AGROBACTERIUM-MEDIATED GENE TRANSFER RESULTS MAINLY IN TRANSGENIC PLANTS TRANSMITTING T-DNA AS A SINGLE MENDELIAN FACTOR

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ABSTRACT

Forty-four independent transformed tobacco plants were obtained from a cocultivation experiment with *Agrobacterium tumefaciens* strains carrying modified Ti-plasmids. The transformed plants were either self-fertilized or crossed with nontransformed plants or with other transformed plants. The segregation of a phenotypic marker (kanamycin resistance) in the progenies of these plants was determined. In 40 cases out of 44, the segregation of the kanamycin resistance marker is consistent with Mendelian genetics. Among these 40 clones, 35 contain a single kanamycin resistance locus. The five others segregate two independent resistance loci. In two of the single insert clones, the segregation ratio after selfing indicates that the T-DNA insertion may have caused a recessive lethal mutation.

Agrobacterium tumefaciens is a phytopathogenic bacterium that induces oncogenesis of infected tissues through gene transfer. This bacterium is able to introduce and stably integrate a part of its endogenous Ti plasmid (for Tumor inducing), the T-DNA (Transferred-DNA), into the plant cell genome. The expression of T-DNA genes in the plant cell results in the formation of a plant tumor (via the production of phytohormones) and the production of new metabolic products collectively called opines. pTi-encoded catabolic functions enable the bacterium to use opines as substrate for its growth. For a review, see GHEYSEN *et al.* (1985).

The Ti plasmids have been modified for several years to be used as vectors for the genetic engineering of plants. Transformed plants have been regenerated first from tumors, then from cocultivation experiments with Ti plasmids (WULLEMS *et al.* 1981; DE GREVE *et al.* 1982; ZAMBRYSKI *et al.* 1983). Mendelian transmission of the T-DNA in the progeny of these plants is possible since OTTEN *et al.* (1981), DE BLOCK *et al.* (1984), WALLROTH *et al.* (1986)

Abbreviations: Km^R = kanamycin-resistant, Km^S = kanamycin-sensitive, χ^2 = chi-square, Ery^R = erythromycin-resistant, Cml^R = chloramphenicol-resistant, Rif^R = rifampicin-resistant, *nos* = nopaline synthase, *aph(3')II* = aminoglycoside phosphotransferase II, *P* = probability of a greater χ^2 for one degree of freedom.

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and others have given examples of transformed individuals with progeny that showed a 3:1 segregation ratio after self-fertilization, or a 1:1 segregation ratio in crosses with untransformed plants.

We constructed transformed tobacco plants (*Nicotiana tabacum*) with several modified Ti plasmids. All Ti plasmids used had a T-DNA carrying a chimeric nopaline synthase-aminoglycoside phosphotransferase II (*nos-aph*(3′)*II*) gene. Expression of the *nos-aph*(3′)*II* gene in transformed plant cells makes them kanamycin-resistant (Km^R) HERRERA-ESTRELLA *et al.* 1983; FRALEY *et al.* 1983; BEVAN, FLAVELL and CHILTON 1983; DE BLOCK *et al.* 1984). We report here the segregation of kanamycin resistance in the F_1 progeny of 44 independently transformed resistant plants. This is, to our knowledge, the first genetic analysis of a relatively large sample of transformed plants.

Segregation data allow the determination of the number of T-DNA inserts present in the transformed clones and the detection of recessive lethal mutations due to T-DNA integration. The importance of the data presented is not only the confirmation of the Mendelian inheritance on a more significant number of plants but also the indication that, in the majority of the transformed clones, the T-DNA segregates as a single locus. The latter is an advantage in the construction of transgenic plants and is essential when using the T-DNA as an insertion mutagen or as a gene-tagging sequence.

