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Limited-Host-Range Plasmid of *Agrobacterium tumefaciens*: Molecular and Genetic Analyses of Transferred DNA

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A tumor-inducing (Ti) plasmid from a strain of *Agrobacterium tumefaciens* that induces tumors on only a limited range of plants was characterized and compared with the Ti plasmids from strains that induce tumors on a wide range of plants. Whereas all wide-host-range Ti plasmids characterized to date contain closely linked oncogenic loci within a single transferred DNA (T-DNA) region, homology to these loci is divided into two widely separated T-DNA regions on the limited-host-range plasmid. These two plasmid regions, T_A-DNA and T_B-DNA, are separated by approximately 25 kilobases of DNA which is not maintained in the tumor. The T_A-DNA region resembles a deleted form of the wide-host-range T_L-DNA and contains a region homologous to the cytokinin biosynthetic gene. However, a region homologous to the two auxin biosynthetic loci of the wide-host-range plasmid mapped within the T_B-DNA region. These latter genes play an important role in tumor formation because mutations in these loci result in a loss of virulence on *Nicotiana* plants. Furthermore, the T_B-DNA region alone conferred tumorigenicity onto strains with an intact set of *vir* genes. Our results suggest that factors within both the T-DNA and the *vir* regions contribute to the expression of host range in *Agrobacterium* species. There was a tremendous variation among plants in susceptibility to tumor formation by various *A. tumefaciens* strains. This variation occurred not only among different plant species, but also among different varieties of plants within the same genus.

Virulent strains of *Agrobacterium tumefaciens* can induce tumorous growth on many dicotyledonous plants. These strains all contain a large tumor-inducing plasmid (Ti plasmid), a part of which is transferred to, integrated, and stably maintained within the nuclear genome of tumor tissue (3, 11, 35). The transcribed regions of the wide-host-range (WHR) plasmid transferred DNA (T-DNA) have been examined extensively by insertion mutagenesis (14, 27, 28, 37, 38) and Northern blot hybridization (15, 48). This T-DNA codes for oncogenic functions which are required for maintenance of the tumorous phenotype and for opines which the inciting bacterium can utilize as sole carbon and nitrogen sources (3, 11, 35). The oncogenic loci of the T-DNA regions have been designated *tms* and *tmr* (14). The *tms* loci code for two enzymes of auxin synthesis (19, 23, 40); the *tmr* locus codes for the first enzyme of cytokinin biosynthesis (1, 2). There is also some evidence that the *tml* locus (14, 38) and transcript 5 (11, 21) can affect tumor morphology, although the functions of these loci remain unclear. Another region of the Ti plasmid, designated *vir*, is required for tumor formation but is not found integrated into plant tumor DNA (13, 37).

Host range is primarily determined by the particular Ti plasmid harbored by the infecting strain (30, 45). Mutagenesis of the WHR T-DNA and *vir* regions has indicated that both of these regions can contribute to host specificity (14, 18, 22, 31, 38). Thus far, studies have focused on the WHR Ti plasmids, all of which share a highly conserved common DNA region within the T-DNA (7, 10) and allow tumor formation on a wide range of plant species. We chose to characterize a limited-host-range (LHR) Ti plasmid because it reportedly shares little homology to this common DNA region (43) and because it codes for a very restricted host range (26, 45). Whereas WHR Ti plasmids express virulence on a wide variety of dicotyledonous plants, the LHR Ti plasmids are unable to induce tumors on many plant species commonly used in crown gall studies. LHR plasmid pTiAg162, described here, is typical of LHR Ti plasmids found in *Agrobacterium* biotype III strains associated with grapevines (*Vitis* species) (25, 26). Although the only natural host for the original isolate, Ag162, may be *Vitis* species, in the laboratory some *Nicotiana* species are also permissive hosts. Solution hybridization experiments have shown that the LHR and WHR Ti plasmids share only about 15% overall homology, indicating that they are widely divergent examples of tumor-inducing plasmids (43). Recent studies have shown that there are two T-DNA regions on LHR Ti plasmid pTiAg63 (5). Our efforts were directed at identifying the determinants required for tumor formation in LHR Ti plasmid pTiAg162 to compare these determinants with those of WHR plasmid pTiA6. Through such a direct comparison we have begun to understand some of the factors which control host range expression in *A. tumefaciens*.

MATERIALS AND METHODS

Bacterial strains and plasmids. LHR plasmid pTiAg162 and WHR plasmid pTiA6 have been described previously (26, 41, 43). The LHR and WHR strains used in this study were isogenic, differing only in Ti plasmid content. Cosmid clones of the LHR plasmid have also been described (24). *A. tumefaciens* strains were maintained on AB minimal medium (46) supplemented with the appropriate antibiotics (200 μg of carbenicillin and 100 μg of kanamycin per ml). *Escherichia coli* HB101 (24) was cultured on L-agar medium (33) with the appropriate antibiotics (200 μg of carbenicillin, 50 μg of kanamycin, and 15 μg of tetracycline per ml). Bacterial conjugation was obtained on nutrient agar medium by the triparental mating procedure of Ditta et al. (12).

