Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 9486–9490, December 1990
Genetics

Sexual hybridization of *Lycopersicon esculentum* and *Solanum rickii* by means of a sesquidiploid bridging hybrid

(tomato/evolution/phylogeny/germ plasm/wide hybridization)

JOSEPH W. DEVERNA*, CHARLES M. RICK†, ROGER T. CHETELAT*†, BRENDA J. LANINI*, AND KEVIN B. ALPERT*

*Campbell Institute of Research and Technology, Route 1, Box 1314, Davis, CA 95616

Contributed by Charles M. Rick, August 20, 1990

ABSTRACT **A sesquidiploid hybrid having two genomes of *Lycopersicon esculentum* and one of *Solanum lycopersicoides* served as a pistillate bridging parent in crosses with *Solanum rickii* to produce *L. esculentum* × *S. rickii* hybrid progeny. Of the four progeny obtained, one (GH2754) was diploid and three were aneuploid with extra *S. lycopersicoides* chromosomes. The hybrids had morphological features of both parents, but attributes of the wild parent dominated. The hybrid nature of the four progeny was confirmed by isozyme, restriction fragment length polymorphism, and cytological analyses. A mean of 9.15 bivalents was observed in pollen mother cells of GH2754. A high level of pollen abortion was seen in all hybrids. Crosses of the hybrids with staminate *S. rickii* yielded one backcross individual, revealing a very low, but certain level of female fertility. Colchicine treatment of GH2754 generated one promising amphidiploid hybrid, which exhibited strong preferential chromosome pairing (94% of the examined cells had 24 bivalents) and appreciable pollen fertility (43% stainable). Chromosome pairing, isozyme, and restriction fragment length polymorphism data support a very close relationship between the two *Solanum* spp. and a much greater distance between them and *L. esculentum*, but the data do not discriminate between them in respect to their distances from the latter. The cytological and molecular observations, previous reports of successful transfer of traits from *S. lycopersicoides* to *L. esculentum*, and our hybridization of *L. esculentum* × *S. rickii* suggest good prospects for gene transfer from *S. rickii* to *L. esculentum*.**

The immense and highly diversified *Solanum* (nightshade) genus is generally acknowledged to be most closely related and ancestral to *Lycopersicon* (tomatoes) (1, 2). Series *Juglandifolia*, subsection *Potatoe*, section *Petota*, of the genus *Solanum* is the group most closely affiliated and probably ancestral to *Lycopersicon*—conclusions based on morphological, genetic, and cytological evidence. Chromosomes of *Potatoe*, as evidenced by studies with *Solanum tuberosum*, are homosequential with those of *Lycopersicon* except for four major inversions, according to restriction fragment length polymorphism (RFLP) mapping (3). Within this subsection, *Juglandifolia* exhibits the greatest resemblance and contains the only species that have been sexually hybridized with *Lycopersicon*; the hybrids exhibit total homosequentiality of chromosomes (4, 5). The first species of this series to be thus hybridized is *Solanum lycopersicoides* Dun. (6). We have recently obtained hybrids between *Lycopersicon esculentum* Mill. (cultivated tomato) and *Solanum rickii* Corr., which is closely related to *S. lycopersicoides*. The purpose of this article is to report the fashion in which these hybrids were generated, their salient cytogenetic features, and their bearing on phylogeny of this group.

S. rickii (genomic constitution herein designated R, species designated RR) is an endangered species with an exceedingly limited geographic distribution in the northern Atacama Desert of Chile. It was first collected in 1957 and described and named by Correll (7). Its attribute of currently greatest economic interest is the capacity to survive the extreme aridity of its native habitat. It hybridizes very easily with *S. lycopersicoides* (genomic constitution designated S), and the resulting RS progeny grow vigorously and show no signs of sterility (C.M.R. and J.W.D., unpublished observation). *S. lycopersicoides* hybridizes with *Lycopersicon esculentum* (genomic constitution designated L), yielding LS hybrids, albeit with some difficulty. The apparent strong compatibility of SS and RR, weak compatibility of LL and SS, and the availability of sesquidiploid hybrids (designated LLS) (8) containing two sets of LL chromosomes and one set of SS chromosomes led us to attempt the LLS × RR bridging cross. The utilization of a sesquidiploid hybrid as a bridging medium has, to our knowledge, never been reported before.