MATERIALS AND METHODS

Materials: Protoplasts for the cocultivation experiment were prepared from sterile tobacco plants (*Nicotiana tabacum*, cv. Petit Havana, line SR1; MALIGA, SZ.-BREZNOVITS and MÁRTON 1973). The bacterial strains used were C58C1 derivatives. The plasmids harbored by the transforming strains were derived from pGV3850 (ZAMBRYSKI *et al.* 1983) by cointegration of intermediate vectors. All intermediate vectors were derived from the plasmids pGV778 and pGV819, which are described elsewhere (F. BUDAR *et al.*, unpublished results) (see Table 1). All plasmids carry the chimeric *nos-aph*(3′)*II* and the nopaline synthase (*nos*) genes. In addition, pGV2420 contains gene *1* (tryptophane-2-monooxygenase; VAN ONCKELEN *et al.* 1985) of the octopine T-DNA, and pGV2441 and pGV2442 contain gene *2* (coding for an amidohydrolase; SCHRÖDER *et al.* 1984) of the octopine T-DNA. pGV2420, pGV2441 and pGV2442 are described elsewhere (F. BUDAR *et al.*, unpublished results).

The cocultivation, following a method modified from MÁRTON *et al.* (1979), and the regeneration of kanamycin-resistant plants will be described elsewhere (F. BUDAR *et al.*, unpublished results). Briefly, transformed microcolonies derived from the cocultivated protoplasts were selected on kanamycin (50 mg/liter) containing medium. The transformation efficiency obtained in this experiment varied between 3 and 9% (number of Km^R colonies for 100 microcolonies plated on the medium). Plants were regenerated from the transformed colonies. A single plant was isolated from each colony, so that each of the 44 plants results from an independent transformation event. Nopaline was detected in transformed colonies as described by LEEMANS *et al.* (1981) (see Table 2).

Crosses were performed in the greenhouse (BURK and CHAPLIN 1979). The seeds were harvested before the opening of the capsule to ensure absence of microbial contamination.

Kanamycin resistance assay on seedlings: Seeds are germinated on hormone-free half-strength LINSMAIER and SKOOG (1965) medium containing 50 mg/liter of kanamycin. Km^R plantlets develop normally on this medium. Sensitive individuals cannot form true leaves; their cotyledons become white, and the seedlings finally die. Km^R and kanamycin-sensitive (Km^S) seedlings are counted 3 weeks to 1 month after germination.

TABLE 1

Plasmids used in transformation experiments

Plasmid	Intermediate vector	Description of intermediate vector	Genes expressed in plant cells
pGV2420	pGV814	*Eco*RI-*Cla*I fragment of octopine T-DNA in *Eco*RI-*Cla*I sites of pGV819	Chimeric *nos-aph*(3′)*II* gene nopaline synthase, gene *1*
pGV2439	pGV827	Substitution of *Sal*I fragment of pLGV23neo[a] carrying nopaline T-DNA right border in pGV819	Chimeric *nos-aph*(3′)*II* gene nopaline synthase
pGV2440	pGV828	Substitution of *Sal*I fragment of pLGV23neo[a] carrying nopaline T-DNA right border in pGV778	Chimeric *nos-aph*(3′)*II* gene nopaline synthase
pGV2441	pGV823	Fragments *Hin*dIII-22 and -38 of octopine T-DNA in *Hin*dIII site of pGV819	Chimeric *nos-aph*(3′)*II* gene nopaline synthase, gene *2*[b]
pGV2442	pGV824	Fragments *Hin*dIII-22 and -38 of octopine T-DNA in *Hin*dIII site of pGV819	Chimeric *nos-aph*(3′)*II* gene nopaline synthase, gene *2*[b]

[a] HERRERA-ESTRELLA *et al.* (1983).
[b] Gene *2* is in opposite orientations in pGV823 an pGV824.

RESULTS AND DISCUSSION

Seeds from (1) self-fertilization (2) crosses between transformed and untransformed plants and (3) crosses between transformed plants were assayed for germination on a kanamycin-containing medium. The segregation of sensitive and resistant seedlings is shown in Tables 3, 4 and 5.

