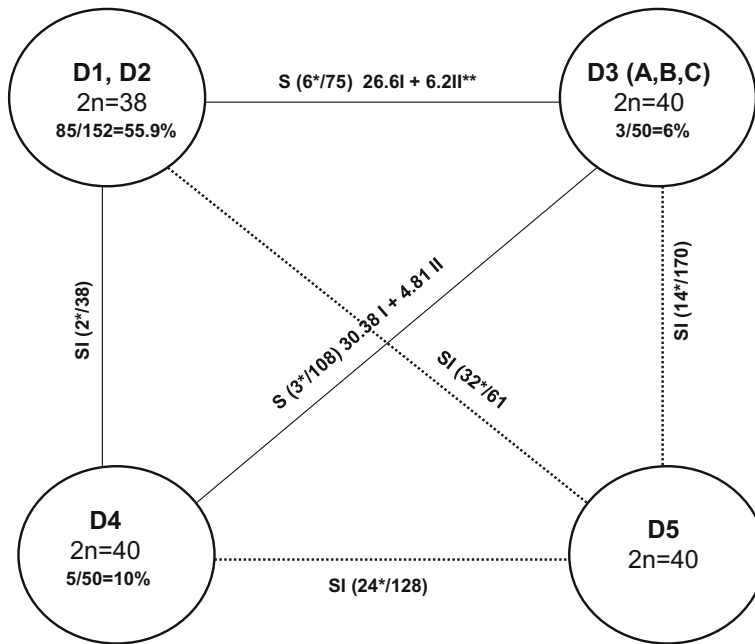


Fig. 2.12 Summary of genomic relationships among five groups of aneuploid ($2n = 38$; D1 and D2) and diploid ($2n = 40$; D2 (A, B, C), D4, and D5) *G. tomentella* based on crossability rate and meiotic chromosome pairing in F_1 hybrids. The within-group

crossability rate (%) is shown inside the circle. The between-group crossability rates (number of pod set/total number of flowers pollinated) are shown in the parentheses. *, number of aborted pods; **, Singh et al. (1988); S sterile; SI seed inviability

et al. (1988) assigned it the E-genome symbol. The D4 isozyme group *G. tomentella* contains PI 441000 (D₃-genome) and has a close affinity cytogenetically with A-genome species but is distinct from other D isozyme groups (Grant et al. 1984a; Singh et al. 1988). Although the D4 isozyme group is morphologically distinct from the A-genome species, it does have long and narrow leaves and a longer pod length that is a characteristic feature of A-genome species and that distinguishes it from other diploid *G. tomentella* accessions (Singh et al. 1998b). The histone H3-D gene sequence also grouped D4 isozyme accessions with A-genome species (Brown et al. 2002), and the D4 isozyme group *G. tomentella* was classified as *Glycine syndetika* B.E. Pfeil and Craven (Pfeil et al. 2006). The A₆-genome symbol was assigned to PI441000 (Singh and Chung 2007; Singh et al. 2007b). No viable hybrid plants were produced in crosses between accessions of the D5 and D1, D2, D3, and D4 isozyme groups (Fig. 2.12).

2.4.2 Origin of Polyploid Complexes of *Glycine tabacina* and *G. tomentella*

Of the 26 species of the subgenus *Glycine*, *G. hirticaulis*, *G. tabacina*, and *G. tomentella* contain $2n = 40$ and $2n = 80$ chromosomes (Table 2.3). Furthermore, *G. tomentella* consists of aneuploid ($2n = 38$) and aneutetraploid ($2n = 78$) accessions. Tetraploid *G. hirticaulis* has restricted geographical distribution in the Northern Territory of Australia. *Glycine tomentella* accessions with $2n = 80$ chromosomes are also distributed in Taiwan, and it was designated as *G. dolichocarpa* Tateishi and Ohashi (Tateishi and Ohashi 1992) and species status was verified by seed protein patterns (Hsieh et al. 2001). On the other hand, aneutetraploid ($2n = 78$) *G. tomentella* is found in Australia and Papua New Guinea and tetraploid ($2n = 80$) *G. tomentella* is distributed in Australia, Papua New Guinea, Philippines, Timor Island of Indonesia, and

Taiwan. Aneutetraploid and tetraploid tomentellas are allopolyploid and exhibit diploid-like meiosis (Singh and Hymowitz 1985a) and complexes of multiple origins (Putievsky and Broué 1979; Singh et al. 1987b, 1989, 1992b; Singh and Hymowitz 1985b; Doyle and Brown 1989; Kollipara et al. 1994; Doyle et al. 1999, 2004; Hsing et al. 2001; Brown et al. 2002; Rauscher et al. 2004). Classification of the polyploid *G. tabacina* and *G. tomentella* accessions into discretely defined, reproductively isolated groups using various methods (morphological, cytogenetic, biochemical, and molecular) helps in better understanding the origin of the species complex (Doyle et al. 1990c; Kollipara et al. 1994).