On the basis of previous experience (8–11) with LS, LLSS, and LLS hybrids, our expectations from the progeny of the LLS × RR cross were diploid LR and LR with extra S chromosomes. The prospects for gene transfer from LR to LL would depend, as in previous experience with SS (9–12), on the ability to utilize LR hybrids directly, crossing LL with LLRR, or on the ability to derive usable progeny from LLS × LLRR crosses. Once an avenue for gene transfer from RR to LL has been devised, traits of horticultural importance are likely to be found in this scantily evaluated wildling.

MATERIALS AND METHODS

Stocks of *Lycopersicon pennellii* (LA716), *S. lycopersicoides* (LA1964), and *S. rickii* (LA1974) were provided by the Tomato Genetics Stock Center at University of California, Davis. The cultivar UC82B, or closely related stocks, was used as the *L. esculentum* parent. Previously reported hybrids were also used and are described as follows: GH266, an LLS hybrid (8); GH195, a 4*x* LLSS hybrid (13); backcross derivatives of UC82B × (UC82B × *L. pennellii*), selected for their ability to bypass stylar barriers on pistillate LLS and LS (11).

Greenhouse culture and plant hybridizations were done by standard procedures except that LLS hybrids, known to be male sterile, were not emasculated prior to hybridization. Embryos from crosses of LLS × RR were dissected, plated on HLH medium (14), and rooted on R1/2N (15). Polyploidization of LR hybrids by colchicine, chromosome counting, and pollen viability (scored as the percentage of 1000

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Abbreviations: RFLP, restriction fragment length polymorphism; PMC, pollen mother cell.

†Present address: Department of Vegetable Crops, University of California, Davis, Davis, CA 95616.

pollen grains stainable with acetocarmine) were carried out as described (8).

Isozyme analysis was performed as described (11). RR, LR, and SS individuals were scored for 17 isozyme loci: *Aco-1*, *Adh-1*, *Est-1*, *Got-2*, *Got-3*, *Got-4*, *Mdh-4*, *6Pgdh-1*, *6Pgdh-2*, *Pgi-1*, *Pgm-2*, *Prx-1*, *Prx-2*, *Prx-3*, *Prx-7*, *Skdh-1*, and *Tpi-2*. The chromosomal locations of these markers are known from the tomato isozyme gene linkage map (16).

Total plant DNA was isolated as described (17). Resuspended DNA was digested with the restriction enzymes *Hin*dIII, *Msp* I, *Pst* I, and *Xba* I (Bethesda Research Laboratories) according to the manufacturer's specifications. Electrophoresis of plant DNA, Southern blotting, and hybridization and random hexamer labeling of probes were as described (17), except that whole plasmids, including inserts, were labeled as probes and the hybridization solution contained 5% dextran sulfate.

RFLPs were identified between LL, RR, SS, LLSS, LLS, and the LR hybrids (GH2753, GH2754, GH2755, and GH2843) by using the following 30 mapped tomato genomic probes (18) kindly provided by S. D. Tanksley of Cornell University, Ithaca, NY: TG3, TG6, TG8, TG16, TG18, TG19, TG20, TG28, TG30, TG32, TG34, TG36, TG37, TG42, TG45, TG46, TG54, TG60, TG61, TG63, TG65, TG68, TG73, TG88, TG94, TG96, TG114, TG122, TG123, and TG194. Of the 120 possible probe by restriction enzyme combinations, 86 combinations resulted in patterns discernible by Southern analysis.

Each isozyme and RFLP marker was rated as to its ability to uniquely distinguish L, R, and S alleles ("allele score"). A score of 0 indicates that a polymorphism did not exist between the three parents. A score of 1 indicates those cases where alleles of L = R but R ≠ S; a score of 2, where alleles of L ≠ S but S = R; a score of 3, where L = S but S ≠ R; a score of 4, where all three parental alleles could be distinguished. It should be noted that in categories 1, 2, 3, and 4, although certain parental alleles were by themselves distinguishable, heterozygotes were not always identifiable in the hybrids due to additivity of bands in common with both parents. Also, allele variability within RR and SS was observed for some loci; in these instances, markers were classified according to the most frequent or likely allele.

