Xyloglucan is the predominant hemicellulose in primary cell walls of dicots and several monocots. Enzymes that cleave the xyloglucan backbone at unbranched glucose residues have been identified in glycoside hydrolases (GH) families 5, 7, 12, 16, 44, and 74. In this work three GH12 glucanases from *Aspergillus clavatus* (*AclaXegA*), *A. zonatus* (*AspZo*) and *A. terreus* (*AtEglD*) were studied. *AclaXegA* is strictly specific for xyloglucan while *AtEglD* and *AspZo* cleave beta-glucan and xyloglucan. The phylogenetic tree of Aspergilli GH12 enzymes reveals two well-defined clades representing endoglucanases and xyloglucanases. *AspZo* was included in the xyloglucanases group based on phylogenetic and structural characteristics, as well as *AclaXegA*, but based on functional analysis its enzymatic activity was characteristic of promiscuous endoglucanases such as *AtEglD* *AspZo* supposedly represent a link between two group of enzymes. The main objective of this work was to understand why some GH12 enzymes are strictly specific for xyloglucan hydrolysis. We characterized these enzymes by biochemical studies and Molecular Dynamics Simulations (MDS). The first question was why *AclaXegA* has no activity on beta-glucan? By MDS we conclude that the region of *AclaXegA* that accommodates the substrate reducing-end is primarily composed by non-polar residues and the unbranched glucose of beta-glucan does not bind properly. However, *AtEglD* and *AspZo* are primarily composed by polar residues in the equivalent region and properly bind unbranched glucoses. The second question was what is the key residue for xyloglucan hydrolysis? The residue Y111, localized at the loop 1 and conserved in all the endoglucanases analyzed, is essential for the catalysis of xyloglucan by *AtEglD*. Moreover, the Y111 has equivalent residues in *AspZo* (Y30) and *AclaXegA* (Y19). In summary, *AspZo* has a deletion in the loop1 (corresponding to Y111 in *AtEglD*) but has an equivalent residue that determine the hydrolysis of xyloglucan (Y30), as well as *AclaXegA* (Y19).

**Key words:** Xyloglucanase; GH12; Xyloglucan.