2.4.2.1 *Glycine tabacina* ($2n = 80$)

Diploid *G. tabacina* is indigenous to Australia. A tetraploid ($2n = 80$) cytotype is found sympatrically with diploids in Australia, but 80-chromosome tabacinas are distributed also in the islands of the south Pacific (New Caledonia, Vanuatu, Fiji, Tonga, Niue) and west central Pacific (Taiwan, Ryuku, Marianas) (Singh et al. 1992b). Morphological observations (Costanza and Hymowitz 1987), cytogenetic investigation (Singh et al. 1987b, 1992b), and molecular studies (Doyle et al. 1990a, b, 1999) have shown two distinct groups in the 80-chromosome *G. tabacina*. It is an allopolyploid complex of multiple origins. One group contains adventitious roots, while the other group has long and narrow leaves (like A-genome species) and has no adventitious roots. All the intraspecific F_1 hybrids within each group showed normal meiosis and seed fertility. However, F_1 hybrids between two groups were sterile owing to disturbed meiosis. At metaphase-I, a model chromosome association of $40I + 20II$ was recorded (Singh et al. 1987b), indicating that both groups have one genome in common and differ for the second genome.

Singh et al. (1992a, b) proposed, based on cytogenetics, that the 80-chromosome *G. tabacina* without adventitious roots is a complex, probably synthesized from A-genome (*G. canescens*, *G. clandestina*, *G. argyrea*, *G. syndetika*) and *G. tabacina* with adventitious roots evolved through segmental allopolyploidy from

B-genome (*G. latifolia*, *G. microphylla*, *G. tabacina*, *G. stenophita*) or by allopolyploidy having one of the B-genome species and second unknown genome donor species. Doyle et al. (2000) included *G. stenophita* into B-genome group; however, cytogenetic relationship with other B-genome species has not been determined.

Doyle et al. (1999) suggested, based on sequencing of histone H3-D locus, the multiple origins with gene exchange among lineage increase the genetic base of a polyploid and help better colonization of polyploid *G. tabacina* relative to its diploid progenitors. Hybridization is unlikely in a highly self-inbreeder in the nature; however, F_1 hybrids among B-genome species are completely fertile (Putievsky and Broué 1979; Newell and Hymowitz 1983; Grant et al. 1984b; Singh and Hymowitz 1985c). Since B-genome species are sympatric (Doyle et al. 1999), adventitious root trait is controlled by recessive gene (Singh et al. 1988) and Bowman Birk Inhibitor (BBi) is present in A-genome species including 80-chromosome *G. tabacina* without adventitious roots but absent in B-genome species and 80-chromosome *G. tabacina* with adventitious roots (Kollipara et al. 1997).

2.4.2.2 *Glycine tomentella* ($2n = 78, 80$)

Diploid-like meiosis, isozyme banding patterns among the accessions and meiotic pairing in intraspecific and interspecific F_1 hybrids, wide geographical distribution, and aggressive and vigorous growth habit suggest that 78- and 80-chromosome tomentellas are of allopolyploid origin and are polyploid complexes (Singh and Hymowitz 1985a).

Genomic complexes within species can be determined by obtaining intraspecific hybrids involving parental accessions of diverse morphology, cytology, and geographical origins. Meiotic pairing and molecular results in intraspecific plants of 78-chromosome tomentellas have revealed three complexes (designated based on isozyme; T1, T5, and T6; Doyle and Brown 1985). Hybrids within groups showed normal chromosome pairing. All hybrids between genomic complexes showed one

common genome (EE-genome; 38-chromosome *G. tomentella*), and this was verified by molecular methods (Kollipara et al. 1994). This suggests that some complexes have one genome common and differ for the second genome. T1 group aneutetraploid tomentella predominates and is distributed in Queensland, and one accession in Papua New Guinea. T5 group is found in New South Wales, while T6 group is found in Western Australia. Thus, these isozyme groups are geographically isolated and may have originated independently with an aneudiploid ($2n = 38$; E-genome) as common genome donor.