RESULTS

Hybrids of SS × RR were derived from seed without *in vitro* manipulations. Chromosomes in the first division of pollen mother cells (PMCs) of RS paired almost entirely as bivalents (Table 1 and Fig. 1*A*). The bivalents were most often observed as rings, but occasional rods were seen (Fig. 1*A*). Chromosome heteromorphy between S and R was observed for the nucleolar organizing homologues (chromosome 2) (Fig. 1*A*). During division I pairing, only 1 of 43 cells possessed an unpaired chromosome set. In the second division, chromosome behavior was entirely normal as evidenced from observations of 120 PMCs at prophase II and 6 PMCs at metaphase II. Ninety-four percent of RS pollen was viable based on acetocarmine stainability. Overall, the high male fertility and regular behavior of chromosomes observed during meiosis in RS is that expected for crosses within or between two closely related species.

Pollinations (349 in total) of LLS × RR resulted in 234 fruit from which embryo rescue was attempted. Fruit for embryo culture were collected from 19 to 68 days after pollination. Of the 30 embryos obtained, only 4 resulted in mature plants. These plants were obtained only from embryos that were cultured at or prior to 28 days after pollination, suggesting that embryos are subject to dysgenesis at later stages. Only one of the resulting progeny was diploid (GH2754). Two hybrids (GH2755 and GH2843) were found to have extra chromosomes by means of cytological observations and RFLPs. GH2753 was not evaluated cytologically, but extreme aneuploidy was confirmed by RFLP analysis (see below).

As expected, the four hybrids obtained from the LLS × RR matings exhibited morphological traits intermediate between LL and RR. The hybrids were clearly dominated by features of RR: cream-colored dialytic anthers, pale-green glossy foliage, deeply serrated leaves, indeterminate growth habit, and compound inflorescence (Fig. 2). With respect to features of the inflorescence (Fig. 2*A*), notable exceptions were GH2753, which produced buds but never bloomed, and GH2843, whose inflorescence was a simple cyme (not shown). GH2754, of most interest because it should be devoid of those problematic features associated with aneuploidy, had dialytic anthers with a slightly exserted stigma (Fig. 2*C*), as did its tetraploid counterpart (Fig. 2*C*). The proportion of stainable pollen was low (1–2%) in all LR hybrids. The hybrids, except GH2753, grew vigorously and were easy to maintain; GH2754 was also propagated in the field, where it grew and flowered profusely. Fruit from the parents and tetraploid LLRR hybrid are shown in Fig. 2*D*.

Cytological analysis of L and R chromosome pairing behavior focused on GH2754. The extent of pairing was essentially the same in diakinesis and metaphase of the first division (Table 1): the mean numbers of bivalents per cell were 9.2 and 9.1, respectively; the remainder were almost entirely univalents. Occasional cells were observed with complete pairing (Fig. 1*B*) or regular separation of homo-

Table 1. Chromosome pairing features of *Lycopersicon* and *Solanum* hybrids

	Number of PMCs					
	LR (GH2754)		LLRR (GH2754X)		RS	
Number of bivalents	Diakinesis	Metaphase	Diakinesis	Metaphase	Diakinesis	Metaphase
4	1					
5						
6						
7	1	6				
8	7	28				
9	33	40				
10	19	25				
11	1	8			1	
12	1	3			29	13
21			2			
22			5	2		
23			25	11		
24			88	50		
Total PMCs	63	110	120	63	30	13
Average no. of bivalents	9.2	9.1	23.7	23.8	12.0	12.0