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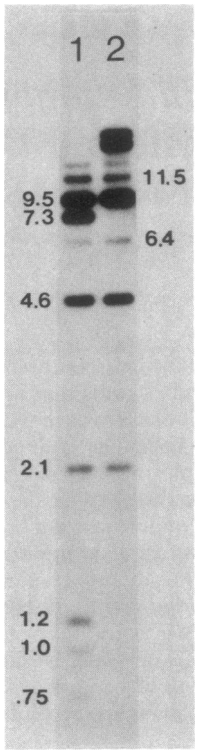

FIG. 1. Southern blot analysis of pLHRΔ11. Total *Agrobacterium* DNA was isolated from the LHR strain (lane 1) and pLHRΔ11 (lane 2) and digested with *Bam*HI. Southern blots were probed with nick-translated pVCK431 and visualized by autoradiography. The numbers to the left and right are kilobase markers.

Tumor lines. The LHR strain was inoculated onto sterile, decapitated plants, and the resulting tumors were cultured axenically by methods previously described (20). The two tumor lines described in this manuscript were derived from the LHR strain inoculated onto *Vitis vinifera* cv. Cabernet sauvignon and *Nicotiana glauca* plants and were named CS806 and Ag856, respectively.

DNA isolation and restriction enzymes. Purified plasmid DNA was isolated by the methods of Currier and Nester (8) or Birnboim and Doly (4). Plant DNA was isolated by methods previously described (29). Restriction enzymes were obtained from Bethesda Research Laboratories, Inc., and reaction conditions were those recommended by the supplier.

DNA hybridizations. DNA fragments were separated electrophoretically and transferred to nitrocellulose (42). Radiolabeled probe was prepared by nick translation (32) with DNA polymerase I (New England Nuclear Corp.) and DNAse I (Worthington Diagnostics). Plant genomic DNA hybridizations were done as described by Thomashow et al. (44). Southern blot (42) hybridizations of plasmid DNA were as previously described (43), with high-stringency washes at 65°C in 0.3× SSC ($\sim T_m$, −17°C) and low-stringency washes at 65°C in 6× SSC ($\sim T_m$, −42°C) (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate).

Construction of mutants. Transposon insertions into cosmid-cloned DNA were obtained by methods already described (14), and the resulting mutations were recombined into the Ti plasmid by the marker exchange procedure (39). Deletions were constructed in vitro by partial digestion of cosmid DNA carrying a Tn*5* insertion with *Bam*HI and subsequent ligation and transformation. Kanamycin-resistant clones were screened in *E. coli* for the loss of desired restriction fragments and then reintroduced into *A. tumefaciens* by conjugation. Marker exchange selecting for the kanamycin-resistance determinant of Tn*5* resulted in a deletion of the appropriate fragments of Ti plasmid DNA. Each deletion began at the *Bam*HI site within the Tn*5* insertion and extended outward into the adjacent Ti plasmid DNA.

All transposon insertions and deletions were verified by Southern blot hybridization, an example of which is shown in Fig. 1 for the derivative pLHRΔ11. A successful deletion was verified by showing that specific restriction fragments were lost while flanking fragments were maintained. Total *Agrobacterium* DNA was digested with *Bam*HI, and the fragments were separated by agarose gel electrophoresis and then transferred to nitrocellulose. Southern blots were probed with nick-translated pVCK431 (24) and visualized by autoradiography. pLHRΔ11 lacked the 7.3-, 1.0-, 1.2-, and 0.75-kilobase (kb) *Bam*HI fragments and retained the flanking 9.5-, 2.1-, and 4.6-kb *Bam*HI fragments (Fig. 1 and Fig. 2). It should also be noted that pVCK431 hybridized to the 11.5- and 6.4-kb *Bam*HI fragments within pVCK418 and to a 13-kb *Bam*HI fragment in an unknown region of the plasmid (Fig. 1). Furthermore, this probe hybridized to fragments from the coresident plasmid pPH1JI, although this was due simply to vector homology and has been described previously (14).

Virulence assays. Virulence was assayed by wounding and inoculating plants as described previously (38, 47). Virulence was scored as positive or negative based on the formation of a tumorous response 4 to 8 weeks after infection. *Vitis* plant inoculations were performed at the basal end of stem cultures grown on 0.5× MS^- medium (34) in scintillation vials. The results of these inoculations were recorded after 1 month of incubation and a minimum of three successive transfers on MS^- medium. Plant hosts included *Kalanchoë diagremontiana*, *Nicotiana tabacum* var. xanthi, *Nicotiana rustica*, *N. glauca*, *Vitis* sp. cv. Seyval, and *Vitis labruscana* cv. Steuben.

