Theor Appl Genet (2001) 103:212–218

R.J. Singh · H.H. Kim · T. Hymowitz

Distribution of rDNA loci in the genus *Glycine* Willd.

Received: 10 October 2000 / Accepted: 6 December 2000

Abstract The objective of this study was to examine the distribution of rDNA loci in the genus *Glycine* Willd. by fluorescent in situ hybridization (FISH) using the internal transcribed spacer (ITS) region of nuclear ribosomal DNA as a probe. The hybridized rDNA probe produced two distinct yellow signals on reddish chromosomes representing two NORs in 16 diploid (2n=40) species. Aneudiploid (2n=38) and aneutetraploid (2n=78) *Glycine tomentella* Hayata also exhibited two rDNA sites. However, the probe hybridized with four chromosomes as evidenced by four signals in two diploid species (*Glycine curvata* Tind. and *Glycine cyrtoloba* Tind.) and tetraploid (2n=80) *G. tabacina* (Labill.) Benth. and *G. tomentella*. Synthesized amphiploids (2n=80) of *Glycine canescens* F. J. Herm. (2n=40) and the 40-chromosome *G. tomentella* also showed four signals. This study demonstrates that the distribution of the rDNA gene in the 16 *Glycine* species studied is highly conserved and that silence of the rDNA locus may be attributed to amphiplasty during diploidization and speciation.

Keywords Soybean · *Glycine* · rDNA · Fluorescent in situ hybridization · Polyploidy

Introduction

Soybean [*Glycine max* (L.) Merr.] is an economically important legume but is not considered as a model crop for cytogenetic studies (Singh and Hymowitz 1999). It contains a high (2n=40) chromosome number (Karpechenko 1925; Veatch 1934). All chromosomes are symmetrical at mitotic metaphase, with chromosome size ranging from 1.42 µ*M* to 2.8 µ*M* (Sen and Vidyabhusan 1960) and a lack morphological distinguishing landmarks. Occasionally, a pair of satellite (SAT) chromosomes (nucleolus organizers, usually known as NORs) are visible by Feulgen staining (Palmer and Heer 1973). Giemsa C-banding failed to characterize individual soybean chromosomes because a single centromeric band was recorded in metaphase chromosomes (Ladizinsky et al.1979).

Mitotic metaphase chromosomes of *Glycine soja* Sieb. and Zucc., a wild annual progenitor of the soybean, and 16 wild perennial species of the subgenus *Glycine* Willd., are karyotypically indistinguishable (Singh, unpublished data). However, pachytene chromosome analysis of an interspecific F_1 hybrid between soybean and *G. soja* created the first soybean chromosome map (Singh and Hymowitz 1988). This pioneering contribution laid the foundation for the production of primary trisomics (Xu et al. 2000b) and led to the association of the classical linkage map and several molecular maps with specific chromosomes in order to develop a universal map for the soybean.

Molecular cytogenetics in the soybean has lagged behind maize, wheat, barley, rice and tomato. Fluorescent in situ hybridization (FISH) was sporadically applied to locate rDNA sites in interphase cells (Skorupska et al. 1989) and metaphase chromosomes (Griffor et al. 1991). Genomic relationships among 16 wild perennial species have been determined based on cytogenetic (interspecific crossability rate, meiotic chromosome pairing, hybrid viability) and molecular methods (Hymowitz et al. 1998). Wild perennial species grow in diverse geographical areas and under a wide range of climatic conditions (Table 1), and harbor traits for resistance to pests and pathogens (Singh and Hymowitz 1999). The utilization of cytological molecular markers for re-confirming the biosystematic results in the genus *Glycine* is hampered due to the small and symmetrical mitotic metaphase chromosomes.

Communicated by B.S. Gill

R.J. Singh (✉) · T. Hymowitz
Department of Crop Sciences, University of Illinois, Urbana, IL 61801, USA
e-mail: r-singh@uiuc.edu

H.H. Kim
Department of Biology, Korean Sahmyook University, Seoul, 139–742, South Korea

Table 1 Number of rDNA Loci in the genus *Glycine*. *, Illinois number; + plant introduction number. Abbreviations: LAT, Latitude; LONG, Longitude; WA, Western Australia; QLD, Queensland; SA, South Australia; V, Victoria; PNG, Papua New Guinea; IL, Illinois

