



FIGURE 6.13
Glyphosate and its mode of action. Glyphosate, acting as an analog of phosphoenolpyruvate, inhibits the formation of 5-enolpyruvylshikimate 3-phosphate, a precursor of aromatic amino acids.

transgenic plants will promote the use of safer, more biodegradable herbicides, perhaps in smaller amounts. One example involves the herbicide glyphosate, which inhibits 5-enolpyruvylshikimate 3-phosphate synthase – an enzyme involved in the biosynthesis of aromatic amino acids – by acting as a structural analog of phosphoenolpyruvate (PEP; Figure 6.13).

This enzyme was purified from crop plants and sequenced, and DNA probes corresponding to its amino acid sequence were synthesized. These probes were used to isolate cDNA for the enzyme from the cDNA library of a plant cell line known to overproduce 5-enolpyruvylshikimate 3-phosphate synthase. The cDNA was then cloned behind the strong CaMV 35S promoter and ahead of the nopaline synthetase terminator, and the gene complex was introduced into plant cells (e.g., petunia) via a disarmed Ti plasmid vector. The transgenic plants produced a much higher level of the target enzyme and thus were significantly more resistant to glyphosate (Figure 6.14). These results were encouraging because glyphosate has very low toxicity to animals and is rapidly degraded in soil.

An obvious improvement to this strategy is to use DNA coding for a glyphosate-resistant mutant enzyme for the construction of transgenic plants. Early experiments using the genes from glyphosate-resistant mutant



FIGURE 6.14
Production of glyphosate-resistant plants. The gene for 5-enolpyruvylshikimate 3-phosphate synthase, cloned behind a strong promoter, was introduced into petunia plants as described in the text. Three weeks after these transgenic petunia plants (top) and unaltered control plants (bottom) were sprayed with Roundup (a pesticide containing glyphosate), the control plants were dead but the transgenic, resistant plants were completely healthy. [From Shah, D. M., et al. (1986). Engineering herbicide tolerance in transgenic plants. *Science*, 233, 478–481; with permission.]