The data presented in these tables can be interpreted by either of two main hypotheses:

H_1: The resistance marker segregates as a single Mendelian factor, in which case self-fertilization can lead to one of the following situations:

H_{1a}—Integration of the T-DNA (containing the Km^R gene) induces a mutation that is lethal when homozygous: the expected segregation ratio is 2 Km^R:1 Km^S.

H_{1b}—The homozygotes for the T-DNA are viable: the expected segregation ratio is 3 Km^R:1 Km^S.

H_2: The resistance marker segregates as determined by two independent Mendelian loci, because of the presence of T-DNAs at two loci in the genome, far enough from one another. After self-fertilization, the expected segregation ratio is 15 Km^R:1 Km^S.

In the case of crosses with an untransformed plant (Table 4), the first hypothesis (H_1) predicts a segretation ratio of 1 Km^R:1 Km^S, and the second hypothesis (H_2) a segregation ratio of 3 Km^R:1 Km^S. In the case of crosses between transformed plants (Table 5), when both plants satisfy the first hy-

TABLE 2

Main characteristics of transformed plants

Plant no.	Agrobacterium strain used in the transformation experiment	Phenotypic markers	
		Km^R	Nopaline synthesis
	C58C1 Ery^R Cml^R (pGV2420)		
2420-1		+	−
2420-2		+	+
2420-3		+	+
2420-4		+	+
2420-5		+	+
2420-6		+	+
2420-7		+	+
2420-13		+	+
2420-14		+	+
	C58C1 Rif^R (pGV2439)		
2439-1		+	−
2439-2		+	+
2439-3		+	+
2439-4		+	−
2439-5		+	−
2439-10		+	−
2439-12		+	+
2439-15		+	+
2439-19		+	−
	C58C1 Rif^R (pGV2440)		
2440-3		+	+
2440-4		+	−
2440-5		+	+
2440-6		+	−
2440-7		+	+
2440-9		+	−
2440-10		+	−
2440-15		+	+
2440-16		+	−
2440-17		+	+
2440-19		+	−
2440-20		+	−
	C58C1 $Ery^R Cml^R$ (pGV2441)		
2441-4		+	+
2441-7		+	−
2441-8		+	+
2441-11		+	−
2441-12		+	+
2441-13		+	+
2441-14		+	+
	C58C1 Ery^R Cml^R (pGV2442)		
2442-1		+	+
2442-5		+	+
2442-6		+	+
2442-7		+	+
2442-8		+	+
2442-13		+	+
2442-14		+	−

pothesis ($H_{1\text{-}1}$), the expected segregation ratio is 3 Km^R:1 Km^S; and when one parent satisfies the first and the other the second hypothesis ($H_{1\text{-}2}$), the expected segregation ratio is 7 Km^R:1 Km^S. (It seems that none of our crosses involves two plants satisfying H_2, in which case the expected segregation ratio is 15 Km^R:1 Km^S.)

We calculated the χ^2 value for each hypothesis in each segregation table. We decided to reject an hypothesis at the 5% risk ($P < 0.05$). The retained hypotheses are indicated in the last column of each table.

As can be seen in Tables 3, 4 and 5, 40 of 44 plants show a segregation of the resistance marker that can be explained by either of the two hypotheses described above. For all the plants that are involved in different crosses, the results are consistent between crosses.

For no progeny was it necessary to introduce the hypothesis of more than two independent integrations. Furthermore, none of the plants produces progeny giving a result intermediate between H_{1b} and H_2. Such a result would imply a partial linkage between two resistance loci.

In two cases (2439-19 and 2441-13) the hypothesis H_{1a}, involving the presence of a lethal mutation linked to the T-DNA insertion, was retained.

Five plants (2420-2, 2439-12, 2440-7, 2440-20 and 2441-14) segregate two independent resistance loci. This segregation can be interpreted as the result of two independent transformations of the same cell.