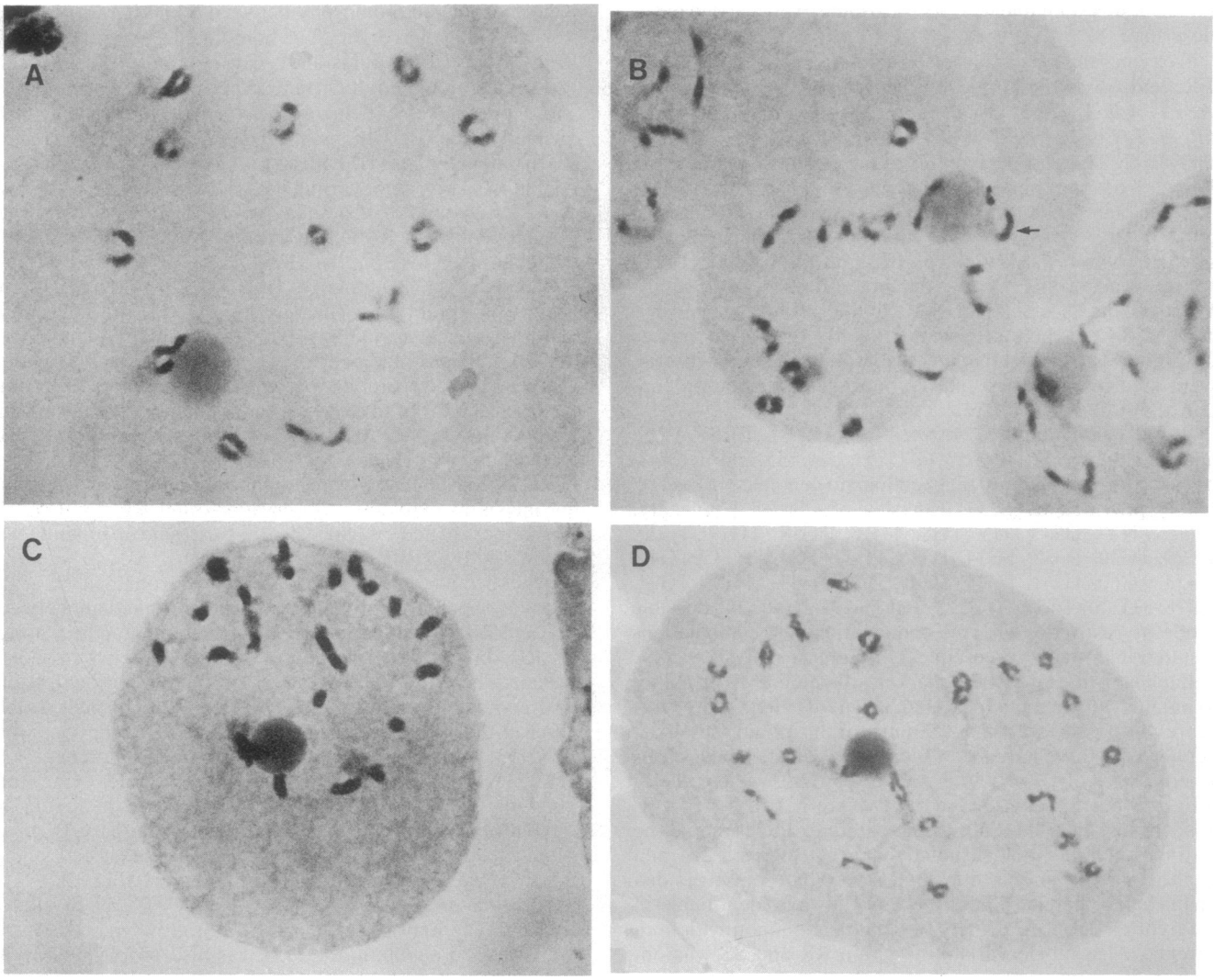

FIG. 1. Photomicrographs of PMCs of RS (*A*), LR (GH2754) (*B* and *C*), and LLRR (GH2754X) (*D*). (×≈1550.) (A) Association of 12 bivalents. (*B*) Rare diakinesis figure showing 12 bivalents and a heteromorphic nucleolar pair (arrow). (*C*) Typical diakinesis figure showing disrupted pairing (7 bivalents, 14 univalents). (*D*) Regular pairing behavior showing 24 bivalents and L and R pairs associated with the nucleolus.

logues, but, in general, pairing was incomplete (Fig. 1*C*). Occasional regular meiotic pairing behavior opens the possibility for a low frequency of viable gametes, especially with the megagametophyte. Nearly all of the bivalents were rods (Fig. 1 *B* and *C*) in contrast to prevailing ring bivalents in both parents, revealing greatly reduced chiasma formation. The nucleolar pairs (chromosome 2) were consistently heteromorphic (Fig. 1*B*), as were several other chromosome pairs. The high frequency of univalents was reflected in the presence of lagging chromosomes in 86% of the anaphase and telophase cells. In all of these features, the cytology of the LR hybrids is remarkably similar to that of LS (6).

To evaluate the female fertility of GH2754, pollinations of it with RR and with first generation backcross derivatives of *L. pennellii* were utilized. Numerous pollinations with the *L. pennellii* derivatives failed to produce progeny; however, pollinations with RR resulted in one individual that strongly resembled RR. The success of this cross suggests that introgression of RR traits through pistillate LR is possible, but the utilization of a bridging hybrid, capable of bypassing the stylar incompatibility of LR and readily hybridizable with LL, is required.