Species	Code	2n	Genome	Accessions	rDNA loci	LAT	LONG	Collection site
Subgenus *Glycine*								
G. albicans	ALB	40	I	889*	1	–14.82	125.83	0.5 km E Amex Mining Camp, WA
G. arenaria	ARE	40	H	505204+	1	–15.40	128.97	50 km W Kununurra, WA
G. argyrea	ARG	40	A_2	505151	1	–25.90	153.08	3 km from Rainbow Beach, QLD
G. canescens	CAN	40	A	440932	1	–26.42	139.55	65 km S Birdsville, SA
G. clandestina	CLA	40	A_1	440958	1	–37.07	148.25	Iandra Rd, Young, NSW
G. curvata	CUR	40	C_1	505166	2	–20.38	145.62	30.5 km E Pentland, Homestead, QLD
G. cyrtoloba	CYR	40	C	440963	2	–20.82	149.28	Brampton Island, Track to lookout, QLD
G. falcata	FAL	40	F	505179	1	–24.88	146.25	Tambo, QLD
G. latifolia	LAT	40	B_1	378709	1	–29.77	151.12	Inverell, NSW
G. latrobeana	LTR	40	A_3	483196	1	–38.05	144.03	5.5 km NW Inverleigh, V
G. microphyllla	MIC	40	B	440956	1	–17.22	145.55	Kairi Res. Stn. QLD
G. pindanica	PIN	40	H_2	1251	1	–17.52	122.27	50 km N Broome, WA
G. tabacina	TAB	40	B_2	373990	1	–30.92	152.58	Kempsey, NSW
G. tomentella	TOM	38	E	440998	1	–26.92	150.12	20 km E Condamine, QLD
G. tomentella	TOM	40	D_3	505222	1	–12.97	143.07	Lockhart River Airport,Near terminal, QLD
G. tomentella	TOM	40	D	441000	1	–18.03	144.85	70 km SE Mt. Garnet, QLD
G. tabacina	TAB	80	BB_1	373992	2	–29.65	150.83	Delungra, NSW
G. tabacina	TAB	80	AB	440996	2	–29.82	153.03	8 km SE Grafton, NSW
G. tomentella	TOM	78	D_3E	483218	1	–20.82	149.28	Brampton Island, QLD
G. tomentella	TOM	80	AD_3	441005	2	–20.45	149.02	Lindeman Island, QLD
G. tomentella	TOM	80	A?	446988	2	–9.50	147.07	3 km from Boroko, PNG
Subgenus *Soja*								
G. soja	SOJ	40	G	81,762	1	ca 42.5	ca 135	Russia via Japan
G. max	MAX	40	G	Clark 63	1	40.11	–88.20	Urbana, IL

The SAT chromosome includes an NOR, the site of the rDNA gene. The labeled rDNA is precisely located on the chromosome and is quantified using FISH. Variation in NORs (rDNA sites) in the Triticeae was attributed to genome dominance, inactivity and suppression (amphiplasty) (Lewis et al. 1996; Linde-Laursen et al. 1996; Taketa et al. 1999), and in *Oryza* to the environment (Fukui et al. 1994). Since wild perennial species inhabit extremely diverse regions (desert to tropical rain forest) of Australia, the main objective of this study was to examine the distribution of rDNA gene in the genus *Glycine* and to ascertain whether variation in the number of rDNA loci is caused by genome dominance or by growing conditions. This investigation encouraged us to locate molecular markers on specific soybean chromosomes with the aid of primary trisomics and tetrasomics.

Material and methods

Plant materials

The *Glycine* species used in this study, the three letter code, the 2n chromosome numbers, the genome, the plant introduction identification number, and the collection sites are listed in Table 1. Information on latitude (LAT) and longitude (LONG) helps locate precisely each *Glycine* species collection site used in this study. Wild perennial *Glycine* species were collected from the desert (ARE), tropical rain forest (MIC, TOM), tropical grass lands (ALB, ARG, CUR, CYR, FAL, PIN), temperate forest (CAN, LTR, TAB, LAT) and open Eucalypt forest (CLA). *Glycine hirticaulis* Tind. & Craven and *Glycine lactovirens* Tind. & Craven were not studied due to the lack of seed. One synthesized amphiploid (AA D_3D_3) from an interspecific F_1 hybrid of CAN (AA) and TOM (D_3D_3) was also included. Soybean cv Clark 63, was bred at Urbana, Illinois (temperate region), and *G. soja* (PI 81762) was introduced into the United States from Japan. The latter accession was initially collected from the North East region of Russia. These accessions were grown for several generations in the greenhouse of the University of Illinois, Urbana-Champaign, and used for establishing genomic relationships among *Glycine* species by cytogenetic and molecular methods (Singh and Hymowitz 1985; Singh et al. 1988, 1992; Kollipara et al. 1997).

Methods

Mitotic chromosomes of the accessions listed in Table 1 were stained according to the Feulgen procedure described by Singh (1993) and Singh et al. (1998), and by the FISH method of Ahmad et al. (1999) with some modifications described below:

Seeds of wild perennial *Glycine* species and *G. soja* were scarified and germinated at room temperature (RT) in Petri plates on moist filter paper. Seeds of soybean cv Clark 63 were germinated in sterilized vermiculite in the greenhouse and kept moist with distilled water. Actively growing roots were collected and pre-treated in 0.5% 8-hydroxyquinoline for 5 h at 16°C. Roots were fixed in a freshly prepared mixture of 3 (95% ethanol):1 (propionic acid) and stored in a refrigerator for at least 24 h. Roots were washed twice (5-min each) with 0.01 M CA-SC buffer (2 parts 0.01 M citric acid+3 parts 0.01 M sodium citrate; pH 4.5) and were allowed to sit in buffer for 30 min at RT. Roots were softened for 1 h at 37°C in an enzyme solution composed of 0.02 g cellulose “Onozuka” R-10, 0.01 g pectinase (Sigma # P-2401) in 1 ml of CA-SC buffer. Roots were washed twice (5-min each) with 1 ml of CA-SC buffer and kept in a refrigerator for 1 to 2 h. A root tip was cut and squashed on a clean slide under a clean cover slip with a drop of 45% acetic acid. Slides were scanned for prometaphase and metaphase chromosome spreads by a phase-contrast lens. Cover slips were removed after dipping slides in liquid nitrogen for a few seconds. Slides were treated with 45% acetic acid for 10 min at RT and air-dried overnight at RT.