In four cases (plants 2439-4, 2440-4, 2440-15, 2441-12) our hypotheses are unable to account for the segregation of the Km^R marker. These plants have been retested, and their Km^R phenotype was confirmed. In all these cases, an excess of sensitive seedlings (compared to what is expected with hypothesis H_{1a}, which maximizes their proportion) is obtained. We have no definitive explanation for these abnormalities. They can, however, be sorted into two groups. Two sets of progenies (from plants 2440-15 and 2441-12), although presenting an excess of sensitive types, gave clear-cut results: Km^R or Km^S plantlets were easily recognizable. The other two sets (from plants 2439-4 and 2440-4) gave intermediate phenotypes, and it was difficult to sort out Km^R and Km^S plants. Therefore, we tested the progeny of 2440-4 on a lower concentration of Km (25 mg/liter) and we obtained the same result (2 Km^R among 95 seedlings).

CONCLUSION

The genetic analysis of several plants resulting from our transformation experiment confirms preliminary results obtained earlier (OTTEN *et al.* 1981; WULLEMS *et al.* 1981; MEMELINK, WULLEMS and SCHILPEROORT 1983; BARTON *et al.* 1983; DE BLOCK *et al.* 1984). T-DNA genes are, in the majority of cases, conserved during meiosis and are expressed in the progeny. In most cases the segregation of the T-DNA marker can be interpreted within the framework of Mendelian genetics. For all plants involved in different crosses, consistent results were obtained.

We encountered only four cases where the transmission or the expression of the gene into the progeny was not achieved as expected. Several explana-

TABLE 3

Segregation of the Km^R marker in seeds from self-fertilization of transformed plants

