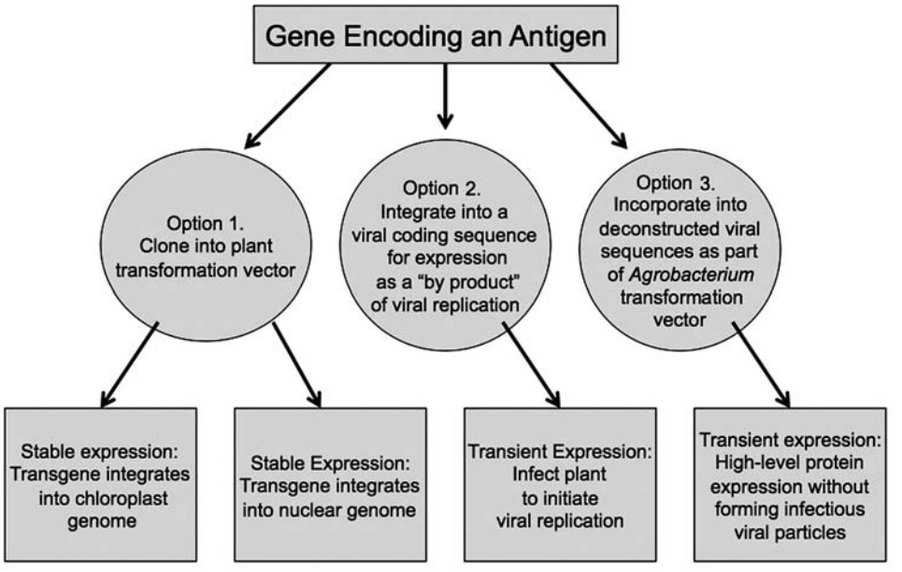

Figure 1 A summary of the various strategies now in use to cause antigen expression and accumulation in plants. Antigen-encoding genes derived from pathogens are often resynthesized to optimize their expression in plant cells (codon usage, removal of cryptic introns, etc.). The most widely utilized option for expression of these genes has been to create transgenic plants (Option 1) in which either the chloroplast or the nuclear genome is stably transformed so that each cell in the resulting transgenic plant is a potential biomanufacturing center for the protein immunogens (2,3,7,8,11,34). In these plants, gene expression can either be constitutive or induced by exogenous agents. In Option 2, single-stranded RNA virus genomes are converted to DNA for manipulation in bacterial plasmids, a new gene encoding the antigen is inserted under a viral promoter control, and RNA is transcribed and used as the infectious agent to initiate viral genome amplification with the resulting concomitant production of the antigen (35–37). A variant of this approach is the engineering of the viral coat protein to cause expression of fused epitopes on the surface of the virus (37). In Option 3, viral RNA is also converted into a DNA sequence, engineered with a new gene, but the DNA is then moved into *Agrobacterium*; the bacterium is then infiltrated into leaves to express RNA from the DNA sequence and achieve ''deconstructed virus'' replication with the concomitant expression of the desired antigen (35,38–44).

The concept of using plant biotechnology to produce subunit vaccines has evolved over the last decade and a half of research. The initial focus was on the production of antigens in a food crop and then utilizing this ''edible vaccine'' as a means of oral immunization (1,3,6,7). The concept has been validated in preclinical and human clinical trials; both serum and mucosal antibody responses to food-delivered antigens have been documented in animals and humans (2,12–32). While these observations have stimulated a high level of academic interest, there has been no comparable corporate acceptance of ''food-delivered vaccines'' (2,11,33,34). Factors such as variability of antigen content in plant tissues, uncertainty of antigen stability during storage and transport, and a lack of clear path to licensure of plant-made vaccines have been perceived as major obstacles to introduction of a product. As a result, the focus of many plant-made vaccine efforts since about 2005 has moved to integrate plant-based antigen production with traditional downstream processing to yield purified immunogens that can be formulated and delivered in conventional vaccine systems. Since processing costs are directly related to antigen expression levels in cells or tissues, plant biotechnology efforts have largely concentrated on finding methods to enhance antigen accumulation. Chief among the emerging strategies to achieve high levels of antigen accumulation has been a switch from using transgenic approaches to transient, viral vector–based approaches. These changes in strategies and techniques are discussed in the following sections. The various options for achieving antigen accumulation in plants are summarized in Figure 1.

PLANT-DERIVED VACCINES IN CLINICAL TRIALS

The concept of producing subunit vaccine antigens in transgenic plants was first published in the scientific literature by describing the expression of hepatitis B surface antigen (HBsAg) in tobacco plants (13). Following the publication of their pioneering paper, the group headed by Arntzen and Mason continued to develop the concept of plant-based vaccines and reported their work in a succession of papers. The initial report focused on the expression and structure of the plant-produced HBsAg, which assembled into 22-nm virus-like particles (VLPs) similarly to the yeast-derived commercial vaccine antigen. Partially purified and concentrated tobacco-derived HBsAg was used in parenteral immunization experiments in mice, demonstrating its ability to invoke the expected B and T lymphocytic responses (14). To further prove that plant-derived HBsAg could stimulate mucosal immune responses following oral delivery, the group refocused their attention to expression in potato tuber. Surprisingly, the plant-derived material proved superior to the yeast-derived antigen in both priming and boosting immune responses in mice (15). Success in these preclinical trials led to phase I studies with potatoes expressing HBsAg (16).