RESULTS

Identification of T-DNA regions. Overlapping cosmid clones which encompass the entire plasmid were used as probes to identify the regions of LHR plasmid Ag162, which were maintained in axenic tumors formed on *N. glauca* and *V. vinifera* cv. Cabernet sauvignon (Fig. 3). Probes were hybridized to Southern blots of fractionated tumor and normal DNA. Probes covering two plasmid regions hybridized with tumor DNA from both *Vitis* and *N. glauca* plants. The two plasmid regions included in the cosmid clones pVCK418 and pVCK431 were separated by more than 25 kb of DNA that was not found in transformed tissue. The leftward region, designated T_A-DNA, covered approximately 5 to 7 kb, and the rightward region, designated T_B-DNA, spanned approximately 20 kb of plasmid DNA. Autoradiographs of Southern blots of normal and tumor DNA isolated from *N. glauca* and *Vitis* sp. and digested with *Hin*dIII are shown in Fig. 4. For each blot, lanes 5 and 6 represent reconstructions which were intended to mimic one and five copies per diploid *N. glauca* genome, respectively. pVCK418 and pVCK431 hybridized to tumor DNA sequences and not to untransformed normal *Vitis* (lanes 1) or *N. glauca* (lanes 3) DNA.

Internal fragments within the tumor are recognized by their comigration with specific fragments in the reconstruc-

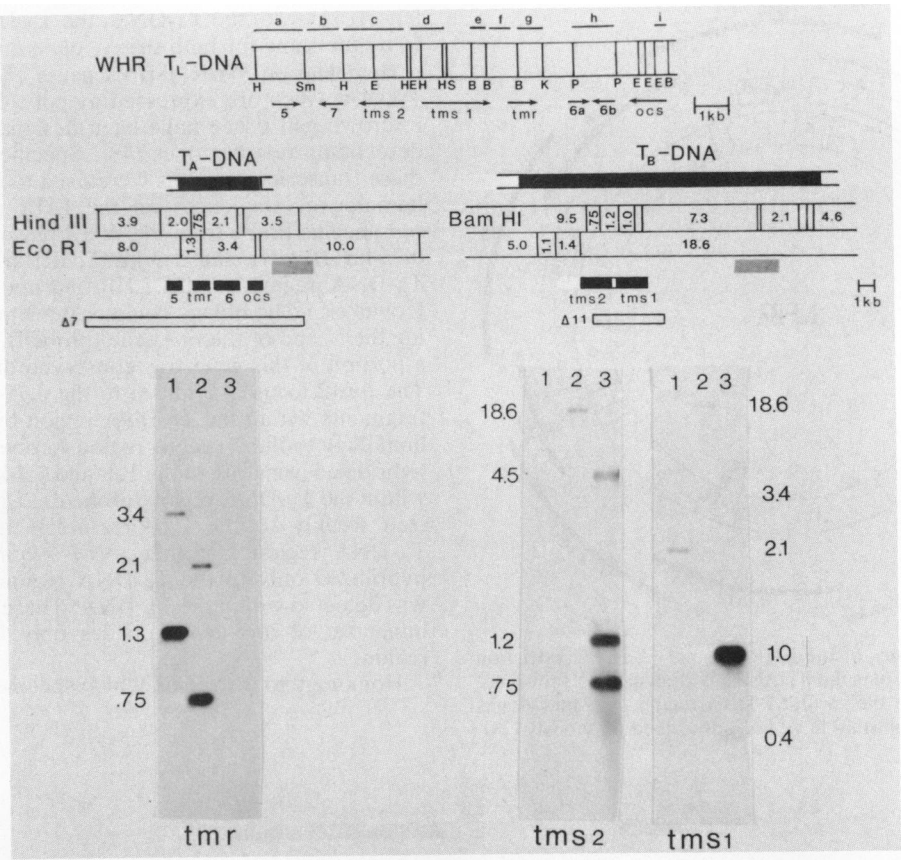

FIG. 2. Analysis of LHR T-DNA regions. A restriction map for the WHR T_L-DNA region is shown for *Hin*dIII (H), *Sma*I (Sm), *Eco*RI (E), *Sal*I (S), *Kpn*I (K), *Pvu*II (P), and *Bam*HI (B). Below this map the transcriptional organization of the WHR T-DNA is shown, and above the map the probes used in this analysis are shown. Probes were purified from agarose gels. Also shown are maps of the LHR T-DNA regions. Black bars below the LHR T-DNA restriction map indicate the position of homology within the LHR T-DNA to each of the WHR probes. Also shown are the results of probing the LHR T-DNA regions with probes specific for the *tmr*, *tms-1*, and *tms-2* loci of the WHR plasmid. For the *tmr* probing, lanes 1 and 2 represent *Eco*RI and *Hin*dIII digests, respectively, of pVCK418. Lane 3 represents an *Xho*I digest of pVCK431. For each of the *tms* probings, lane 1 represents a *Hin*dIII digest of pVCK418 and lane 2 represents an *Eco*RI digest of the entire LHR plasmid. Lane 3 represents a *Bam*HI-*Sal*I double digest of the 9.1-kb *Sal*I fragment cloned into the vector pBR322. The details of this analysis are in the text. The open bars designate the regions of the LHR plasmid which are deleted in the derivatives pLHRΔ7 and pLHRΔ11. The gray bars represent the region of homology between the two T-DNA regions. The numbers to the left and right of the gels are kilobase markers.