Based on isozyme banding patterns, Doyle and Brown (1985) separated 80-chromosome *G. tomentella* accessions into three (T2, T3, T4) groups. They did not examine accessions from Indonesia. Kollipara et al. (1994) assigned T7 group to accessions from Indonesia. Cytogenetics, total seed protein profiles, protease inhibitor activity band profiles, immunostained banding patterns, and RFLP analysis clearly identified four distinct groups (T2, T3, T4, and T7) in 80-chromosome *G. tomentella* (Kollipara et al. 1994). Meiotic chromosome pairing at diakinesis-metaphase-I in the F_1 hybrids within isozyme groups was normal and the plants were completely fertile, but hybrids between groups were sterile. All F_1 hybrids between $T2 \times T3$, $T3 \times T4$, and $T2 \times T7$ were sterile; presumably as a consequence of disturbed meiotic chromosome pairing. Chromosome pairing results suggest one common genome in the T2, T3, T4, and T7 groups. Accessions from Indonesia (T7) did not show complete genome affinity with T3 group (accessions from Cooktown, Qld, Port Moresby, PNG, and Gratto Creek, Western Australia). However, maximum chromosome association of $30II + 20I$ at metaphase-I was recorded, and it produced few cleistogamous pods and mature seeds. This suggests that accessions of T7 probably carry the same genome and geographical isolation played a major role in their divergence. An independent origin cannot be proposed because *Glycine* species with diploid cytotypes have not been identified in Indonesia.

A way to ascertain the ancestors of 78- and 80-chromosome tomentellas is to synthesize

amphidiploids from their putative parental species (Singh et al. 1998a). Aneuploidtetraploid (DDEE, AAEE; $2n = 78$) and allotetraploid (AADD; $2n = 80$) were produced by somatic chromosome doubling of $2n = 39$ and $2n = 40$ F_1 hybrids. Meiotic chromosome pairing in the synthesized amphiploid was diploid-like and produced normal pods and seeds. Synthesized amphidiploids were hybridized with accessions of tomentellas of T1, T2 ($2n = 78$), and T2 ($2n = 80$) isozyme groups. Meiotic pairing was normal and fertile (Singh et al. 1989), and molecular results verified the cytogenetic results (Kollipara et al. 1994). Rauscher et al. (2004) reported that the genome donors of T2 *G. tomentella* are diploid *G. tomentella* of D3 and D4 isozyme groups. The D4 (PI44100) isozyme showed genomic affinity with *G. clandestina* (PI505161) (Singh et al. 1987). Pfeil et al. (2006) taxonomically classified PI441001 as *G. syndetika*, and Singh et al. (2007) assigned it genome symbol A_6 (Table 2.3). Cytogenetics and molecular studies of Kollipara et al. (1994) support *G. canescens* or any A-genome species including *G. syndetika* as possible genome donor to T2 tomentella.

Kollipara et al. (1994) concluded “both aneutetraploid (T1, T5, T6) and tetraploid accessions (T2, T3, T4, and T7) may have originated by independent events. Reconstruction of hypothesized ancestors through synthesis of artificial hybrids appears to provide an excellent way to analyze polyploid complexes such as those of *G. tomentella*. However, it is also important to understand the diversity among diploid donors of these polyploids.” Rauscher et al. (2004) verified this statement by molecular studies.

2.4.3 Chromosomal Aberrations-Structural Aberrations

Chromosomal structural changes such as deficiencies, duplications, interchanges, and inversions have not been systematically produced, identified, and used in physical genetic mapping in soybean. An interchange of spontaneous origin

in soybean (Sadanaga and Grindeland (1984) has been used to locate the w_1 (white flower) locus on the satellite chromosome (chromosome 13). Palmer et al. (1987) surveyed 56 *G. soja* accessions from China and the Russia that also included PI81762 studied by Singh and Hymowitz (1988). They concluded that these accessions have a single similar or identical interchange. Singh and Hymowitz (1988) examined an interspecific F_1 hybrid of soybean $\times$ PI81762 and observed one quadrivalent that was always associated with the nucleolus. Mahama et al. (1999) identified six of the possible 20 reciprocal translocation lines. Mahama and Palmer (2003) identified that classical linkage groups (CLGs) six and eight are the same by reciprocal translocations.

Inversions (paracentric and pericentric) have neither been produced nor used in physical mapping of the soybean genome. Study has been limited to identifying a paracentric inversion in soybean $\times$ *G. soja* hybrid (Ahmad et al. 1977; Palmer et al. 2000). Wild perennial *Glycine* species with similar genomes are differentiated by a paracentric inversion (Singh 2003) as the majority of the sporocytes showed normal pairing at metaphase-I (Fig. 2.9a), but at anaphase-I, a chromatin bridge and an acentric fragment were observed (Fig. 2.9b).