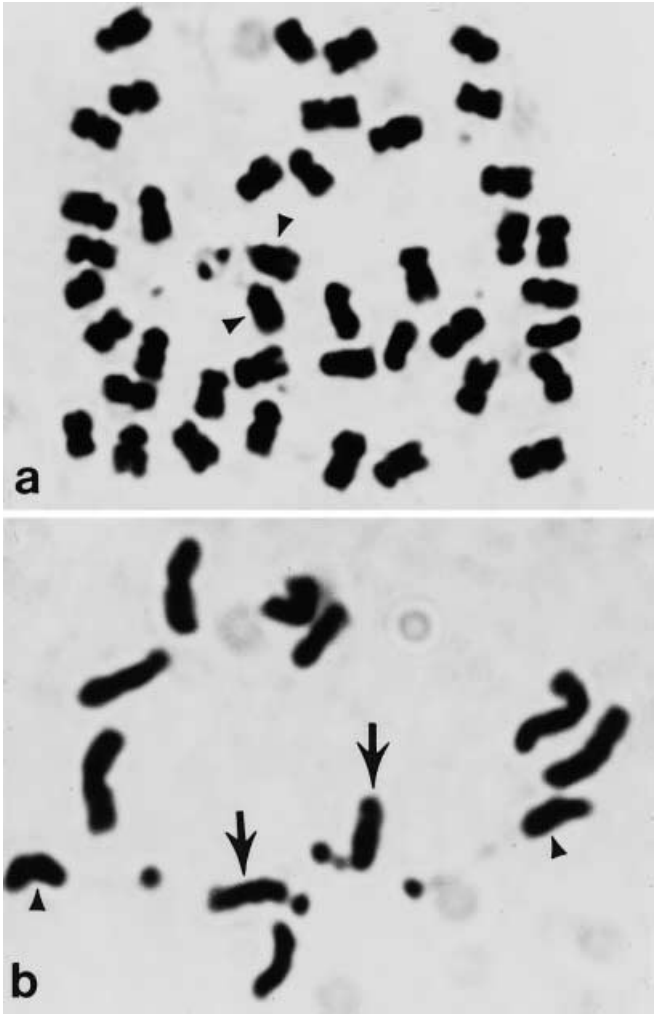

Fig. 1a, b Feulgen-stained mitotic metaphase chromosomes of *G. tomentella* (**a**), and *G. cyrtoloba* (**b**). **a** A metaphase plate of 40-chromosome *G. tomentella* (PI 505222) showing one pair of SAT (chromosome 13) chromosomes with NORs (*arrowheads*); **b** A partial metaphase plate of *G. cyrtoloba* (PI 440963) showing two pairs of SAT chromosomes with NORs. The visual karyomorphology of one pair is chromosome 13 (*arrowheads*) and the second pair (*arrows*) is an unidentified chromosome

A total of eight slides were processed in a single batch at a given time using Coplin jars. Each slide was treated with 100 µl of RNAse (30 µg/ml in 2×SSC; 1×SSC=0.15 M Nacl+0.015 M sodium citrate; pH 7.0). The RNAse solution on the specimen was overlaid with rectangular plastic cover slips (24 mm×24 mm) and the slides were incubated in a moist chamber at 37°C for 1 h. The slides were then subjected to denaturation with 70% formamide in 2×SSC for 2 min at 70°C, followed by dehydration at –20°C in a series of 70%, 80%, 95% (5-min each) and 100% (30 s) ethanol. The hybridization solution (400 µl) consists of 10 µl of a biotin-labeled ITS region (ITS1, 5.8 S, ITS2; approximately 700 nucleotides) probe DNA (BioPrime DNA Labeling System, Life Technologies, Gaithersburg, Md.; cat # 18094–011), 17 µl of unlabeled nonspecific ssDNA (salmon sperm DNA), 200 µl of 50% formamide, 40 µl of 20×SSC, 80 µl of 50% dextron sulfate and 50 µl of TE buffer. Each slide was treated with a 50-µl solution and was overlaid with a plastic cover slip and incubated at 80°C for 8 min followed by overnight incubation at 37°C in a moist chamber. The post-hybridization steps, such as washing, fluorescent labeling, amplification, and visualization of fluorescent signals, were the same as those described by Ahmad et al. (1999). FISH slides were observed with a Zeiss Axioskop microscope equipped with neutral density filters. Photographs (×100 oil immersion lens) were taken by using Fujichrome (color slide) Sensia II film, ASA 400.