Plant	Observed segregation		χ^2 value for hypothesis			Retained hypothesis[a]
	Km^R seedlings	Km^S seedlings	H_{1a} (2:1)	H_{1b} (3:1)	H_2 (15:1)	
2420-1	78	22	5.8 ($0.01 < P < 0.025$)	0.5 ($0.25 < P < 0.5$)	42.3 ($P << 0.005$)	H_{1b}
2420-3	112	37	4.8 ($0.025 < P < 0.05$)	0 ($P > 0.9$)	87.8 ($P << 0.005$)	H_{1b}
2420-5	121	49	1.6 ($0.1 < P < 0.25$)	1.3 ($0.25 < P < 0.5$)	147.8 ($P << 0.005$)	H_1
2420-6	108	29	9.1 ($P << 0.005$)	1.1 ($0.25 < P < 0.5$)	52.0 ($P << 0.005$)	H_{1b}
2420-7	82	36	0.4 ($0.5 < P < 0.75$)	1.9 ($0.1 < P < 0.25$)	118.5 ($P << 0.005$)	H_1
2420-13	215	85	3.4 ($0.05 < P < 0.1$)	1.8 ($0.1 < P < 0.25$)	249.7 ($P << 0.005$)	H_1
2420-14	417	121	28.5 ($P << 0.005$)	1.8 ($0.1 < P < 0.25$)	242.2 ($P << 0.005$)	H_{1b}
2439-1	134	43	6.5 ($0.01 < P < 0.025$)	0.05 ($0.75 < P < 0.9$)	98.4 ($P << 0.005$)	H_{1b}
2439-2	213	77	6.0 ($0.01 < P < 0.025$)	0.4 ($0.5 < P < 0.75$)	204.0 ($P << 0.005$)	H_{1b}
2439-3	87	21	9.4 ($P << 0.005$)	1.8 ($0.1 < P < 0.25$)	32.1 ($P << 0.005$)	H_{1b}
2439-4	0	201	402.0 ($P << 0.005$)	603.0 ($P << 0.005$)	3015.0 ($P << 0.005$)	None
2439-5	397	122	22.6 ($P << 0.005$)	0.6 ($0.25 < P < 0.5$)	263.8 ($P << 0.005$)	H_{1b}
2439-10	89	29	4.1 ($0.025 < P < 0.05$)	0 ($P > 0.9$)	67.6 ($P << 0.005$)	H_{1b}
2439-12	344	19	129.0 ($P << 0.005$)	75.6 ($P << 0.005$)	0.6 ($0.25 < P < 0.5$)	H_2
2439-15	220	60	17.8 ($P << 0.005$)	1.9 ($0.1 < P < 0.25$)	110.0 ($P << 0.005$)	H_{1b}
2439-19	140	67	0.1 ($0.75 < P < 0.9$)	6.0 ($0.01 < P < 0.025$)	241.0 ($P << 0.005$)	H_{1a}
2440-3	169	68	2.3 ($0.1 < P < 0.25$)	1.7 ($0.1 < P < 0.25$)	203.7 ($P << 0.005$)	H_1
2440-4	1	179	354.0 ($P << 0.005$)	532.0 ($P << 0.005$)	2668.1 ($P << 0.005$)	None
2440-5	76	29	1.5 ($0.1 < P < 0.25$)	0.4 ($0.5 < P < 0.75$)	81.8 ($P << 0.005$)	H_1
2440-6	224	85	4.7 ($0.025 < P < 0.05$)	1.0 ($0.25 < P < 0.5$)	238.3 ($P << 0.005$)	H_{1b}
2440-7	154	6	63.0 ($P << 0.005$)	38.5 ($P << 0.005$)	1.7 ($0.1 < P < 0.25$)	H_2
2440-9	169	47	13.0 ($P << 0.005$)	1.2 ($0.25 < P < 0.5$)	88.7 ($P << 0.005$)	H_{1b}
2440-10	203	61	12.4 ($P << 0.005$)	0.5 ($0.25 < P < 0.5$)	128.0 ($P << 0.005$)	H_{1b}
2440-15	33	33	8.3 ($P << 0.005$)	22.0 ($P << 0.005$)	215.6 ($P << 0.005$)	None
2440-16	117	41	3.9 ($0.025 < P < 0.05$)	0.1 ($0.75 < P < 0.9$)	104.6 ($P << 0.005$)	H_{1b}
2440-17	72	32	0.3 ($0.5 < P < 0.75$)	1.8 ($0.1 < P < 0.25$)	106.7 ($P << 0.005$)	H_1
2440-19	105	27	9.9 ($P << 0.005$)	1.5 ($0.1 < P < 0.25$)	45.5 ($P << 0.005$)	H_{1b}
2440-20	231	17	78.2 ($P << 0.005$)	43.5 ($P << 0.005$)	0.2 ($0.5 < P < 0.75$)	H_2

2441-4	116	42	3.2 ($0.05 < P < 0.1$)	0.2	($0.5 < P < 0.75$)	111.5 ($P \ll 0.005$)	H_1
2441-7	257	81	13.4 ($P \ll 0.005$)	0.2	($0.5 < P < 0.75$)	181.0 ($P \ll 0.005$)	H_{1b}
2441-8	120	39	5.5 ($0.01 < P < 0.025$)	0	($P > 0.9$)	90.7 ($P \ll 0.005$)	H_{1b}
2441-11	207	75	5.8 ($0.01 < P < 0.025$)	0.4	($0.5 < P < 0.75$)	199.2 ($P \ll 0.005$)	H_{1b}
2441-12	83	204	184.0 ($P \ll 0.005$)	325.0	($P \ll 0.005$)	2058.7 ($P \ll 0.005$)	None
2441-13	62	34	0.2 ($0.5 < P < 0.75$)	5.6	($0.01 < P < 0.025$)	139.4 ($P \ll 0.005$)	H_{1a}
2441-14	99	8	32.2 ($P \ll 0.005$)	17.5	($P \ll 0.005$)	0.3 ($0.5 < P < 0.75$)	H_2
2442-5	208	68	9.4 ($P \ll 0.005$)	0	($P > 0.9$)	159.3 ($P \ll 0.005$)	H_{1b}
2442-6	386	137	12.0 ($P \ll 0.005$)	0.4	($0.5 < P < 0.75$)	355.1 ($P \ll 0.005$)	H_{1b}
2442-7	130	41	6.7 ($0.005 < P < 0.01$)	0.1	($0.75 < P < 0.9$)	91.7 ($P \ll 0.005$)	H_{1b}
2442-8	52	10	8.3 ($P \ll 0.005$)	2.6	($0.1 < P < 0.25$)	10.3 ($P \ll 0.005$)	H_{1b}
2442-13	321	87	26.5 ($P \ll 0.005$)	2.9	($0.05 < P < 0.1$)	158.2 ($P \ll 0.005$)	H_{1b}
2442-14	229	85	5.5 ($0.01 < P < 0.025$)	0.7	($0.25 < P < 0.5$)	232.3 ($P \ll 0.005$)	H_{1b}