2.4.4 Chromosomal Aberrations—Numerical Changes

2.4.4.1 Autopolyploidy

Darlington and Wylie (1955) proposed $\times = 10$ as the basic chromosome number of the genus *Glycine*. Haploid ($2n = 20$) (Crane et al. 1982), triploid ($2n = 60$) (Chen and Palmer 1985), and tetraploid ($2n = 80$) (Sen and Vidyabhusan 1960) soybean plants have been reported. Haploid and triploid are completely sterile, and tetraploid soybean produces few one or two large seeded pods. Tetraploid soybean has no commercial value. Tetraploid $\times$ diploid crosses in natural population have failed to produce an autotriploid (Sadanaga and Grindeland 1981), an excellent source for producing primary trisomics

(Singh 1998a). However, Chen and Palmer (1985) identified autotriploids from the progenies of male sterile lines, but the derived autotriploid was not used to produce primary trisomics. Xu et al. (2000b) found a hypertriploid ($2n = 3x + 1 = 61$) plant from a cross T31 [a homozygous recessive glabrous (*pp*)] $\times$ T190-47-3 (an unidentified primary trisomic). The hypertriploid plant produced 98 selfed seed, and the chromosome numbers of the plants ranged from $2n = 50$ to 69. Chromosome number in hypertriploid $\times$ diploid seeds ranged from $2n = 44$ to 56. These lines were not used in producing primary trisomics either.

2.4.4.2 Aneuploidy—Trisomics

Primary Trisomics

Primary trisomics in soybean (an individual with normal chromosome complements plus an extra complete chromosome; $2n = 2x + 1 = 41$) have been isolated from the progenies of asynaptic and desynaptic mutants (Palmer 1976; Gwyn et al. 1985; Xu et al. 2000c). Gwyn et al. (1985) examined four primary ($2n = 41$) trisomics (Tri A, B, C, D) and observed that they were similar to the diploid ($2n = 40$). Singh and Hymowitz (1991) identified, by using pachytene chromosome, Tri A as Triplo 5, Tri C as Triplo 1, Tri D as Triplo 4, and Tri S (Skorupska et al. 1989) as Triplo 13. Triplo 13 contains 3 satellite chromosomes. Ahmad et al. (1992) identified four new primary trisomics (Triplo 2, 3, 10, and 14), and Xu et al. (2000c) isolated 12 additional primaries from 37 aneuploid lines ($2n = 41, 42, 43$) and tentatively identified 20 simple primary trisomics that were designated, based on the length of pachytene chromosome, as Triplo 1 (contains the longest chromosome) to Triplo 20 (the shortest chromosome). These aneuploid lines originated from the progenies of asynaptic and desynaptic mutants that were supplied by the late Reid Palmer (USDA/ARS, Iowa State University, Ames, IA, 50011-1010).

At metaphase-I of meiosis, a majority of the microsporocytes in primary trisomics exhibit 1III + 19II or 20II + 1I. Average female transmission of 20 soybean primary trisomics was

42% with a range of 27 (Triplo 20) to 59% (Triplo 9). Female transmission rate has been estimated from the hybrid population (Xu et al. 2000c). This may be the reason for high female transmission rate of the extra chromosome in primary trisomics of soybean; heterozygosity often favors the higher female transmission rate (Singh 2003).

Primary trisomics of soybean have been used to associate a few classical genetic markers. Three marker genes *Eu1* (seed urease), *Lx1* (lipoxygenase-1), and *P2* (puberulent) were located on chromosome 5, 13, and 20, respectively (Xu et al. 2000c). Zou et al. (2003a, b) associated yellow leaf mutant *y10* with chromosome 3 by primary trisomics method. Griffin et al. (1989) located *fr1* gene on CLG 12 which was separated from *Ep* gene with a distance of 41.4 ± 0.8 cM, and Jin et al. (1999) associated CLG 12 with molecular linkage group (MLG) K. Root fluorescence gene (*fr1*) showed trisomic ratio with Triplo 9 (216:14) and disomics ratio with Triplo 1 (23:5), 2 (21:7), 3 (25:4), 4 (32:6); 5 (23:6), 6 (19:5), 12 (27:8), and 15 (39:10). This clearly demonstrates that the *fr1* is located on chromosome 9 which contains MLG K and CLG 12.

Hedges and Palmer (1991) used five primary trisomics (A, B, C, D, and S) to locate several isozyme loci. *Dial* (diaphorase) showed trisomics ratio with trisomic D which is chromosome 4 of Singh and Hymowitz (1988, 1991). Thus, chromosome 4 has a genetic marker because none of the MLGs has been associated with chromosome 4 (Zou et al. 2003b).