tion. Junction fragments are defined as noninternal fragments which are formed by joining plasmid and plant sequences or rearranged plasmid sequences. *Hin*dIII fragments of 2.1 and 0.75 kb were present as internal fragments in the T_A-DNA region in the *N. glauca* and *Vitis* sp. tumor lines (Fig. 4a). These internal fragments were present in four to five copies in the *N. glauca* tumor as judged by the five-copy reconstruction, and four different junction fragments were observed (Fig. 4a, lane 4). The number of copies of T-DNA could not be accurately judged in the *Vitis* tumor because the genome size of the species studied was not known. However, there appeared to be many junction fragments in low copy, suggesting that multiple copies of T_A-DNA were in this tumor (lane 2). The strong hybridization to a 7.5-kb *Hin*dIII fragment (lane 2) resulted from cross hybridization between the two T-DNA regions. This cross homology was seen in the reconstruction (lane 6) in which hybridization was detected to the 7.5-kb *Hin*dIII fragment within the T_B-DNA region. The homology between the two T-DNA regions is also evident in Fig. 1 as described above. Here, pVCK431 hybridized to the 11.5- and 6.4-kb *Bam*HI fragments at the right end of the T_A-DNA region and to a 13-kb *Bam*HI fragment from an unidentified region of the plasmid. We further characterized this cross homology by probing pVCK418 and pVCK431 against each other by Southern blotting (data not shown). In this way, we accurately mapped the conserved sequences shared by these two clones. These regions are designated by gray bars in Fig. 2.

The T_B-DNA region found within pVCK431 was present in more copies than was the T_A-DNA region in the *Vitis* tumor. Internal *Hin*dIII fragments of 8.5, 1.1, and 7.5 kb were present in high copy numbers, and seven junction fragments were present with an intensity equivalent to one to two copies for the *N. glauca* genome (Fig. 4b, lane 2). At least seven additional junction fragments were present with an intensity greater than the five-copy reconstruction for the *N. glauca* genome. One additional fragment of 3.6 kb was present at approximately five copies per diploid cell. This 3.6-kb fragment could be either an internal or a junction fragment. In the *N. glauca* tumor, the 8.5-, 1.1-, and 7.5-kb internal *Hin*dIII fragments were present in three to four copies, and six junction fragments hybridized to yield an intensity nearly equal to the one-copy reconstruction (Fig. 4,

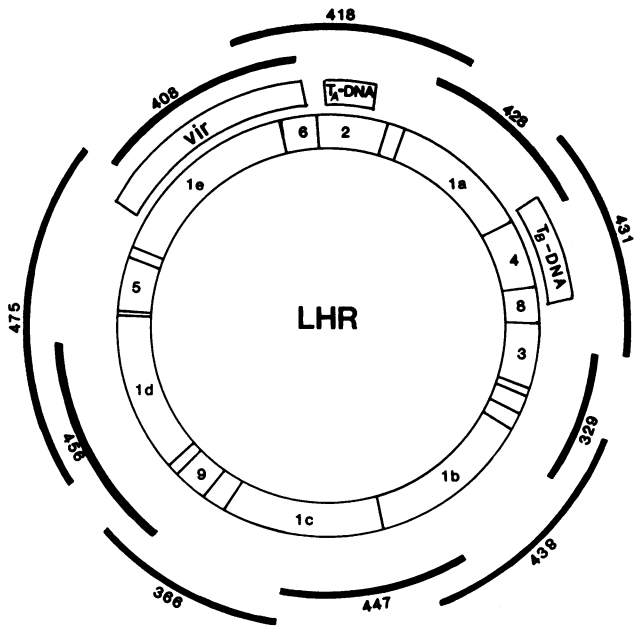

FIG. 3. Cosmid clones of the LHR plasmid. A *Kpn*I restriction map of the 231-kb LHR plasmid pTiAg162 is shown. Also shown are the relative positions of the *vir* and T-DNA regions. Cosmid clones which encompass the plasmid have been described previously (24).

lane 4). As with the T_A-DNA, the T_B-DNA region appeared to be the same for both the *N. glauca* and *Vitis* tumors.

Homology to WHR T-DNA genes. The regions of WHR T-DNA which are expressed as polyadenylated transcripts in crown gall tissue have been designated 1 to 7 based on descending relative sizes (48). Specific probes for each of these transcribed regions were used to identify and map the homologous sequences on the LHR plasmid (Fig. 2). A *tmr*-specific probe from the WHR plasmid hybridized to the 0.75-kb *Hin*dIII and 1.3-kb *Eco*RI fragments within the T_A-DNA region of the LHR plasmid (Fig. 2, probe g). However, no homology could be detected to a clone containing the 5′ end of the *tmr* gene (probe f), indicating that only a portion of this gene was conserved on the LHR plasmid. The *tms-2* locus hybridized to the 0.75- and 1.2-kb *Bam*HI fragments within the T_B-DNA region but did not show any homology to the T_A-DNA region (probe c). The *tms-1* locus hybridized primarily to the 1.0- and 0.4-kb *Bam*HI fragments within the T_B-DNA region (probe d). This locus also hybridized weakly to the 2.1-kb *Hin*dIII fragment within the T_A-DNA region. Another *tms-1*-specific probe (probe e) hybridized only to the T_B-DNA region, and no homology was detected within the T_A-DNA. These data suggest that an intact set of *tms* genes resides only within the T_B-DNA region.