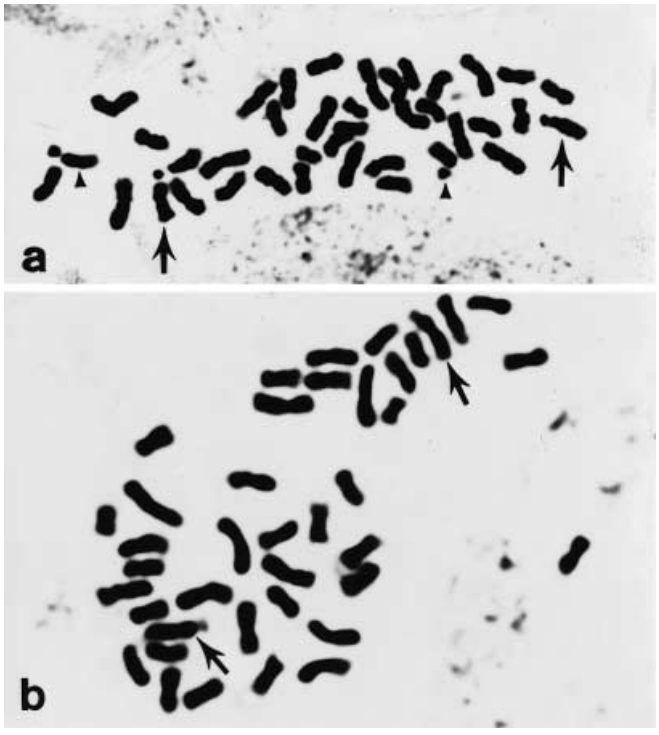

Fig. 2a, b Karyotypic comparison of complete metaphase cells of CYR and CUR after Feulgen staining. **a** A metaphase plate of CYR with 36+4 SAT chromosomes. Two of the SAT (*arrowheads*) chromosomes involve chromosome 13, not visible in CUR (**b**), while a pair of SAT (*arrows*) chromosomes is common to both CYR and CUR. The remaining chromosomes are alike

Results

Distribution of NORs in the genus *Glycine* by Feulgen staining

Karyomorphology at mitotic metaphase of 16 diploid (2n=40) species, listed in Table 1, of the genus *Glycine* did not express morphological distinguishing landmarks after Feulgen staining. Most of the chromosomes were metacentric and occasionally a pair of satellite (SAT) chromosomes was observed (Fig. 1a). The SAT chromosome has been designated as chromosome 13 by Singh and Hymowitz (1988) following pachytene chromosome analysis of an interspecific F_1 hybrid of *G. max* (MAX) and *G. soja* (SOJ). The nucleolous organizer region (NOR) is often not visible in over-contracted mitotic metaphase chromosomes. The kinetochore in all prometaphase chromosomes was usually flanked by heterochromatin.

Glycine cyrtoloba (CYR) clearly showed four SAT chromosomes (Figs. 1b, 2a); one pair was chromosome