Gardner et al. (2001) associated *Rps1-K* (*Phytophthora sojae*) with chromosome 3 by primary trisomics because this gene is dominant and showed 2:1 (trisomics ratio) with Triplo 3 and disomic ratio (3:1) with 9 other primary trisomics. MLG N is associated with chromosome 3. This demonstrates that primary trisomics are an excellent cytogenetic tool to associate genes and linkage maps physically to the chromosomes. Seeds of primary trisomics are unavailable and are likely perished because they have not been deposited in the USDA soybean germplasm collection (<https://npgsweb.ars-grin.gov/gringlobal/site.aspx?id=24>).

Monosomics

In soybean, monosomics ($2n - 1 = 39$; an individual lacking one chromosome is called monosomic) have been isolated from the progenies of synaptic mutant (Skorupska and Palmer 1987) and Triplo 3 and Triplo 6 (Xu et al. 2000a). Skorupska and Palmer (1987) isolated two plants with $2n = 39$ and 92 seedlings with $2n = 40$ from 1380 seeds; only 94 seeds germinated. Morphologically, mono-3 was smaller with reduced vigor, while mono-6 was similar to the disomics. Female transmission in mono-3 was 6.5%, while mono-6 was not transmitted among 105 plants. The transmission rate in monosomics is sporadic in soybean. It was concluded that monosomics in soybean are viable and fertile and can be produced; however, no systematic effort is being made to isolate monosomics in this economically important crop.

Tetrasomics

In soybean, tetrasomics ($2n + 2 = 42$; an individual carrying two extra homologous chromosomes in addition to its normal somatic chromosome complement is called tetrasomics) are identified in low frequencies from the selfed progenies of primaries ($2n = 41$) (Singh and Chung 2007). Tetrasomic 13 plants were weak and died prematurely. Gwyn and Palmer (1989) observed, based on morphological measurement, that tetrasomics and double trisomics ($2n + 1 + 1$) could be distinguished accurately from their disomics sibs. Tetrasomics mostly breed true, and occasionally related trisomics ($2n = 41$) and diploids ($2n = 40$) are identified. Most primary trisomics plants are produced from tetrasomics $\times$ disomics crosses. Tetrasomics in the soybean are unique cytogenetic stock because they are not viable in diploid crops such as maize, barley, rice, and tomato. It is sad that primary trisomics and tetrasomics stock of soybean are unavailable and probably they have been lost forever.

2.4.5 Chromosome Mapping

Chromosome, genetic, and cytogenetic maps in the model economically important crops such as rice, maize, barley, wheat, and tomato were

developed first, and molecular maps followed. By contrast, several molecular maps have been developed first in the soybean and these maps were not associated with the chromosomes by primary trisomics.

2.4.5.1 Chromosome Map

Although the precise chromosome number of soybean was determined in 1925 (Karpechenko 1925), several chromosome maps have been developed. However, none of the papers convincingly identified individual somatic metaphase chromosomes because the chromosomes are symmetrical and only a pair of nucleolus organizer chromosome is identified in one of the best chromosome spreads. Singh and Hymowitz (1988) constructed a chromosome map of soybean by using pachynema chromosomes (Figs. 2.7 and 2.8). This pioneering research has set the stage to produce all possible primary trisomics in the soybean (Xu et al. 2000c).

2.4.5.2 Classical Linkage Groups

A genetic linkage map with 20 linkage groups, designated as CLGs of soybean, has been proposed (Palmer et al. 2004). Each of the classical linkage groups 2, 3, 12, 13, 15, 16, 18, 20, (21?) has two qualitative trait loci. Thus, the genetic linkage map of soybean is not saturated with classical markers as compared to other economically important crops. Mahama and Palmer (2003) associated CLGs 6 and 8 on the same linkage groups using translocation stocks. Translocation tester sets involving 20 soybean chromosomes have not been produced so far.

2.4.5.3 Cytogenetic Map

By using SSR markers from 20 MLGs and primary trisomics, Zou et al. (2003b) associated 11 MLGs with the 11 chromosomes and they failed to associate nine MLGs with the remaining chromosomes. It is possible that the trisomic set is not complete. Segregation distortion is common using primary trisomics and SSR markers, and this may be due to the preferential selection of gametes containing certain genotypes (Zou et al. 2006).