Homology to the region which specifies transcript 5 of the

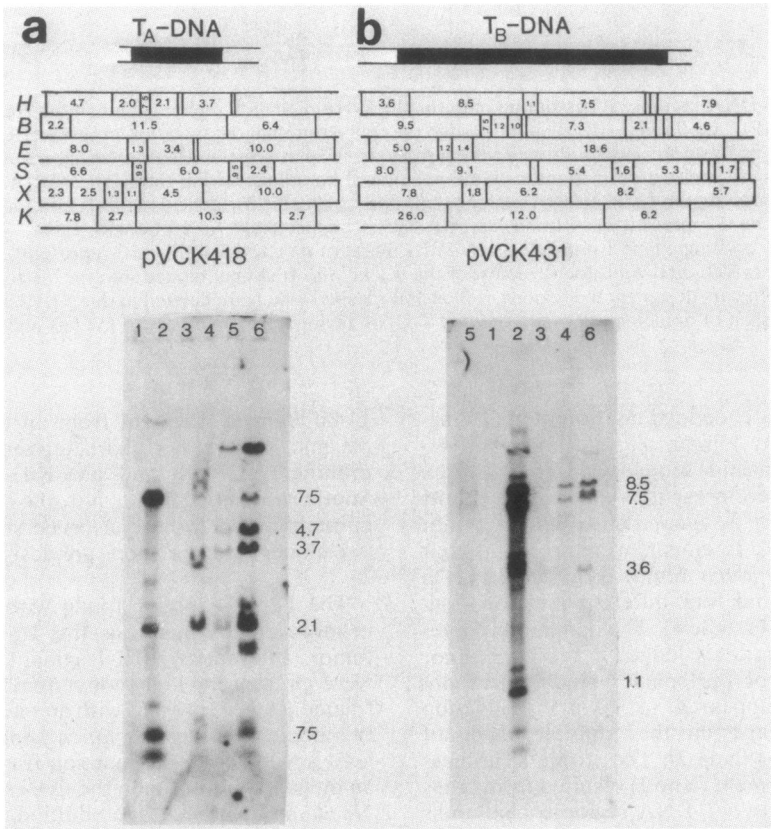

FIG. 4. Genomic Southern blotting of tumor lines. *Hin*dIII (H), *Bam*HI (B), *Eco*RI (E), *Sal*I (S), *Xho*I (X), and *Kpn*I (K) restriction sites are designated for the T_A-DNA and T_B-DNA regions which are encompassed by the cosmid clones pVCK418 and pVCK431. Southern blots were prepared and hybridized as described in the text. Blots were probed with pVCK418 (a) and pVCK431 (b). Lanes (both a and b): 1 and 3, normal, untransformed DNA control; 2, *Vitis* sp. tumor DNA; 4, *N. glauca* tumor DNA; 5 and 6, one- and five-copy reconstructions, respectively, based on the size of the *N. glauca* genome. Details are discussed in the text. The numbers to the right of the gels are kilobase markers.

WHR T-DNA was observed in the 2.0-kb *Hin*dIII and 8.0-kb *Eco*RI fragments in the T_A-DNA region (Fig. 2, probe a). A probe specific for transcript 7 did not hybridize to the LHR plasmid (probe b). A probe covering transcripts 6a and 6b, designated *tml* (14), hybridized to the 2.1-kb *Hin*dIII and 3.4-kb *Eco*RI fragments within the T_A-DNA region (probe h). A faint hybridization signal was also detected at the position of the 2.1-kb *Bam*HI fragment within the T_B-DNA region. An octopine synthase-specific probe (transcript 3) hybridized strongly to the 3.5-kb *Hin*dIII and 10.0-kb *Eco*RI fragments within T_A-DNA (Fig. 2). This probe was also homologous to the 2.0-kb *Hin*dIII and 1.3-kb *Eco*RI fragments, also in the T_A-DNA region.

Mutant characterization. If both T-DNA regions are necessary for tumorigenesis, then deletions within either region should affect virulence. We assayed deletion derivatives of the LHR plasmid on three cultivars of *Nicotiana* plants (*N. rustica*, *N. glauca*, and *N. tabacum*) and on two cultivars of *Vitis* plants (*Vitis* sp. cv. Seyval and *V. labruscana* cv. Steuben). A deletion encompassing all of the T_A-DNA region and extending about 5 kb beyond the left end of this region was constructed with pVCK418. This mutation was incorporated into the LHR Ti plasmid by marker exchange (see above), and the strain harboring the deleted plasmid (pLHRΔ7 [Fig. 2]) was assayed for virulence (Table 1). This strain formed an apparently wild-type tumor on *N. rustica* and on *V. labruscana* cv. Steuben, indicating that the entire T_A-DNA region is dispensable for tumor formation on these plants. In contrast, this same deletion led to an avirulent phenotype on *N. glauca* and *Vitis* sp. cv. Seyval.

