In the absence of *N*-glycosylation, mAbs are more susceptible to thermal and chemical denaturation and to proteolytic degradation. For example, denaturation of three different mAbs due to high temperatures was protected by approximately 10% due to glycosylation (Ionescu et al., 2008; Stanley et al., 2009; Zheng et al., 2011). Furthermore, the susceptibility to chemical denaturation via guanidine hydrochloride of the same three nonglycosylated mAbs was increased by 0.6 M, and their susceptibility to preotolytic degradation by papain was reduced over a 24-h incubation period (Zheng et al., 2011). Thermal and chemical denaturation of mAbs is of particular interest to downstream processing since both parameters may contribute to complications due to aggregation and diminished shelf life (Zheng et al., 2011).

Despite the high degree of conservation in glycosylation patterns in eukaryotes, *N*-glycosylation mutants of *Arabidopsis thaliana* show no abnormal morphology. However, improper *N*-glycosylation in humans has been correlated with the pathogenesis of various acquired and immunological diseases (Nagels et al., 2012; Strasser et al., 2004b; Varki and Freeze, 2009; Xue et al., 2013). With regard to therapeutic proteins, proper *N*-glycosylation is of particular concern as it is required to maintain the pharmaceutical efficacy of the therapeutic; improper *N*-glycosylation may result in diminished therapeutic efficacy and could also lead to immunogenicity in the patient.

17.4.3 *N*-Glycan Profiles of Plants and Mammals

Kingdoms *Plantae* and *Animalia* share core glycan structures but differ in accessory glycan linkages in the following ways: (1) as shown in Figure 17.2C, mammalian *N*-glycans contain α1,6-fucose linked to the proximal *N*-acetylglucosamine; the ends of the branched *N*-glycans are capped with a penultimate β1,4-galactose and a terminal α2,6-*N*-acetylneuraminic acid (sialic acid), though mAbs are not typically sialylated at asparagine-297, and (2) as shown in Figure 17.2A, plant *N*-glycans contain a bisecting β1,2-xylose linked to the core mannose; α1,3-fucose linked to the proximal *N*-acetylglucosamine; and terminal β1,3-galactose (Bosch et al., 2013). The presence of β1,2-xylose and α1,3-fucose is of particular concern for plant-produced pharmaceuticals, as these two sugars are potentially immunogenic to humans.

17.4.4 Immunogenicity of β1,2-Xylose and α1,3-Fucose

Antibodies against biopharmaceuticals, including mAbs, have been detected in the serum of patients in both preclinical and clinical trials (Malucchi and Bertolotto, 2008; Subramanyam, 2008). These antidrug antibodies result in the premature clearance of biopharmaceuticals in patients, thereby reducing the efficacy of the drug, though in some instances no adverse effects on therapeutic efficacy were reported, as in the case of recombinant human growth hormone (Malucchi and Bertolotto, 2008).

In mAbs, two potential structural domains contribute to its potential immunogenicity. Both foreign complementarity-determining regions (CDRs) on humanized antibodies and glycans that contain xenogeneic sugars, such as β1,2-xylose and α1,3-fucose, can elicit a host immune response (van de Weert and Møller, 2008). Although the humanization of murine mAbs by replacement of framework regions