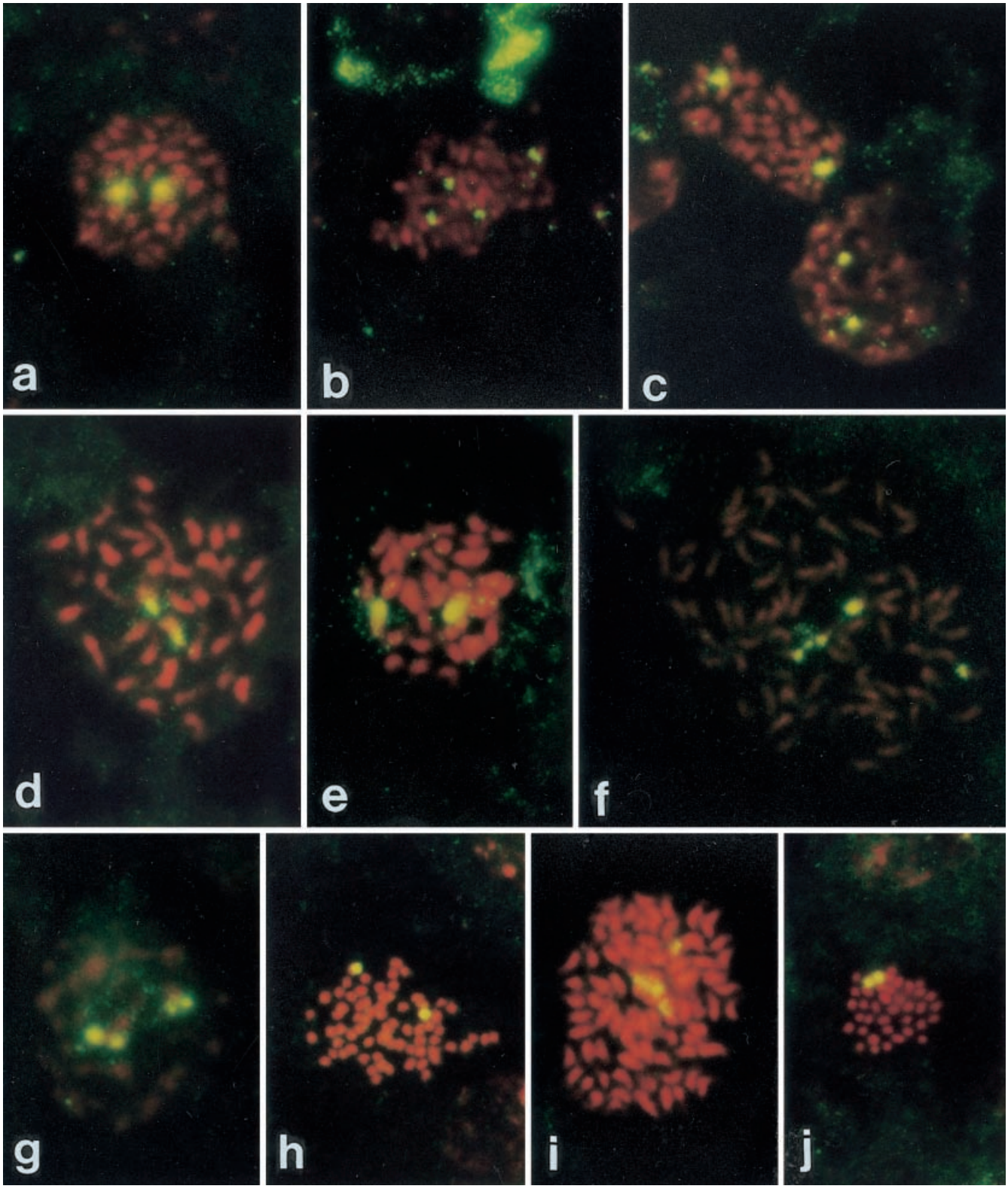

Fig. 3a–j FISH of mitotic metaphase and interphase from seven species of the genus *Glycine*. **a** CAN; **b** CUR; **c** MIC; **d** 40-chromosome TOM; **e** 38-chromosome TOM; **f** 80-chromosome TAB; **g** 80-chromosome TOM; **h** 78-chromosome TOM; **i** Synthesized amphiploid (2n=80) of CAN and TOM (2n=40); **j** MAX cv Clark 63

13 (arrow heads) and the other pair (arrows) was karyotypically unidentified. However, the NORs of chromosome 13 were absent at metaphase in *Glycine curvata* (CUR) but the other SAT pair that was present in CYR (Fig. 2a, arrows) was visible in CUR (Fig. 2b; arrows). We confirmed these results by examining cytologically

three additional accessions of CUR (IL 793, IL836, IL1306) and CYR (IL1312, IL1313, IL1318) after Feulgen staining.

Feulgen stain failed to exhibit the expected four SAT chromosomes at metaphase of the 80-chromosome *Glycine tabacina* (TAB) and the 78- and 80-chromosome *Glycine tomentella* (TOM). Mitotic-chromosome cells selected for precise chromosome counts were highly contracted and NORs were not detectable.

Distribution of rDNA in the genus *Glycine* by FISH

Molecular cytogenetic methods, and particularly FISH, determine precisely the number of NORs (rDNA loci) in an organism. Table 1 shows the distribution of rDNA loci in the genus *Glycine* as determined by FISH. Two bright yellow signals on reddish chromosomes were observed in 14 diploid wild perennial *Glycine* species (Fig. 3a, c, d, e, j) and the 78-chromosome TOM (Fig. 3h). The rDNA probe hybridized with four chromosomes of CUR (Fig. 3b) and CYR. The expression of four signals in CUR was unexpected because two NORs were recorded from Feulgen staining. However, both species are alike taxonomically and genomically (Table 1). The intensity and sharpness of hybridization signals varied from slide to slide, sample to sample, and cell to cell. Figure 3c contains two cells of MIC. The upper metaphase cell shows two stronger hybridization sites than those recorded in the bottom prometaphase cell.

The rDNA probe hybridized with four chromosomes of the 80-chromosome TAB [with (PI 373992) and without (PI 440996) adventitious roots (Fig. 3f)] and TOM (Fig. 3i). Adventitious roots are a morphological distinguishing trait for B-genome species (Costanza and Hymowitz 1987) and nature of their inheritance is recessive (Singh et al. 1992). One chromosome expressed the strongest site of hybridization than the other three chromosomes (Fig. 3f, g). A cautionary note is for Fig. 3f which showed one weakest signal observed occasionally. This is not a rDNA site but is the stretched secondary constriction region that fluoresced. Signals were terminal as observed clearly in a prometaphase cell (Fig. 3d). The entire chromosome exhibited a strong hybridization signal in condensed small metaphase chromosomes (Fig. 3a).