Another derivative, pLHRΔ11, had the region of T_B-DNA that was homologous to the WHR *tms* loci removed (Fig. 2). The results of inoculating this strain are given in Table 1. This deletion resulted in avirulence on both *N. rustica* and *N. glauca*, indicating that the *tms* loci of the LHR plasmid play a critical role in tumor formation on these plants. In contrast, the deletion in pLHRΔ11 did not appear to affect tumor formation on *Vitis* plants, as strains harboring this plasmid induced tumors. This indicated that loci in addition to the *tms* genes contribute to oncogenesis by the LHR plasmid. Tn*5* insertions in and around the region that shared homology to the WHR *tms* loci allowed these functional loci to be precisely localized. Only those insertions which mapped within the *tms* homology region (Fig. 2) affected oncogenicity. Tn*5* insertions were isolated in the region of the LHR plasmid that shared homology to the WHR octopine synthase locus (Fig. 2). These strains gave rise to apparently wild-type tumors which did not synthesize octopine.

TABLE 1. Mutational analysis of LHR T-DNA region[a]

Plants	Strain			
	LHR	Δ7	Δ11	WHR
N. tabacum	−	−	−	+
N. glauca	+	−	−	+
N. rustica	+[b]	+[b]	−	+
Vitis sp. cv. Seyval	+	−[c]	+	+
V. labruscana cv. Steuben	+	+	+	−[d]

[a] Virulence was assayed on a variety of host plants for the LHR and WHR strains and for two deletion derivatives of the LHR strain.
[b] Roots from tumor.
[c] Very attenuated.
[d] Hypersensitive response.

TABLE 2. Functional analysis of T_A-DNA and T_B-DNA regions[a]

Plants	Strain or plasmid		
	LHR	LBA4404(pVCK431)	LBA4404(pVCK418)
N. glauca	+	+	−
N. rustica	+[b]	+[b]	−
Kalanchoë spp.	−	+[b]	−
Vitis sp. cv. Seyval	+	+	+/−[c]
V. labruscana cv. Steuben	+	+	+

[a] Cosmid clones (24) covering the LHR T_A-DNA (pVCK418) or T_B-DNA (pVCK431) regions were assayed for their ability to confer tumorigenicity when present in an avirulent strain containing the WHR *vir* region (LBA4404) (36). The results of inoculating the same plants with the LHR strain are also shown.
[b] Roots from tumor.
[c] Very attenuated.

Functional analysis of LHR T-DNA regions. A binary vector system was used to assess the role of each T-DNA region in tumor formation. It has previously been shown that the *vir* region of a WHR plasmid is capable of mobilizing the T-DNA region into plant cells in a *trans* configuration (9, 17). Strain LBA4404 harbors a deletion derivative of WHR plasmid pTiACH5 (36) and contains the entire WHR *vir* region but none of the T-DNA sequences. Cosmid clones of the LHR plasmid containing the T_A-DNA region (pVCK418) or the T_B-DNA region (pVCK431) were idependently mobilized into LBA4404. The two strains, LBA4404(pVCK418) and LBA4404(pVCK431), were assayed for their ability to induce tumors on a variety of host plants (Table 2). Strain LBA4404(pVCK431) induced tumors on *N. glauca* (Fig. 5B) and *N. rustica* and on *Kalanchoë* stems (Fig. 6), indicating that the T_B-DNA region alone was sufficient to induce a tumorous response. Strain LBA4404(pVCK418) was unable to induce tumor formation, indicating that the T_A-DNA region was not sufficient for tumor induction on any of these host plants (Fig. 5A). However, when strains LBA4404(pVCK418) and LBA4404(pVCK431) were coinoculated onto *N. glauca*, tumors developed which were larger than those induced by the inoculation of LBA4404(pVCK431) alone (Fig. 5C), suggesting that the T_A-DNA region contributed to the tumorous response. Furthermore, strain LBA4404(pVCK418) induced tumors on *V. labruscana* cv. Steuben, indicating that the T_A-DNA region is sufficient for tumor formation on this host (Table 2).