2.5 Wide Hybridization

2.5.1 Genetic Resources

Harlan and de Wet (1971) developed the concept of three gene pools—primary (GP-1), secondary (GP-2), and tertiary (GP-3) based on the success rate of hybridization among/between species. The clear understanding of taxonomic and evolutionary relationships between a cultigen and its wild relatives is a prerequisite for the exploitation of the primary, secondary, and tertiary gene pools.

2.5.1.1 Soybean GP-1

Soybean GP-1 consists of biological species that can be crossed to produce vigorous hybrids that exhibit normal meiotic chromosome pairing and possess total seed fertility. Gene segregation is normal, and gene exchange is generally easy. ARS GRIN maintains 20073 accessions of *G. max* and 1181 accessions of *G. soja* as of May 24, 2017 (<https://npgsweb.ars-grin.gov/gringlobal/site.aspx?id=24>).

2.5.1.2 Soybean GP-2

GP-2 species can hybridize with GP-1 easily, and F_1 plants exhibit at least some seed fertility (Harlan and de Wet 1971). *Glycine max* is without GP-2 because no known species has such a relationship with soybean. It is possible that species in the soybean GP-2 do exist in Southeast Asia where the *Glycine* genus may have originated. However, it is merely a speculation and extensive plant exploration in this part of the world is required to validate this assumption.

2.5.1.3 Soybean GP-3

GP-3 is the third outer limit of potential genetic resource. Hybrids between GP-1 and GP-3 are lethal, or completely sterile, and gene transfer is not possible or requires radical techniques (Harlan and de Wet 1971). Based on this definition, GP-3 includes the 26 wild perennial species of the subgenus *Glycine*. These species are indigenous to Australia, and various surrounding

islands and are geographically isolated from *G. max* and *G. soja*. Table 2.3 shows the *Glycine* species and their $2n$ chromosome numbers, nuclear genomes, and geographical distributions. The USDA Soybean Germplasm Collection, Urbana, Illinois, as of May 24, 2017, maintains 1006 accessions of the 19 wild perennial species (http://www.ars-grin.gov/cgi-bin/npgs/html/site_holding.pl?SOY).

2.5.2 Intersubgeneric Hybridization

The twenty-six wild perennial species of the genus *Glycine* subgenus *Glycine* have not been exploited in soybean breeding programs. These species are extremely diverse morphologically, cytologically, and genomically, grow in very diverse climatic and soil conditions, and have a wide geographical distribution (Singh and Hymowitz 1999). Wild perennial *Glycine* species have great potential for soybean improvement. They are a rich source of agronomically useful genes and alleles (Chung and Singh 2008). However, extensive and reproducible screening for abiotic and biotic stresses of 26 wild perennial *Glycine* species are lacking. Schoen et al. (1992) studied resistance of *G. tomentella* to 3 Australian isolates of soybean leaf rust and found PI 441001 ($2n = 78$), used in this study, resistant to all three races. It has been demonstrated that resistance to soybean rust in PI441001 has a chemical basis; a chemical inhibits the growth of fungus spores (Damla et al. 2008).

Ladizinsky et al. (1979b) initiated producing intersubgeneric hybrids between soybean and five wild perennial species of the subgenus *Glycine* and concluded “there would be little chance that these wild species could be exploited for breeding purposes.” Since 1979, several researchers have attempted to hybridize wild perennial *Glycine* species with the soybean, but only a few sterile intersubgeneric F_1 hybrid combinations have been achieved by using embryo rescue method (Table 2.6). Pod abortion in intersubgeneric

hybridization is a post-hybridization problem (Singh and Hymowitz 1987). Pod retention can be achieved by spraying a growth hormone mixture (100 mg GA_3 + 25 mg NAA + 5 mg Kinetin/L distilled water) once a day for 19 days. The developing pods should be removed 19–21 days post-pollination, and the immature seeds should be extracted aseptically and cultured onto a seed maturation medium (Singh 2007). Thus far, only Singh et al. (1990, 1993) have successfully produced backcross-derived fertile progenies from the soybean and a wild perennial, *G. tomentella* ($2n = 78$). Monosomic alien addition lines (MAALs) and modified diploid ($2n = 40$) lines are being isolated and identified (Singh et al. 1998a; Singh 2007; Singh and Nelson 2014, 2015).