GH2754 was treated with colchicine, yielding six LLRR individuals. The usual instability was observed in such convertants, but after several months, some of the clones stabilized and one (GH2754X) was selected for more intensive study (Fig. 2*B*). Its chromosome number was consistently $2n = 48$, and no evidence of chromosomal chimerism was observed. Nearly all of its chromosomes associated in pairs (Fig. 1*D*); the mean number of pairs in diakinesis and metaphase was 23.7 and 23.8, respectively, and the great majority of cells showed 24 bivalents (Table 1). This consistent pairing behavior was regularly confirmed by occasional meiotic figures showing the regular behavior of L (smaller of two pairs) and R nucleolar organizing pairs (Fig. 1*D*). Lagging chromosomes were seen in only 7 of 117 (6%) figures examined. This orderly chromosome behavior was consistent with the observed 43% pollen stainability and reminiscent of that of LLSS hybrids (6, 19).

Seventeen isozyme and 30 RFLP markers covering the 12 tomato homologues were used to assay hybridity, chromosome dosage, and genetic recombination. Markers not showing L, R, or S allele polymorphisms (category 0) included four isozyme loci (*Prx-1*, *Prx-2*, *Prx-7*, and *6Pgdh-1*) and three RFLP loci (TG30, TG46, and TG65); therefore, of the 47 markers assayed, 40 had useful polymorphisms for distinguishing two or more of the parental alleles. Chromosomal markers with a score of 4 (loci where all three parental alleles could be distinguished) were most informative, and of the 23 markers in this category, only 3 markers could not distinguish

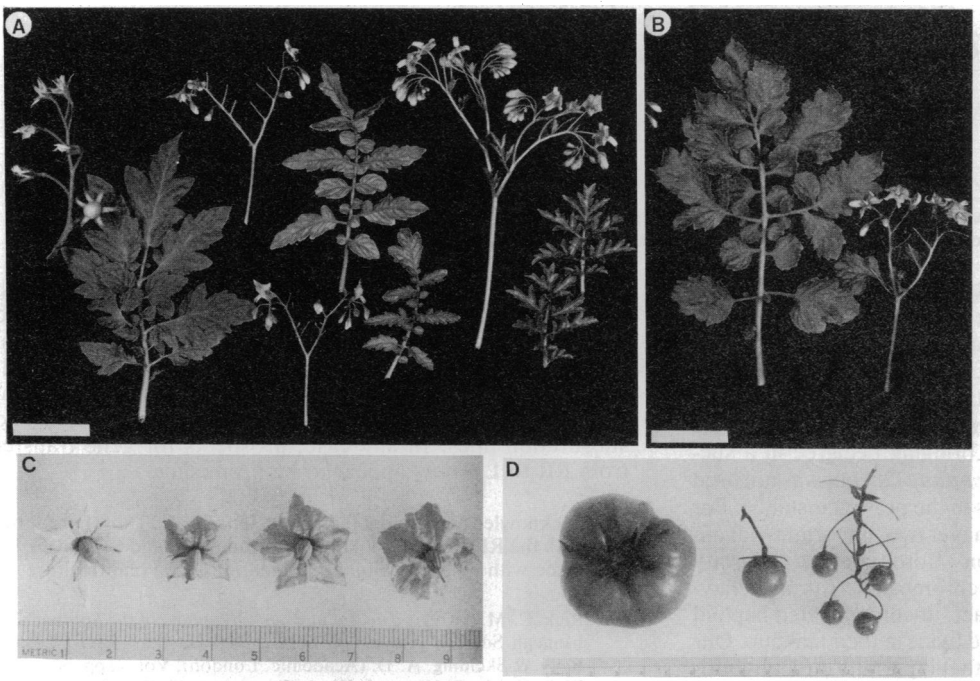

FIG. 2. Morphology of parents (LL and RR) and resulting LR and LLRR hybrids. (*A*) Inflorescence and leaves of LL, LR (GH2754), and RR (from left to right). (*B*) Leaf and inflorescence of LLRR hybrid. (*C*) Flowers of LL, LR, LLRR, and RR (from left to right). (*D*) Fruit from LL, LLRR, and RR (from left to right). (*A* and *B*, bar = 4 cm.)

all three parental alleles in the hybrids due to similarity in banding patterns (or band additivity).

