Food allergy is being reported more commonly in many countries, especially among children, which may, in the long run lead to nutritional deficiencies and growth delay as well as psychological and psychosocial burdens, as strict avoidance of food is the only solution available. However, there are a number of promising therapeutic strategies currently being investigated for the treatment of food allergies. Allergen–specific approaches, such as various forms of immunotherapy, have been a major focus of investigation and appear to be promising methods of desensitization. More recently, the addition of anti–IgE monoclonal antibodies (mAbs) to immunotherapy regimens has been studied. Early work with antigen–fixed leukocytes in a murine model has shown promise in inducing tolerance, as have vaccines containing modified recombinant food proteins co–administered with heat–killed Escherichia coli. Non–specific approaches include a Chinese herbal formulation, anti–IgE mAbs, and Trichuris suis ova therapy. The array of treatment modalities currently being investigated increases the likelihood of finding one or more effective therapies for the treatment of food allergy (Henson and Burks, 2012).

The failure to develop oral tolerance or a loss of oral tolerance has been hypothesized to be the primary problem in food allergy. The GI tract plays a critical role in the development of oral tolerance, as it is the largest immunologic organ in the body. The GI tract must perform a balancing act, processing ingested food into a form that can be absorbed and used for energy and growth, while at the same time preventing the entry of harmful pathogens into the circulation. It accomplishes this through both physiologic and immunologic mechanisms, and disruption of any of these pathways may lead to breakdown in oral tolerance induction (Kumar et al., 2012).
The physiologic barrier is comprised of a single-cell layer of columnar epithelial cells joined by tight junctions and covered with a mucous layer that collectively works to keep the internal sterile environment separate from the outside world. Normally, when food is ingested, luminal and brush border enzymes, bile salts, and gastric acids break down food proteins, rendering them less immunogenic. These same factors also serve to destroy pathogens. Alterations in gastric pH with the use of antacids have been shown to impede gastric protein digestion, thereby inducing a higher risk for food sensitization. In mice, antacid treatment with sucralfate induced changes in the structure of the gut epithelium and villi, as well as an increase in eosinophils and mucus-producing cells in the intestine. Additionally, altered intestinal permeability, whether genetically predetermined or as seen in various disease states as well as during the newborn period, may promote sensitization via increased exposure to intact proteins (Sicherer and Sampson, 2010).

Another allergen-specific approach being investigated is the administration of a vaccine containing recombinant modified proteins. This approach has been pursued because modifying the antigenic epitopes can diminish the risk of immediate allergic reactions during immunotherapy. This is accomplished with point mutations introduced by site-directed mutagenesis or with protein polymerization, leading to improvements and development of immunotherapeutic strategies; major bottlenecks for further diffusion of allergen specific immunotherapy can be observed (Calderon et al., 2012). New extract preparations and especially vaccines containing molecular components require validation. The complexity of possible component combinations requires novel bioinformatic approaches. Studies exploring cost-effectiveness of immunotherapeutic treatments for allergic rhinitis and asthma are still lacking and should be assessed in relation to the various health systems across the world. The macro-economic impact of allergies and the long-term cost-effectiveness of allergen specific immunotherapy need further detailed evaluation. There is a need for extensive studies to bring promising new
biotechnological innovations, such as biological agents, vaccines of modified allergen molecules and engineered components for allergy diagnosis, closer to clinical practice.

Another modification of molecules introduced into the allergy research is application of allergenic peptides. The peptides lose the features of IgE-binding epitopes while retaining features of the T lymphocyte epitopes. Studies aimed to target exclusively the T cell receptor (TCR) are supposed to increase the efficacy of the immune tolerance induction and to reduce the IgE-mediated side effect of the immunotherapy. The use of recombinant DNA technology appears to provide a realistic means of achieving improvements in obtaining precisely defined preparations. The genetic modifications result in hypoallergenicity of the allergen derivatives i.e., recombinant allergens and peptides. Natural allergen extracts can thus be substituted by pure recombinant wild-type allergens or single recombinant fusion proteins consisting of several wild-type allergens (Jutel et al., 2012).