H_{1a}: The kanamycin resistance marker segregates as one Mendelian locus, and homozygotes are nonviable.
H_{1b}: The kanamycin resistance marker segregates as one Mendelian locus, and homozygotes are viable.
H_1: Neither H_{1a} nor H_{1b} can be rejected on the basis of the segregation data.
H_2: The kanamycin resistance marker segregates as two independent Mendelian loci.
[a] Hypotheses were rejected at the 5% risk ($P < 0.05$).

TABLE 4

Segregation of the Km^R marker in seeds from crosses between transformed plants (mother) and untransformed one (father)

Transformed plant crossed	Observed segregation		χ^2 value for hypothesis		Retained hypothesis[a]
	Km^R seedlings	Km^S seedlings	H_1 (1:1)	H_2 (3:1)	
2420-2	114	31	47.5 ($P << 0.005$)	1.0 ($0.25 < P < 0.5$)	H_2
2420-4	77	72	0.2 ($0.5 < P < 0.75$)	43.2 ($P << 0.005$)	H_1
2420-7	45	40	0.3 ($0.5 < P < 0.75$)	22.1 ($P << 0.005$)	H_1
2420-14	63	53	0.9 ($0.25 < P < 0.5$)	26.5 ($P << 0.005$)	H_1
2440-17	52	40	1.6 ($0.1 < P < 0.25$)	16.8 ($P << 0.005$)	H_1
2440-19	78	77	0 ($P > 0.9$)	50.3 ($P << 0.005$)	H_1
2441-7	56	59	0.1 ($0.75 < P < 0.9$)	42.4 ($P << 0.005$)	H_1
2442-14	54	47	0.5 ($0.25 < P < 0.5$)	25.0 ($P << 0.005$)	H_1

H_1: The kanamycin resistance marker segregates as one Mendelian locus.
H_2: The kanamycin resistance marker segregates as two independent loci.
[a] Hypotheses were rejected at the 5% risk ($P < 0.05$).

tions can be invoked for such behavior; (1) expression of the gene is repressed in the seeds; (2) the resistance gene has been lost in the gametes; (3) the T-DNA has been integrated and expressed in the cytoplasmic genome (chloroplastic or mitochondrial), and the cytoplasms of the plants were therefore chimeric: the resistance gene has been diluted or lost during the subsequent divisions; and (4) the T-DNA induces lethal (or sublethal) mutations in the gametes bearing it.

The segregation of two independent kanamycin resistance loci in the progeny of five plants was interpreted as the consequence of independent transformations of the same cell. Each transformation event can result in the integration of one or more tandemly repeated T-DNAs (ZAMBRYSKI *et al.* 1980; HOLSTERS *et al.* 1982). Such tandem repeats will segregate as one Mendelian locus. The number of plants resulting from two transformation events (five plants of 44) is consistent with the transformation efficiency of the experiment (9%).