DISCUSSION

To identify factors which contribute to the expression of host range in *Agrobacterium* species, we have begun to characterize an LHR Ti plasmid. The significance of these data can best be understood by comparing the LHR and WHR Ti plasmids. All of the WHR plasmids which have been studied thus far contain closely linked oncogenic loci within a single T-DNA region. However, in the LHR plasmid, homology to these oncogenic loci is divided into two T-DNA regions which are separated by more than 25 kb of DNA not found in the tumor. Similar data were recently reported in another LHR strain (5). The octopine catabolism functions map in the region which is now shown to separate these two T-DNA regions (24). Thus, at least one set of genes which are expressed and function within the bacterium separates the two T-DNA regions. The two T-DNA regions

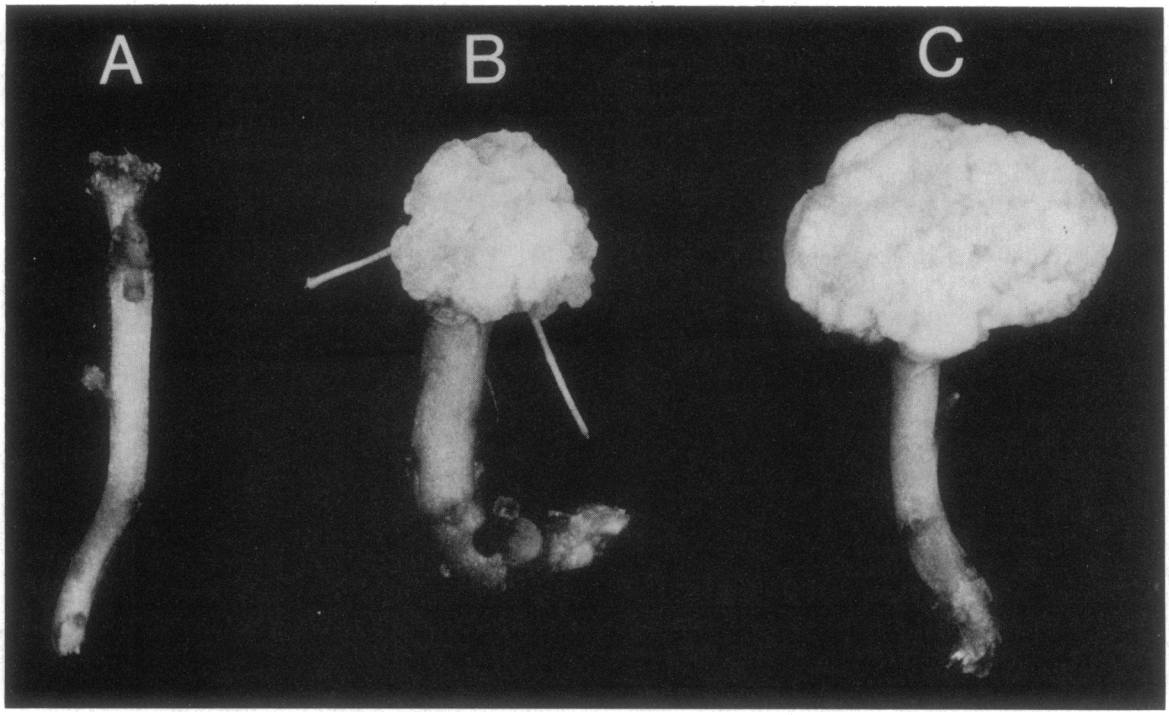

FIG. 5. Functional analysis of LHR T-DNA regions on *N. glauca* plants. Results are shown for inoculating *N. glauca* plants with LBA4404(pVCK418) (A), LBA4404(pVCK431) (B), and LBA4404(pVCK418) coinoculated with LBA4404(pVCK431) (C). Details are discussed in the text.

share common sequences because clones covering each region hybridized to one another. This suggests that a duplication has occurred, an event which may have played a role in the formation of the T-DNA structure of this plasmid.

Our data enabled us to construct a detailed map for the WHR T-DNA genes which are conserved within the LHR T-DNA regions. Superficially, T_A-DNA resembles a deleted form of the WHR T_L-DNA in which all the DNA from gene 7 through the 5′ end of the *tmr* gene is missing. The T_A-DNAregion shares homology to WHR genes 5, 4 (*tmr*), 6, and 3 (*ocs*) in the same relative order (Fig. 4). No homology was detected within the T_A-DNA region to the 5′ end of the *tmr* gene or to genes 7, *tms-2*, and *tms-1*. Although the T_A-DNA region is not required for tumor formation, it may contribute to the tumor phenotype. Because this region shares homology to genes 5 and 6a or 6b of the WHR plasmid, which have previously been shown to influence tumor formation (14, 21), it is possible that these genes are present in the T_A-DNA of the LHR plasmid and contribute to the tumor response. Further studies are needed to identify the specific functions of these loci. The T_A-DNA region also contains a functional octopine synthase gene, since transposon insertions into this locus result in tumors which do not synthesize octopine.

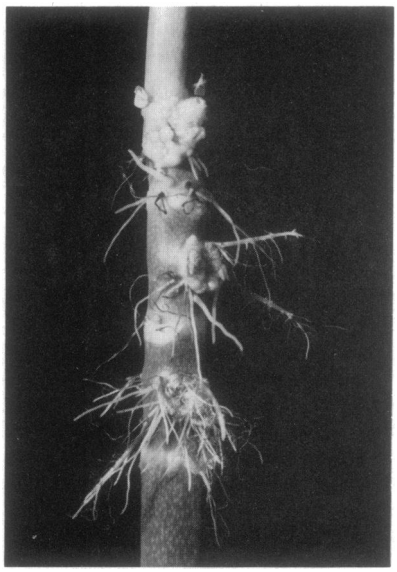

FIG. 6. Inoculation of LBA4404(pVCK431) on *Kalanchoë* stems. Refer to the text for details.