It was expected to record two pairs of hybridization sites in 78-chromosome TOM because it is an allotetraploid (Singh et al. 1989). However, Fig. 3 h showed two strong signals. This suggests that only a single pair of rDNA loci is active in the 78-chromosome TOM. This molecular trait could be useful in identifying the 78-chromosome from the 80- chromosome TOM without counting metaphase spreads. Morphologically, both cytotypes are alike and often it takes a tremendous effort to distinguish them cytologically.

Soybean cv Clark 63 showed a pair of rDNA sites after FISH (Fig. 3j). Both signals were equally strong in interphase and metaphase cells. Similar hybridization sites were recorded in its wild annual progenitor *G. soja*.

Discussion

Fluorescent in situ hybridization is an excellent, reliable, and effective molecular cytogenetic tool for biosystematic studies in *Lens* and *Cicer* (Abbo et al. 1994), *Phaseolus* and *Vigna* (Zheng et al. 1994), *Oryza* (Fukui et al. 1994) and *Hordeum* (Taketa et al. 1999); as well as far physical mapping of molecular markers on chromosomes and genome analysis (Jiang and Gill 1994; Ørgaard and Heslop-Harrison 1994). This report for the first time examines the distribution of NORs after Feulgen staining and of rDNA loci by FISH in the genus *Glycine*. A pair of rDNA loci was observed in 14 diploid (2n=40) wild perennial species of the subgenus *Glycine* and in soybean and its wild annual progenitor *G. soja*. This is congruent with the results obtained after Feulgen staining which also uncovered two SAT chromosomes in these species. Based on pachytene chromosome analysis of a MAX and SOJ interspecific F_1 hybrid, Singh and Hymowitz (1988) assigned number 13 to the SAT chromosome. The NOR in chromosome 13 is in the short arm and is frequently embedded in the nucleolus. Pachytene chromosome lengths of both species are similar. Yanagisawa et al. (1991) identified marker chromosomes in the genus *Glycine* by using a chromosome image analyzing system (CHIAS) after Giemsa N-banding. They examined MAX, SOJ, CAN, CLA, FAL, LAT, TAB and TOM, and found a similar karyotype. By contrast, Ahmad et al. (1984) based on mitotic metaphase chromosome measurement, reported, that *G. soja* chromosomes were about 6–7% smaller than that those *G. max*.

Darlington and Wylie (1955) proposed a x=10 basic chromosome number for the cultivated soybean. Based on this proposal we may hypothesize a putative ancestor with 2n=20 chromosomes for the genus *Glycine* and carrying at least a pair of NORs. However, such a progenitor is either unknown or extinct, or else may be thriving in South East Asia (Cambodia, Laos, Vietnam) and has not been collected and identified so far. Teraploidization (2n=4x=40) through auto (spontaneous chromosome doubling)- or allo (interspecific hybridization followed by chromosome doubling)- polyploidy of the progenitor species either occurred prior to dissemination or after, and cannot be substantiated experimentally because we do not know where the progenitor of the genus *Glycine* originated. The progenitor of the wild perennial species of the subgenus *Glycine* radiated out into several morphotypes depending on the growing conditions in the Australian subcontinent. These species have never been domesticated and remain as wild perennials. By contrast, the pathway of migration from a common progenitor to China is assumed as; wild perennial (2n=4x=40; unknown or extinct) → wild annual (2n=4x=40; *G. soja*) → soybean (2n=4x=40; *G. max*; cultigen). All known species of the genus *Glycine* exhibit diploid – like meiosis and are cleistogamous and inbreeders (Singh and Hymowitz 1985).

Allopolyploidization probably played a major role in the speciation of the genus *Glycine*. This assumption

implies that the 40-chromosome *Glycine* species and the 80-chromosome TAB and TOM are tetraploid and octoploid, respectively. The expression of four rDNA loci in CUR and CYR strongly supports the hypothesis of the allotetraploid origin that was suggested by cytogenetic (Hadley and Hymowitz 1973; Singh and Hymowitz 1985; Xu et al. 2000a) and molecular studies (Lee and Verma 1984; Shoemaker et al. 1996).

Of the 16 wild perennial species of the subgenus *Glycine*, CUR and CYR are the only two species that possess curved pods, while other species harbor straight pods (Tindale 1984, 1986). The species are extremely difficult to hybridize with their relatives as pod abortion 21 days post-pollination is common, and interspecific F_1 plants exhibit seedling and vegetative lethality and total seed sterility (Singh et al. 1988). We can not substantiate experimentally that four dosages of rDNA hinder hybridization with the allied species having two rDNA loci.

Glycine max, *G. soja* and 12 wild perennial *Glycine* species (2n=40) showed one pair of rDNA loci, whereas the 80-chromosome TAB and TOM contain four rDNA loci (Table 1). A pair of rDNA loci was reported in soybean cultivars by Skorupska et al. (1989) and Griffor et al. (1991). It is interesting to note that the 78-chromosome TOM has a single pair of rDNA loci (Fig. 3h). This suggests that the activity of rDNA loci is probably silenced, lost, inactived, or suppressed (amphiplasty) during diploidization. Such a phenomenon is prevalent in the Triticeae (Gerlach et al. 1980; Miller et al. 1983; Linares et al. 1992; Cabrera et al. 1995; Lewis et al. 1996; Linc et al. 1999), as well as in *Arabidopsis* (Maluszynska and Heslop-Harrison 1993), *Allium* (Ricroch et al. 1992) and *Capsicum* (Moscone et al. 1996).