The segregation data for two plants suggest a mutagenic effect of T-DNA insertion, leading to recessive lethal mutations. We cannot exclude an artifact due to suboptimal expression of the kanamycin resistance, leading to an underestimation of the number of resistant seedlings. Therefore, the progeny of these plants will be tested for the presence of plants homozygous for the T-DNA insert. A similar approach will allow us to distinguish between the hypotheses H_{1a} and H_{1b} for the plants for which this was not possible on the basis of the segregation data.

The observation that the majority of the transgenic plants transmits the insert as a single locus stresses the advantage of the Ti plasmid as a gene delivery system and insertion mutagen.

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TABLE 5

Segregation of the Km^R marker in seeds from crosses between two transformed plants

Crossed plants (mother × father)	Observed segregation		χ^2 value for hypothesis		Retained hypothesis[a]
	Km^R seedlings	Km^S seedlings	H_{1-1} (3:1)	H_{1-2} (7:1)	
2420-2 × 2441-7	111	15	11.5 ($P \ll 0.005$)	0 ($P > 0.9$)	H_{1-2}
2420-2 × 2442-14	101	8	18.1 ($P \ll 0.005$)	2.7 ($0.1 < P < 0.25$)	H_{1-2}
2420-4 × 2441-7	54	20	0.2 ($0.5 < P < 0.75$)	14.3 ($P \ll 0.005$)	H_{1-1}
2420-6 × 2441-7	48	13	0.4 ($0.5 < P < 0.75$)	4.3 ($0.025 < P < 0.05$)	H_{1-1}
2420-6 × 2442-14	96	34	0.1 ($0.75 < P < 0.9$)	22.2 ($P \ll 0.005$)	H_{1-1}
2420-7 × 2441-7	68	29	1.2 ($0.25 < P < 0.5$)	26.8 ($P \ll 0.005$)	H_{1-1}
2420-13 × 2441-7	87	26	0.2 ($0.5 < P < 0.75$)	11.4 ($P \ll 0.005$)	H_{1-1}
2420-14 × 2441-7	247	71	1.2 ($0.25 < P < 0.5$)	28.1 ($P \ll 0.005$)	H_{1-1}
2441-7 × 2420-4	48	18	0.2 ($0.5 < P < 0.75$)	13.2 ($P \ll 0.005$)	H_{1-1}
2441-7 × 2420-7	66	19	0.3 ($0.5 < P < 0.75$)	7.5 ($0.005 < P < 0.01$)	H_{1-1}
2441-7 × 2420-13	89	33	0.3 ($0.5 < P < 0.75$)	23.6 ($P \ll 0.005$)	H_{1-1}
2441-7 × 2420-14	50	14	0.3 ($0.5 < P < 0.75$)	5.1 ($0.01 < P < 0.025$)	H_{1-1}
2441-8 × 2420-1	75	26	0 ($P > 0.9$)	16.2 ($P \ll 0.005$)	H_{1-1}
2441-12 × 2420-7	47	32	10.1 ($P \ll 0.005$)	56.7 ($P \ll 0.005$)	None
2442-1 × 2420-3	87	25	0.4 ($0.5 < P < 0.75$)	9.9 ($P \ll 0.005$)	H_{1-1}
2442-7 × 2420-1	82	31	0.4 ($0.5 < P < 0.75$)	23.0 ($P \ll 0.005$)	H_{1-1}
2442-14 × 2420-7	73	28	0.4 ($0.5 < P < 0.75$)	21.4 ($P \ll 0.005$)	H_{1-1}

H_{1-1}: Both plants segregate the kanamycin resistance as one Mendelian locus.
H_{1-2}: One plant segregates the kanamycin resistance as one Mendelian locus and the other as two independent loci.
[a] Hypotheses were rejected at the 5% risk ($P < 0.05$).

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LITERATURE CITED

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