The T_B-DNA region, which shares homology to the *tms-1* and *tms-2* genes of the WHR plasmid, was sufficient to induce tumors when provided with an intact set of *vir* genes. Furthermore, mutations in the loci homologous to *tms* led to avirulence on *Nicotiana* plants, suggesting that these loci play an important role in tumor formation by the LHR plasmid. In contrast to the avirulent phenotype that *tms* mutants of the LHR plasmid produced on *N. rustica* and *N. glauca*, *tms* lesions in the WHR plasmid did not eliminate tumor formation on these plants. This was due primarily to a functional *tmr* locus present in the WHR T-DNA which is sufficient for tumor formation on these plants (38). This difference suggested that the LHR plasmid does not contain a functional *tmr* gene despite the DNA homology. In this regard, the lack of homology to the 5′ end of the *tmr* gene is noteworthy and suggests that a portion of the gene is missing. We have recently determined the nucleotide sequence of the LHR *tmr* region and found that in fact the entire 5′ end of the gene is deleted (M. Yanofsky, B. Lowe,

A. Montoya, B. Krul, M. Gordon, and E. Nester, submitted for publication). We have also observed that on plants such as *N. rustica* the wild-type LHR plasmid induced rooty tumors, a phenotypic characteristic which is analogous to a *tmr* mutation of the WHR plasmid. Similar observations have recently been made (6, 16). Although the LHR *tms* loci are required for tumor formation on *Nicotiana* plants, they are not needed to induce tumors on *Vitis* plants. This indicates that loci in addition to *tms* play a role in oncogenesis by the LHR plasmid. Virulence assays on *Vitis* plants yielded results different form those of similar assays on *Nicotiana* plants, and this emphasizes the importance of assaying mutant strains on their natural host (i.e., *Vitis* plants).

We have described a deletion derivative of LHR plasmid pLHRΔ7 which further reduced the number of plants which are susceptible to tumor formation by the LHR strain. This deletion led to avirulence on *N. glauca* and *Vitis* sp. cv. Seyval plants but had no observable effect on the tumor response on *N. rustica* and *V. labruscana* cv. Steuben. Therefore, a region of the LHR Ti plasmid is required for virulence only on certain plants. The precise location of this region is not known, although it probably maps outside the T_A-DNA region because the T_B-DNA region was sufficient to induce tumors on *N. glauca* and *Vitis* sp. cv. Seyval (see below). Furthermore, a host-specific virulence function, designated *virF*, has been mapped on the WHR plasmid to the left of the T-DNA region (18). This function is required for efficient tumor formation on *N. tabacum* and tomato, but is not needed to induce tumors on *N. rustica* (18). Further analysis should determine whether the LHR plasmid has a functional equivalent to the WHR *virF* locus.

We further characterized the role of the T_A-DNA and T_B-DNA regions in tumor formation by mobilizing individual clones covering each T-DNA region into an avirulent strain which carries the WHR *vir* region. The T_B-DNA region in strain LBA4404(pVCK431) was sufficient to induce tumors when provided with an intact set of *vir* loci. This suggests that for at least some plants, loci within the T_A-DNA are not required for tumor formation. These results also indicate that the WHR *vir* loci can mobilize the LHR T-DNA into plant cells, suggesting that the LHR and WHR plasmids use very similar mechanisms for T-DNA transfer. This is consistent with previous studies which showed that the WHR T-DNA could be mobilized by *vir* loci from a variety of strains (18). Furthermore, strain LBA4404(pVCK431) induced tumors on *K. diagremontiana*, a plant which is resistant to infection by the LHR strain. This indicates that the host range of the LHR strain can be expanded by the WHR *vir* region and suggests that one or more *vir* loci may be deficient on the LHR plasmid.

Our results indicate that there is a tremendous variation among plants in terms of their susceptibility to tumor formation by various *Agrobacterium* spp. strains. This variation occurs not only between different plant species, but also between different varieties of plants within the same genus. The LHR strain was avirulent on *N. tabacum* but induced unorganized tumors on *N. glauca* and rooty tumors on *N. rustica*. The WHR strain induced unorganized tumors on all three of these *Nicotiana* plants. Although the LHR strain is tumorigenic on a wide variety of *Vitis* plants (B. Lowe, Ph.D. thesis, University of Rhode Island, Kingston, R.I., 1985), the WHR strain was avirulent on some of these same plants. This avirulence of the WHR strain was apparently not due simply to the lack of a tumorous response but rather to a hypersensitive response in which the plant cells at the wound site were killed. It will be interesting to determine both the plant and Ti plasmid factors which contribute to this hypersensitive response.

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