INTRODUCTION

In Tunisia, almond (Family: Rosaceae; Genus: Prunus) holds a very important place in agriculture after the olive tree. Almond plantations are spread across all the country and they are characterized by a relatively high genetic diversity (Gouta et al., 2008, 2010).

Almond studies concerned mainly its ecology as well as its physiology. Little work has concerned the metabolism of almond seeds and their germination. We are interested by enzymes involved in germination (Bahri, 2012) and in particular in the study of alpha amylase.

Alpha amylases (EC 3.2.1.1) are α-(1-4) D-glucan glucohydrolases which catalyze (α-(1-4) linkages in starch and any related oligosaccharides to produce D-glucose, D-maltose and a small amount of maltodextrins (Mérière, 1985; Graber, 1989). They have been classified in the family of glycosyl hydrolases 13: GH13 (Davies and Henriass, 1995). Amylases from microorganisms have been extensively studied (Ben Abdelmalek et al., 2009; Kant, 2009).

Protein structure homology modeling has become a routine technique to generate 3D model for proteins when experimental structures are not available (Blasi et al., 2014). Plant amylases are generally considered to be involved in the metabolism of germinating seedling and Biotechnology (Khalid, 2013). Another species, like Prunus persica, of the same genus and in the same family as the almond tree (Drielwanger, 2002) has been much-studied (Han et al., 2015).

To understand almond alpha-amylase mechanism, we combined bioinformatics and biochemistry. In a first step and in the absence of an almond alpha-amylase sequence in the databases, our aim is to propose, for the first time, a Prunus persica alpha amylase fold by homology modeling with Phyre 2 server (Kelley and Sternberg, 2009).

MATERIAL & METHODES

1. Plant material: Samples of Tunisian almond seeds (Prunus amygdalus Mill., var. “Tunio”), were collected on 2012 and kindly provided by the “Olive Tree Institute” (Sfax-Tunisia).

2. Germination: Almond seeds were germinated at 26 ±1°C in the darkness at different stages of germination (Bahri, 2012).

3. Activity assay: The alpha-amylase was extracted and identified by the “glucose oxidase” method (Dinggo, 1975).

4. Homology modelling:
   - The primary structure of alpha-amylase from Prunus persica (UniProt database, code: M5V6U6).
   - The primary structure of our target protein was submitted to the program server (Phyre 2: Protein Homology/analogy Recognition Engine) which returned a list of candidate 3D structures along the alignment of the target sequences with the corresponding template sequence.
   - The 3D model was selected based on the quality of the alignment.
   - The stereochemical assessment was then achieved by constructing the Ramachandran Plot (Sheik, 2002; Kelley and Sternberg, 2009).

CONCLUSION

For the first time:
• An alpha amylase fold has been proposed characterized by the well known beta/alpha fold.

• Alpha-amylase structure seems to be preserved between two different phylogenetic species (Prunus persica and Hordeum vulgare).

• This folding was also observed in an animal species: Sus scrofa.

In order to preserve hydrolysis function, could alpha amylase from germinating seedlings (Prunus amygdalus Mill.), has a conserved beta/alpha fold like Prunus persica and as described in microorganisms (Ben Abdelmalek et al., 2009; Tayyaba et al., 2014). Further investigations would allow us to determine it.

RESULTS

1. Three dimensional structure of Prunus persica α-amylase:
   - The sequence contains 401 residues (Fig. 1).
   - The template used for the construction of the model is an orthologous protein sequence of Hordeum vulgare alpha amylase (PDB code: 2QPU), a plant member of the Poaceae family.
   - The template selection by Phyre 2 is highly trusted as judged by the confidence level score (100) which shows that both the target and the template sequences are homologous with an identity value of 65%.
   - The structure shows a beta/alpha fold with small deviation from the template 3D coordinates even in the loop regions.
   - The model presents 9 alpha helices surrounding a hydrophobic core, consisting mainly on a beta sheet layer. The enzyme also presents two other exposed beta sheets on the protein surface with 5 and 2 strands.

Fig. 1. Three dimensional structure of Prunus persica: (a) Ribbon representation of three dimensional structure of Prunus persica alpha amylase; strands are shown in yellow and helices in purple. (b) Active site of Prunus persica three dimensional structure.

The Ramachandran Plot (Fig. 2)

• 96% of all φ/Ψ angles residues are located in the favored regions.
• 4% in the allowed regions
• no residue located in the outlier zone of the Ramachandran Plot.

Fig. 2. Prunus persica Ramachandran Diagram.

This result is in agreement with that described by Stensson (1994) in which diverse alpha amylases contains a characteristic catalytic (β/α)-barrel domain.

2. Presence of alpha-amylase activity in almond germinating seedlings:

In a previous biochemical study (unpublished data) we have identified the presence of an alpha amylase during the germination of almond (Prunus amygdalus Mill) seedlings. The enzyme showed an optimal activity at the 4th step of germination and has kinetic parameters Vmax = 2.5 U/l and Km = 6.32 mM.

References