Although diploid wild perennial species are distinct taxonomically and genomically and grow in diverse agroclimatic geographical regions (desert to tropical rain forest) of Australia (Table 1), only a single rDNA locus predominates. This suggests that the rDNA gene is highly conserved in the genus *Glycine*. On the other hand, a variable number of rDNA loci is common in the genus *Oryza* (Fukui et al. 1994). *Oryza sativa* ssp. *Indica* contains two rDNA loci while in ssp. *Japonica* the number of rDNA loci range from one (mostly) to two. Furthermore, variability was much greater (range 1–3) in the wild perennial diploid species. These authors suggested that the temperate-region cultivars had one rDNA locus while those in tropical and subtropical regions contained two rDNA loci. This pattern was not recorded in the *Glycine* species. This report therefore recommends the physical mapping of molecular markers on the soybean chromosomes by utilizing primary trisomics (Xu et al. 2000b) and tetrasomics using an improved FISH procedure.

Acknowledgments Research was supported in part by the Illinois Agricultural Experiment Station, and a grant from the Illinois Soybean Program Operating Board No. 96–22–168–3HY. We are grateful to Drs. Krishna Kollipara and S. J. Xu for technical advice. We thank the Korean Sahmyook University for granting H. H. K. sabbatical leave to conduct research at the University of Illinois.

References

Abbo S, Miller TE, Reader SM, Dunford RP, King IP (1994) Detection of ribosomal DNA sites in lentil and chickpea by fluorescent in situ hybridization. Genome 37:713–716

Ahmad F, Acharya SN, Mir Z, Mir PS (1999) Localization and activity of rRNA genes on fenugreek (*Trigonella foenum-graecum* L.) chromosomes by fluorescent in situ hybridization and silver staining. Theor Appl Genet 98:179–185

Ahmad QN, Britten EJ, Byth DE (1984) The karyotype of *Glycine soja* and its relationship to that of the soybean, *Glycine max*. Cytologia 49:645–658

Cabrera A, Friebe B, Jiang J, Gill BS (1995) Characterization of *Hordeum chilense* chromosomes by C-banding and in situ hybridization using highly repeated DNA probes. Genome 38:435–442

Costanza SH, Hymowitz T (1987) Adventitious roots in *Glycine* subg. *Glycine* (*Leguminosae*): morphological and taxonomic indicators of the B genome. Pl Syst Evol 158:37–46

Darlington CD, Wylie AP (1955) Chromosome atlas of flowering plants. George Allen and Unwin, Ruskin House, Museum Street, London

Fukui K, Ohmido N, Khush GS (1994) Variability in rDNA loci in the genus *Oryza* detected through fluorescence in situ hybridization. Theor Appl Genet 87:893–899

Gerlach WL, Miller TE, Flavell RB (1980) The nucleolus organizers of diploid wheat revealed by in situ hybridization. Theor Appl Genet 58:97–100

Griffor MC, Vodkin LO, Singh RJ, Hymowitz T (1991) Fluorescent in situ hybridization to soybean metaphase chromosomes. Plant Molec Biol 17:101–109

Hadley HH, Hymowitz T (1973) Speciation and cytogenetics. In: Caldwell BE (ed) Soybeans: improvement, production, and uses. Agron Monogr 16 ASA, Madison Wisconsin, pp 97–116

Hymowitz T, Singh RJ, Kollipara KP (1998) The genomes of the *Glycine*. Plant Breed Rev 16:289–317

Jiang J, Gill BS (1994) Nonisotopic in situ hybridization and plant genome mapping: the first 10 years. Genome 37:717–725

Karpechenko GD (1925) On the chromosomes of Phaseolinae (in Russian with an English summary). Bull Appl Bot Genet Plant Breed 14:143–148

Kollipara KP, Singh RJ, Hymowitz, T (1997) Phylogenetic and genomic relationships in the genus *Glycine* Willd. based on sequences from the ITS region of nuclear rDNA. Genome 40: 57–68

Ladizinsky G, Newell CA, Hymowitz T (1979) Giemsa staining of soybean chromosomes. J Hered 70:415–416

Lee JS, Verma DPS (1984) Structure and chromosomal arrangement of leghemoglobin genes in kidney bean suggest divergence in soybean leghemoglobin gene loci following tetraploidization. EMBO J 3:2745–2752

Lewis SM, Martínez AJ, Dubcovsky J (1996) Karyotype variation in South American *Elymus* (Triticeae). Int J Plant Sci 157: 142–150

Linares C, Vega C, Ferrer E, Fominaya A (1992) Identification of C-banded chromosomes in meiosis and the analysis of nucleolar activity in *Avena byzantina* C. Koch cv 'Kanota'. Theor Appl Genet 83:650–654