Establishing the protein molecular structure as well as the immune function of a certain natural allergen and its epitopes enabled cloning of allergen proteins with use of recombinant DNA technology. The first recombinant allergens were synthesized with no pre-translational interference in their molecular structure and presented the same or very similar allergenicity as the wild-type proteins, assessed in skin tests, serum IgE-binding assays or basophil degranulation tests. However, some of the recombinant proteins vary in similarity to their native counterparts, as some of the wild-type allergens undergo post translational modifications. The genetic manipulations on allergens and their single epitopes open new prospects for scientific research on immune response as well as for clinical diagnostics and treatment of immune-mediated diseases.
Only some sequences or sections (epitopes) in all allergens are responsible for their immunogenicity and allergenicity. The concept of recombinant allergen–based component–resolved diagnostics (CRD) and immunotherapy (CRIT) was introduced in 1999 by Valenta (Valenta et al., 1999). This approach advocates the use of well–defined allergens (components), for diagnosis of IgE–mediated allergy. Accordingly, application of CRIT is aimed to treat the patient with the clinically relevant allergens from a specific source only.

The use of microarray tests allows a parallel analysis of IgE binding to large numbers of single allergens or peptides. However, the technique has also certain limitations, as some allergenic sources (almond, walnut) or some important allergens in certain allergenic sources are still missing, e.g., peanut Ara h 6 antigen. However, the large amount of data from a single microarray test becomes a challenge for interpretation in terms of its clinical significance. The microarray assay for TLP was performed for different plant TLPs by Palacín et al. (2012).

TLP family genes are known for diversification. As observed in poplar genome, analysis of the poplar TLP family suggests that the expansion of this gene family was followed by diversification, as differences in expression patterns and predicted properties correlate with phylogeny (Petre et al., 2011). Also, by sequence analysis of the *Carica papaya* genome, three different forms of TLPs were identified, where the latex TLP constituent belonged to a well–known form, allowing the molecular modeling of its spatial structure (Looze et al., 2009). A wider phylogenetic analysis of eukaryote TLPs – including plant, animal and fungi sequences shows that TLP gene content and diversity increased markedly during land plant evolution. Mapping the reported functions of characterized TLPs to the eukaryote phylogenetic tree showed that antifungal or glycan–lytic properties are widespread across eukaryote phylogeny, suggesting that these properties are shared by most TLPs and are likely associated with the presence of a conserved acidic cleft in their 3D structure. Petre et al. (2011) established
an exhaustive catalog of TLPs with atypical architectures such as small-TLPs, TLP-kinases and small-TLP-kinases, which have potentially developed alternative functions (such as putative receptor kinases for pathogen sensing and signaling).

TLPs need to be investigated thoroughly for their lesser known functions. Molecular studies of TLP expression, localization and functional activity support a role for TLPs in host defense during pathogen infection. Up-regulation of TLPs has been described in many higher plants infected by pathogens such as bacteria, oomycetes and fungi. Localization studies revealed that plant pathogen-inducible TLPs are secreted into the apoplast. More than 20 TLPs from animals, fungi and plants have been shown to exhibit an antifungal activity, although the mechanisms by which TLPs exert this activity remain unclear. Several antifungal modes of action have been described such as membrane permeabilization, β-glucan binding and degradation, inhibition of enzymes such as xylanase, α-amylase, or trypsin, as well as an apoptosis-inducing mechanism reported in yeast. Other functional properties have been reported for TLPs, including antifreeze activity, protection from abiotic stress and binding to proteins such as actin, viral CMV-1 protein, yeast glycoproteins and G-Protein Coupled Receptor (GPCR) or to hormones such as cytokinins (Liu et al., 2010). All these aspects open up new avenues for carrying out investigations on sapodilla acidic and basic TLPs.

Future perspectives that can be addressed in the field of fruit allergy, based on the important findings from this thesis, can be pointed out briefly. First of all, the total numbers of genes present in the sapodilla TLP family and their chromosomal locations are not known at present in order to assess whether it belongs to a multigene family. The cellular/tissue localization of sapodilla TLPs needs to be explored in relation to their signal sequence and expression profiles in different tissues. Whether the weak β-1,3-glucanase activity of sapodilla acidic TLP correlates to the antifungal activity is yet to be ascertained in terms of
assigning a biological function for this protein. Clinical and immunological cross–reactivity studies of sapodilla–allergic subjects with subjects allergic to other fruits/pollens/vegetables need to be investigated thoroughly to identify other TLP cross–reactive allergens. Finally, from the point of allergenicity features of sapodilla acidic TLP, results from in silico analyses should be extended in order to identify the critical residues in the IgE epitopes for the generation of allergic responses, for which recombinant sapodilla acidic TLP expressed in a heterologous system is absolutely required.