Fig. 3 *A* and *B* illustrates LR hybridity based on banding pattern differences between L, R, and S alleles. Hybrids that were triallelic, thus trisomic, were GH2753 (chromosomes 3, 5, 7, 8, 10, and 11), GH2755 (chromosome 5), and GH2843 (chromosome 7) [for example, GH2753 and GH2843 (Fig. 3*A*) and GH2753 (Fig. 3*B*)]. Evidence for the occurrence of L and S chromosomal recombination (during LLS meiosis) was observed in GH2753 (chromosomes 2, 3, 4, 7, and 12), GH2755 (chromosome 5), and GH2843 (chromosomes 2, 7, and 11); Fig. 3 *C* and *D* illustrates detected recombination in GH2753. GH2755 did not contain L alleles for the two chromosome 11 markers. The common occurrence of recombinational events was not surprising based on pairing behavior of LLS (8). Significantly, no alleles unique to S were observed in GH2754, the only diploid LR progeny and thus of most potential for experimental introgression.

Inferences concerning the degree of relatedness of LL, RR, and SS can be made by evaluating the similarities in allelic frequencies of isozyme and RFLP loci of the three species. The data are restricted by the limited representation of LL (2 genomes = one cultivar), SS (3 genomes = one SS individual plus LLS and LLSS hybrids), and RR (34 genomes = four LR hybrids plus 15 RR individuals). The representation of LL is satisfactory because tomato cultivars are essentially monomorphic for molecular markers (20, 21). The allelic frequency data are summarized in Table 2, in which isozyme and RFLP data are presented separately and in total. The results show that RR and SS are approximately equally divergent from LL, whereas they much more closely resemble each other, having approximately twice as many cases of agreement than either has with LL.

DISCUSSION

Protoplast fusion has been used to asexually hybridize tomato with *S. lycopersicoides* (22), *Solanum nigrum* (23), *S. rickii* (24), and *S. tuberosum* (25). To date, however, there have been no reports of successful use of these hybrids for gene transfer to tomato. By utilizing sexual techniques, however, hybridization and transfer of traits from SS to LL have now been accomplished (2, 9–12, 26).

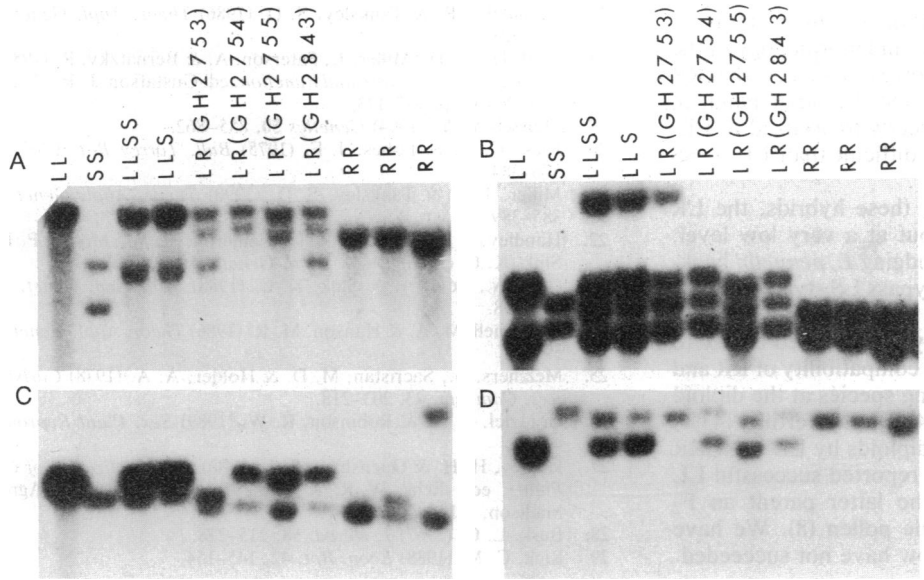

FIG. 3. RFLP analysis of LL, SS, LLSS (GH195), LLS (GH266), LR (GH2753), LR (GH2754), LR (GH2755), LR (GH2843), and RR individuals. Genomic DNA of each genotype was digested with *Xba* I (*A* and *B*) or *Pst* I (*C* and *D*) and probed with the following genomic probes: TG20, chromosome 7 (*A*); TG122, chromosome 10 (*B*); TG34, chromosome 2 (*C*); or TG37, chromosome 4 (*D*). Note the triallelism of GH2753 and GH2843 (*A* and *B*) and the variability of S alleles (*B*) and R alleles (*C*). GH2753 does not possess the L allele of TG34 (*C*) or TG37 (*D*).