Linc G, Friebe BR, Kynast RG, Molnar-Lang M, Köszegi B, Sutka J, Gill BS (1999) Molecular cytogenetic analysis of *Aegilops cylindrica* Host. Genome 42:497–503

Linde-Laursen IB, Seberg O, Frederiksen S, Baden C (1996) The karyotype of *Festucopsis serpentini* (*Poaceae Triticeae*) from Albania studied by banding techniques and in situ hybridization. Pl Syst Evol 201:75–82

Maluszynska J, Heslop-Harrison JS (1993) Molecular cytogenetics of the genus *Arabidopsis* : in situ localization of rDNA sites, chromosome numbers and diversity in centromeric heterochromatin. Ann Bot 71:479–484

Miller TE, Hutchinson J, Reader SM (1983) The identification of the nucleolus organizer chromosomes of diploid wheat. Theor Appl Genet 65:145–147

Moscone EA, Lambrou M, Ehrendorfer F (1996) Fluorescent chromosome banding in the cultivated species of *Capsicum* (*Solanaceae*). Pl Syst Evol 202:37–63

Ørgaard M, Heslop-Harrison JS (1994) Investigations of genome relationships between *Leymus*, *Psathyrostachys* and *Hordeum* inferred by genomic DNA: DNA in situ hybridization. Ann Bot 73:195–203

Palmer RG, Heer H (1973) A root tip squash technique for soybean chromosomes. Crop Sci 13:389–391

Ricroch A, Peffley EB, Baker RJ (1992) Chromosomal location of rDNA in *Allium*: in situ hybridization using biotin- and fluorecein-labelled probe. Theor Appl Genet 83:413–418

Sen NK, Vidyabhusan RV (1960) Tetraploid soybeans. Euphytica 9:317–322

Shoemaker RC, Polzin K, Labate J, Specht J, Brummer EC, Olson T, Young N, Concibido V, Wilcox J, Tamulonis JP, Kochert G, Boerma HR (1996) Genome duplication in soybean (*Glycine* subgenus *soja*). Genetics 144:329–338

Singh RJ (1993) Plant cytogenetics. CRC Press, Boca Raton, Florida

Singh RJ, Hymowitz T (1985) The genomic relationships among six wild perennial species of the genus *Glycine* subgenus *Glycine* Willd. Theor Appl Genet 71:221–230

Singh RJ, Hymowitz T (1988) The genomic relationship between *Glycine max* (L.) Merr. and *G. soja* Sieb. and Zucc. as revealed by pachytene chromosome analysis. Theor Appl Genet 76:705–711

Singh RJ, Hymowitz T (1999) Soybean genetic resources and crop improvement. Genome 42: 605–616

Singh RJ, Kollipara KP, Hymowitz T (1988) Further data on the genomic relationships among wild perennial species (2n=40) of the genus *Glycine* Willd. Genome 30:166–176

Singh RJ, Kollipara KP, Hymowitz T (1989) Ancestors of 80 – and 78 – chromosome *Glycine tomentella* Hayata (Leguminosae). Genome 32:796–801

Singh RJ, Kollipara KP, Hymowitz T (1992) Genomic relationships among diploid wild perennial species of the genus *Glycine* Willd. subgenus *Glycine* revealed by crossability, meiotic chromosomes pairing and seed protein electrophoresis. Theor Appl Genet 85:276–282

Skorupska H, Albertsen MC, Longholz KD, Palmer RG (1989) Detection of ribosomal RNA genes in soybean, *Glycine max* (L.) Merr., by in situ hybridization. Genome 32:1091–1095

Taketa S, Harrison GE, Heslop-Harrison JS (1999) Comparative physical mapping of the 5 S and 18S-25 S rDNA in nine wild *Hordeum* species and cytotypes. Theor Appl Genet 98: 1–9

Tindale MD (1984) Two new Eastern Australian species of *Glycine* Willd. (Fabaceae). Brunonia 7:207–213

Tindale MD (1986) A new North Queensland species of *Glycine* Willd. (Fabaceae). Brunonia 9:99–103

Veatch C (1934) Chromosomes of the soybean. Bot Gaz 96:189

Xu SJ, Singh RJ, Hymowitz T (2000a) Monosomics in soybean: origin, identification, cytology, and breeding behavior. Crop Sci 40:985–989

Xu SJ, Singh RJ, Kollipara KP, Hymowitz T (2000b) Primary trisomics in soybean: origin, identification, breeding behavior, and use in gene mapping. Crop Sci 40:1543–1551

Yanagisawa T, Tano S, Fukui K, Harada K (1991) Marker chromosomes commonly observed in the genus *Glycine*. Theor Appl Genet 81:606–612

Zheng J, Irifune K, Hirai K, Nakata M, Tanaka R, Morikawa H (1994) In situ hybridization to metaphase chromosomes in six species of *Phaseolus* and *Vigna* using ribosomal DNA as the probe. J Plant Res 107:365–369