Limited-host-range plasmid of Agrobacterium tumefaciens: molecular and genetic analyses of transferred DNA.

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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, T1-DNA and T2-DNA, are separated by approximately 25 kilobases of DNA which is not maintained in the tumor. The T1-DNA region resembles a deleted form of the wide-host-range Ti1-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 T2-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 T2-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 infecting 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 S (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 pTiA63 (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 tripapetal 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 BamHI. 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 DNAase 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 (≅Tm, −17°C) and low-stringency washes at 65°C in 6× SSC (≅Tm, −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 Tn5 insertion with BamHI 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 Tn5 resulted in a deletion of the appropriate fragments of Ti plasmid DNA. Each deletion began at the BamHI site within the Tn5 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 BamHI, 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- and 1.2- and 0.75-kilobase (kb) BamHI fragments and retained the flanking 9.5- and 2.1- and 4.6-kb BamHI fragments (Fig. 1 and Fig. 2). It should also be noted that pVCK431 hybridized to the 11.5- and 6.4-kb BamHI fragments within pVCK418 and to a 13-kb BamHI fragment in an unknown region of the plasmid (Fig. 1). Furthermore, this probe hybridized to fragments from the coresident plasmid pPH11, 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 inoculation. Vitis plant inoculations were performed at the base of division sites, 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 Kalanchoe 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 T5-DNA, covered approximately 5 to 7 kb, and the rightward region, designated T9-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 HindIII are shown in Fig. 4. For each blot, lanes 5 and 6 represent recombination products 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-
tion. Junction fragments are defined as noninternal fragments which are formed by joining plasmid and plant sequences or rearranged plasmid sequences. HindIII 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 HindIII 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 HindIII 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 BamHI fragments at the right end of the T_a-DNA region and to a 13-kb BamHI 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 HindIII 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 HindIII 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 KpnI 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).

As with the TA-DNA, the TB-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 HindIII and 1.3-kb EcoRI fragments within the TA-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 BamHI fragments within the TB-DNA region but did not show any homology to the TA-DNA region (probe c). The tms-1 locus hybridized primarily to the 1.0- and 0.4-kb BamHI fragments within the TB-DNA region (probe d). This locus also hybridized weakly to the 2.1-kb HindIII fragment within the TA-DNA region. Another tms-1-specific probe (probe e) hybridized only to the TB-DNA region, and no homology was detected within the TA-DNA. These data suggest that an intact set of tms genes resides only within the TB-DNA region.

Homology to the region which specifies transcript 5 of the

FIG. 4. Genomic Southern blotting of tumor lines. HindIII (H), BamHI (B), EcoRI (E), SalI (S), XhoI (X), and KpnI (K) restriction sites are designated for the TA-DNA and TB-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 HindIII and 8.0-kb EcoRI fragments in the TA-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 HindIII and 3.4-kb EcoRI fragments within the TA-DNA region (probe h). A faint hybridization signal was also detected at the position of the 2.1-kb BamHI fragment within the TB-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 TA-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 (pLHRA7 [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 TA-DNA region is dispensable for tumor formation on these plants. In contrast, this same deletion on an avirulent phenotype on N. glauca and Vitis sp. cv. Seyval.

Another derivative, pLHRA11, had the region of TB-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 pLHRA11 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. Tn5 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. Tn5 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.

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 TA-DNA region (pVCK418) or the TB-DNA region (pVCK431) were independently 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 Kalanchei stens (Fig. 6), indicating that the TB-DNA region alone was sufficient to induce a tumorous response. Strain LBA4404(pVCK418) was unable to induce tumor formation, indicating that the TA-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 TA-DNA region contributed to the tumorous response. Furthermore, strain LBA4404(pVCK418) induced tumors on V. labruscana cv. Steuben, indicating that the TA-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 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

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**TABLE 1. Mutational analysis of LHR T-DNA region**

<table>
<thead>
<tr>
<th>Plants</th>
<th>Strain: LHR</th>
<th>Δ7</th>
<th>Δ11</th>
<th>WHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. tabacum</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>N. glauca</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>N. rustica</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Vitis sp. cv. Seyval</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>V. labruscana cv. Steuben</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

[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.

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**TABLE 2. Functional analysis of TA-DNA and TB-DNA regions**

<table>
<thead>
<tr>
<th>Plants</th>
<th>Strain or plasmid</th>
</tr>
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<tbody>
<tr>
<td>N. glauca</td>
<td>+</td>
</tr>
<tr>
<td>N. rustica</td>
<td>+</td>
</tr>
<tr>
<td>Kalanchei spp.</td>
<td>+</td>
</tr>
<tr>
<td>Vitis sp. cv.</td>
<td>+</td>
</tr>
<tr>
<td>Seyval</td>
<td>+</td>
</tr>
<tr>
<td>V. labruscana cv. Steuben</td>
<td>+</td>
</tr>
</tbody>
</table>

[a] Cosmid clones (24) covering the LHR TA-DNA (pVCK418) or TB-DNA (pVCK431) regions were assayed for their ability to confer tumorigenicty 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.
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, TA-DNA resembles a deleted form of the WHR TL-DNA in which all the DNA from gene 7 through the 5' end of the tmr gene is missing. The TA-DNA region 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 TA-DNA region to the 5' end of the tmr gene or to genes 7, tms-2, and tms-1. Although the TA-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 TA-DNA of the LHR plasmid and contribute to the tumor response. Further studies are needed to identify the specific functions of these loci. The TA-DNA region also contains a functional octopine synthase gene, since transposon insertions into this locus result in tumors which do not synthesize octopine.

The TB-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,

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.

FIG. 6. Inoculation of LBA4404(pVCK431) on Kalanchoë stems. Refer to the text for details.
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 tmr 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 tmr play a role in oncogenesis by the LHR plasmid. Virulence assays on Vitis plants yielded results different from 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 pLHRA7 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 but had no observable effect on the tumor response on N. rustica and V. labrascana 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 Tc-DNA region because the Tc-DNA region was sufficient for inducing 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 Tc-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 Tc-DNA and Td-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 Td-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 Tc-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. diagremoniana, 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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LITERATURE CITED