A schematic diagram to produce reciprocal intersubgeneric hybrid between soybean and *G. tomentella* is shown in Fig. 2.13. The F_1 hybrid plants between soybean cv. Dwight ($2n = 40$) and *G. tomentella*, PI441001 ($2n = 78$) were rescued through embryo culture after 1 year. F_1 plants were vigorous, sterile, and contained $2n = 59$ chromosomes. Chromosomes were doubled (amphidiploid; $2n = 118$) by 0.1% colchicine treatment (Singh 1998a). An amphidiploid plant produced one (mostly) and 2-seeded pods and was backcrossed to Dwight (recurrent parent), and many BC_1 plants with $2n = 79$ chromosomes (40 chromosomes from Dwight and 39 chromosomes from *G. tomentella*) were produced through embryogenesis. All BC_1 plants were totally self-sterile. Backcrossed to Dwight, pod abortion was common but 40 mature seeds were harvested that produced 24 BC_2 plants. Chromosome number in BC_2 plants ranged from $2n = 55$ (40 chromosomes from Dwight + 15 chromosomes from *G. tomentella*) to 59 (40 chromosomes from Dwight + 19 chromosomes from *G. tomentella*). These plants are known as hypotriploids, and all plants were morphologically distinct in ways depending upon the presence of *G. tomentella* chromosome combinations. Amphidiploid plants ($2n = 118$) were induced by colchicine treatment. Amphidiploid $\times$ Dwight (BC_1) was

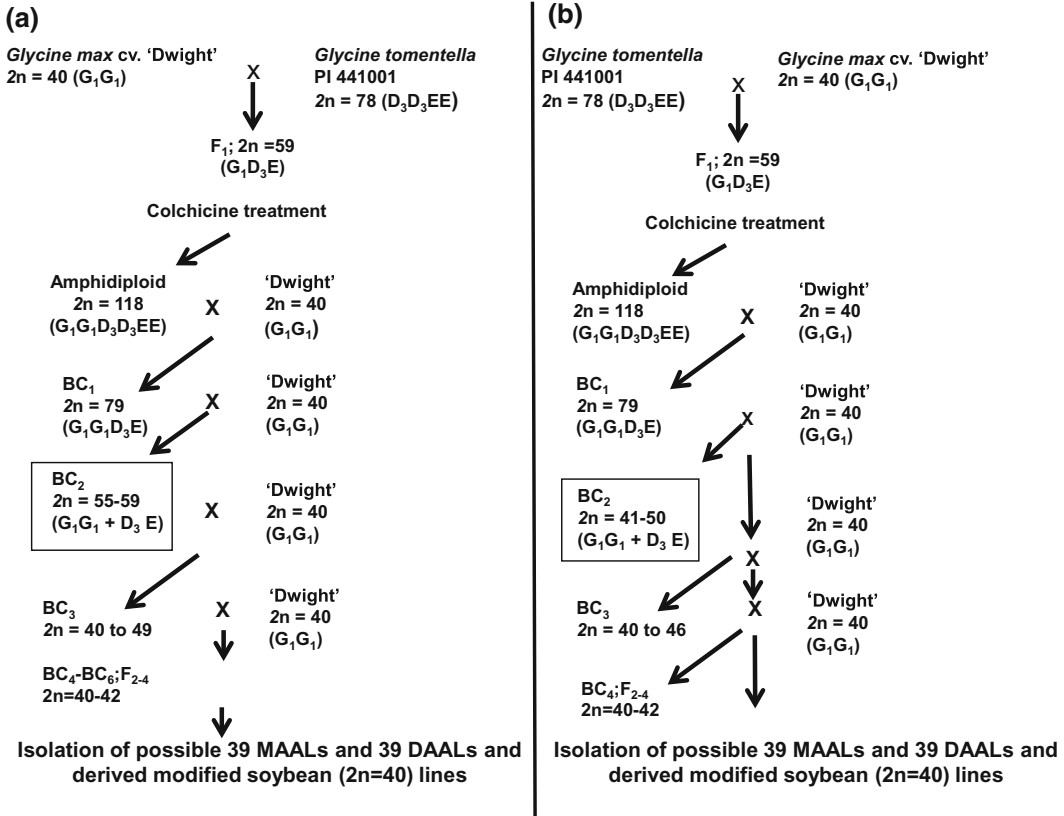


Fig. 2.13 A cytogenetic diagrammatic scheme to produce fertile soybean lines from *G. max* cv. Dwight ($2n = 40$) $\times$ *G. tomentella* ($2n = 78$) (a) and reciprocal hybrid (b). Note, chromosome numbers in BC_2 plants