Table 2. Comparisons of LL, SS, and RR for identity or nonidentity of molecular markers

Analysis	Category*					Total	Genomes compared	Marker allele pattern		Total
	0	1	2	3	4			Identical	Nonidentical	
Isozyme	4	3	8	1	1	17	L vs. R	29	74	103
RFLP	18	4	31	3	30	86	L vs. S	26	77	103
Total	22	7	39	4	31	103	R vs. S	61	42	103

*Allelic observations between LL, RR, and SS: category 0, alleles of L = R = S; category 1, alleles of L = R ≠ S; category 2, alleles of L ≠ R = S; category 3, alleles of L = S ≠ R; category 4, alleles of L ≠ R ≠ S.

The fact that SS × RR hybrids can readily be obtained, that RS hybrids exhibit regular pairing behavior, and the availability of the LLS hybrid led us to attempt LLS × RR hybridizations. Bridging hybridization, as a means of obtaining hybrids, has been utilized by plant breeders in *Cucurbita*, *Gossypium*, *Nicotiana*, and *Solanum* (27). A sesquidiploid hybrid functioned as part of the bridging pedigree in a successful interspecific transfer in *Nicotiana* (28) but was not used to produce a true diploid hybrid as in the present instance. For these purposes, sesquidiploids have two important advantages: (*i*) their hybrid constitution affords greater congruity and (*ii*) thanks to their distinctive chromosome pairing behavior, they yield a large share of intact, uncontaminated haploid gametes of the genome represented twice in their makeup. Our approach is therefore different in that it permits direct hybridization of species that are otherwise noncrossable at the diploid level and can yield hybrids that are free of the third species genome. It also provides opportunities for introgression as well as investigation of chromosome pairing behavior and other evolutionary aspects.

Progeny from the LLS × RR hybridizations were confirmed as LR hybrids by morphological, cytological, and molecular analyses. As expected from previous experience (9, 10), the progeny were both diploid and aneuploid. Experience with L–S and S–R chromosome pairing behavior suggested that pairing in the LR and LLRR hybrids would be similar to that observed for LS and LLSS hybrids (8), as we have confirmed. The cytological behavior observed in the hybrids serves as additional evidence of the close relationship between SS and RR. As to whether SS or RR is more closely affiliated with LL, data presented elsewhere (29) on plant habit and leaf and stem structure suggest that the latter is more closely related. Chromosome pairing data now available for LS (6), LLSS (8, 19), LR, LLRR, and RS indicate clearly that LL has diverged from RR and SS and that RR and SS are closely related, perhaps sibling species. The pairing data, however, do not reveal which of the *Solanum* species is more closely related to LL. These conclusions are also concordant with isozyme and RFLP data. Observations on geographic distribution, autoecology, and morphology of the leaves, inflorescence, and flowers, however, suggest that RR is more closely related to LL (29). On the other hand, in contrast to SS, RR has yet to be directly hybridized to LL, and if possible, it certainly is more difficult than LL × SS hybridizations.

In regard to our ability to utilize these hybrids, the LR hybrid (GH2754) is female fertile but at a very low level. Previous results (11) indicate that bridging *L. pennellii* backcross derivatives can be utilized to bypass LS stylar barriers; this alternative may exist for the LR hybrid but has not been adequately tested. Also, the recently documented ability to transfer traits from SS to LL and the compatibility of RR and SS suggest the use of SS as a bridging species at the diploid level. With the LLRR hybrid GH2754X, pollen fertility (43%) should be adequate to derive sesquidiploids by LL × LLRR hybridizations; previously, we have reported successful LL × LLSS hybridization using for the latter parent an F_2 individual having only 30% stainable pollen (8). We have attempted this objective but until now have not succeeded. Another alternative is LLS × LLRR hybridizations, which have already yielded progeny. This cross is expected to yield LLR sesquidiploids with and without extra S chromosomes. Chromosome pairing in such hybrids should provide additional valuable information concerning phylogenetic relations and might yield progeny consisting of addition and substitution lines. In any case, prospects for gene transfer from RR to LL now appear to be promising.

We acknowledge our indebtedness to Jennifer K. Tauscher for her help with the RFLP assays and to Rosa Buentello and Sofia Ruiz for assistance with embryo cultures, pollinations, and plant culture.

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