developed through immature seed culture, and BC_1 plant contained $2n = 79$ chromosomes. Chromosome number in BC_2F_1 plants ranged from $2n = 55$ to 60 . All BC_2 plants were self-sterile and so were backcrossed to Dwight. Chromosome number in BC_3 plants ranged from $2n = 41$ to 49 . Plants with higher than $2n = 42$ chromosomes were self-sterile and so were backcrossed to Dwight. At the end, we expect to isolate plants $2n = 41$ chromosomes and hope to categorize them into the 39 possible monosomic alien addition lines (MAALs). Progenies of $2n = 41$ chromosome plants segregated about 70% $2n = 40$ chromosome plants, 29% $2n = 41$ chromosome plants, and 1% $2n = 42$ (disomic alien addition lines; DAALs) plants. Transfer of plants from test tube to the field required about 4 years. The modified diploid ($2n = 40$) lines are being

screened for resistance to pests and pathogens. This study has broken the crossability barrier and sets the stage for the exploitation of perennial, wild *Glycine* germplasm so-called a weed from Australia to broaden the genetic base of the cultivated soybean. Materials are distributed to public soybean breeders through material transfer agreement (MTA) (Table 2.6).

Wide hybridization in soybean calls for perseverance, commitment, dedication, patience, and skill. The initial process requires tissue culture expertise, and the post- BC_1 period needs knowledge of cytogenetics and precise record-keeping ability. The handling of soybean chromosomes is required because misidentification of plants from $2n = 40$ to 41 (Sadanaga and Grindeland 1981) may occur if the chromosome spreads are not excellent.

Table 2.6 Progress of wide hybridization in the genus *Glycine*

Number of attempts	Hybrid combinations	References
1	TOM ($2n = 38$) $\times$ CAN ($2n = 40$); F_1 ; $2n = 39 = CT$ ($2n = 78$) $\times$ MAX ($2n = 40$); F_1 sterile	Broué et al. (1982)
2	MAX ($2n = 40$) $\times$ TOM ($2n = 78$); F_1 ; $2n = 59$; sterile	Newell and Hymowitz (1982)
3	MAX ($2n = 40$) $\times$ TOM ($2n = 80$); F_1 ; $2n = 60$; sterile	Newell and Hymowitz (1982)
4	TOM ($2n = 78$) $\times$ MAX ($2n = 40$); F_1 ; $2n = 59$; sterile	Singh and Hymowitz (1985d)
5	MAX ($2n = 40$) $\times$ TOM ($2n = 80$); F_1 embryo ($2n = 64$); no F_1 plant	Sakai and Kaizuma (1985)
6	ARG ($2n = 40$) $\times$ CAN ($2n = 40$); F_1 ; $2n = 40 \times MAX$ ($2n = 40$) = CT ($2n = 80$); sterile	Grant et al. (1986)
7	MAX ($2n = 40$) $\times$ CLA ($2n = 40$); F_1 ; $2n = 40$; sterile	Singh et al. (1987a)
8	MAX ($2n = 40$) $\times$ TOM ($2n = 78$); F_1 ; $2n = 59$; sterile = CT ($2n = 118$)	Newell et al. (1987)
9	TOM ($2n = 78$) $\times$ MAX ($2n = 40$); F_1 ; $2n = 59$; sterile = CT ($2n = 118$)	Newell et al. (1987)
10	CAN ($2n = 40$) $\times$ MAX ($2n = 40$); F_1 ; $2n = 40$; sterile = CT ($2n = 80$)	Newell et al. (1987)
11	MAX ($2n = 40$) $\times$ TOM ($2n = 80$); F_1 ; $2n =$ Not determined	Chung and Kim (1990)
12	MAX ($2n = 40$) $\times$ LAT ($2n = 40$); F_1 ; $2n =$ Not determined	Chung and Kim (1991)
13	MAX ($2n = 40$) $\times$ TOM ($2n = 78$); F_1 ; $2n = 59$; sterile = CT ($2n = 118$)	Bodanese-Zanettini et al. (1996)
14	MAX ($2n = 40$) $\times$ TOM ($2n = 78$); F_1 ; $2n = 59$; CT = ($2n = 118$) $\times$ MAX (BC ₁ -BC ₆); MAALs	Singh et al. (1990, 1993, 1998b)
15	TOM ($2n = 78$) $\times$ Max ($2n = 40$); F_1 ; $2n = 59$; CT = ($2n = 118$) $\times$ MAX (BC ₁ -BC ₄); MAALs	Singh and Nelson (2014)
16	MAX ($2n = 40$) $\times$ TOM ($2n = 78$); F_1 ; $2n = 59$; CT = ($2n = 118$) $\times$ MAX (BC ₁ -BC ₆); MAALs	Singh and Nelson (2015), Singh et al. 2007a)

TOM *G. tomentella*, CAN *G. canescens*, MAX *G. max*, ARG *G. argyrea*, LAT *G. latifolia*, CT Colchicine treatment

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